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Par

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Etudes phytochimique et biologique de cinq plantes de la famille des Solanaceae

Phytochemical study and biological evaluation of five plants belonging to the family Solanaceae

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Résumé

Ce travail de recherche a pour objectifs d'évaluer les activités antibactériennes, antiprolifératives et antioxydantes des extraits de feuilles de *Solanum incanum* L., *S. schimperianum* Hochst, *S. nigrum* L., *Physalis lagascae* Roem. & Schult. et *Withania somnifera* (L) Dunal. Plus précisément, les activités antibactériennes, anti-prolifératives et antioxydantes ont été déterminées à partir des extraits méthanoliques et des fractions de glycoalcaloïdes stéroïdiens (SGAF) de chaque plante. La sensibilité des bactéries, à Gram positif et à Gram négatif, était variable en présence de chacun des extraits (valeurs des IC_{50} dans la gamme de 15 > 1000 µg / mL). L'extrait méthanolique de la feuille de *S. schimperianum* a montré une activité anti-proliférative intéressante contre les lignées cellulaires humaines testées avec des valeurs de CI_{50} variant de 2,69 à 19,83 µg / mL tandis que l'activité la plus élevée des fractions de feuilles (SGAF) de *W. somnifera* a montré des valeurs IC_{50} variant de 1,29 à 5,00 µg / mL. Les fractions SGAF de toutes les espèces ont montré une activité antiradicalaire plus élevée que leurs extraits méthanoliques. La fraction SGAF de *S. schimperianum* a montré l'activité antioxydante la plus forte avec une valeur CI_{50} 3,5 ± 0,2 µg / mL pour le test DPPH et 3,5 ± 0,3 µg / mL pour le test ABTS. L'analyse GC-MS des extraits méthanoliques et des fractions SGAF des espèces étudiées a révélé la présence d'alcaloïdes stéroïdiens, de saponines stéroïdiennes, de stéroïdes et d'autres composés comme des terpènes, des phénols et des alcanes. Leur répartition variait selon les espèces et, ce qui peut fournir des éléments pour évaluer les relations chimiotaxonomiques préliminaires. Douze dérivés de l'acide hydroxycinnamique ont été identifiés dans l'extrait méthanolique de la feuille de *S. schimperianum* et le composé N-caffeoyl agmatine était majoritaire. La présence d'alcaloïdes stéroïdiques comme la solanopubamine et la solanocapsine, ainsi que des dérivés des alcaloïdes 3-amino stéroïdes a été notée. De plus, trois composés, quercétine, kaempférol glycosylé et le β-sitostérol, ont été isolés et identifiés.

Mots-clés: *Solanum* species, *Physalis lagascae, Withania somnifera,* activité antibactérienne, activité anti-proliférative, activité antioxydante, amides de l'acide hydroxycinnamique, alcaloïdes stéroïdiques.

Abstract

This study aimed at the evaluation of *in vitro* antibacterial, antiproliferative and antioxidant activities of methanolic leaf extracts and steroidal glycoalkaloids fractions (SGAFs) of *Solanum incanum* L., *S. schimperianum* Hochst, *S. nigrum* L., *Physalis lagascae* Roem. & Schult. and *Withania somnifera* (L) Dunal. The sensitivity of Gram-positive and Gram-negative bacteria to each extract was variable (IC_{50} values in the range of 15->1000 µg/mL). The methanolic extract of *S. schimperianum* leaf demonstrated interesting anti-proliferative activity against the human cell lines tested with IC_{50} values in the range of 2.69 to 19.83 µg/mL while the highest activity from the SGAFs was obtained from *W. somnifera* leaf with IC_{50} values in the range of 1.29 to 5.00 µg/mL. The SGAFs of all species demonstrated higher scavenging activity than their respective methanolic extracts. The SGAF of *S. schimperianum* displayed the strongest antioxidant activity in both assays with IC_{50} value $3.5 \pm 0.2_{DPPH}$ and $3.5 \pm 0.3_{ABTS}$ µg/mL. GC-MS analysis of methanolic and SGAFs extracts of the studied species revealed the presence of steroidal alkaloids, steroidal saponins, steroids and other compounds like terpenes, phenols and alkanes. Their distribution varied among the species and thus they could provide evidence to assess preliminary chemotaxonomic relationships. Twelve known hydroxycinnamic acid amides were tentatively identified from the methanolic extract of *S. schimperianum* leaf and N-caffeoyl agmatine appeared with the highest intensity. Moreover, the presence of steroid alkaloids solanopubamine and solanocapsine as well as dehydroderivatives of the 3-amino steroid alkaloids was suggested. Furthermore, three compounds quercetin, kaempferol glycoside and *β*-sitosterol were isolated and identified. *In silico* investigation of these three compounds for their potency against cancer revealed that *β*-sitosterol was found to be the most selective compound against human pregnane X receptor (PXR) and gave the highest binding energy (-11.2 kcal/mol). These results suggested that Solanaceae plants endogenous to Sudan could be a potential source of bioactive agents.

Keywords: *Solanum* species, *Physalis lagascae, Withania somnifera,* antibacterial activity, anti-proliferative activity, antioxidant activity, hydroxycinnamic acid amides, steroid alkaloids.

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List of Abbreviations

ABTS: 2,2'-azino-bis (3-ethylbenzthiazoline- 6-sulphonic acid)

ASA: acetylsalicylic acid

CC: column chromatography

CAM: complementary or alternative medicine

Cfu: colony-forming unit

COSY: correlated spectroscopy

d: doublet

dd: double doublet

dt: doublet de triplet

DMSO: dimethyle sulfoxide

DPPH 1: 1-diphenyl-2-picrylhydrazyl

EI-MS: electron impact mass spectra

FAO: Food and Agriculture Organization

Fwt: fresh weight

GC/ MS: gas liquid mass spectroscopy

GLs: glycoalkaloids

HIV: human virus of Ideas

LC-MS: liquid chromatography coupled to an Orbitrap mass spectrometer

m: multiplet

MIC: minimum inhibition concentration

MHD: Mueller Hinton agar

MHB: Mueller Hinton broth

MS: mass Spectra

MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

NCCLS: National Committee for Clinical Laboratory Standards

NMR: nuclear magnetic resonance

NOESY: nuclear Overhauser enhancement spectroscopy

PXR: pregnane X receptor

qd: quadruplet

s: singulet

SPE: solid phase extraction

RNS: reactive nitrogen

ROS: *r*eactive oxygen species

RT: retention time

t: triplet

TLC: thin layer chromatography

TGA: total glycoalkaloid

UV: ultraviolet

WHO: World Health Organization

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Introduction générale

I. Contexte

On trouve dans le monde de très nombreuses plantes parmi lesquelles certaines sont médicinales. Les plantes médicinales sont de plus en plus attractives car elles offrent à l'humanité de nombreux avantages, spécialement dans les domaines de la médecine et de la Pharmacologie. L'aspect médicinal de ces plantes est lié aux constituants chimiques qu'elles renferment qui produisent différentes actions pharmacologiques. Les composés naturels présents dans des plantes telles que les plantes médicinales, les légumes et les fruits agissent avec les nutriments et les fibres pour lutter contre les maladies ou plus particulièrement pour prévenir les maladies (Akinmoladun *et al*., 2007).

Depuis très longtemps, les médicaments à base de plantes sont utilisés pour soulager les symptômes liés à la maladie. Bien que de grands progrès soient observés dans la médecine moderne au cours de ces dernières décennies, les plantes continuent d'apporter une contribution importante aux soins de santé dans le monde. Ce regain d'intérêt pour les plantes médicinales émane de leur utilisation ancestrale en médecine populaire traditionnelle, en particulier dans les pays en développement (Akinmoladun *et al*., 2007).

Le Soudan présente de grandes variations dans sa topographie, son climat, son sol et son hydrologie. Cela a pour conséquence la présence de différents habitats écologiques, de différentes zones de végétation ce qui est favorable pour le développement d'une flore riche. La médecine populaire soudanaise représente un mélange unique de cultures indigènes de traditions islamiques, arabes et africaines. Par conséquent, de nombreux traitements sont utilisés au soudan pour soigner de nombreuses maladies, qu'elles soient épidémiques ou endémiques. En raison de l'énorme richesse végétale au Soudan, ce pays a une longue tradition en médecine populaire. Les remèdes indigènes sont souvent la seule forme de thérapeutique disponible pour les personnes pauvres vivant dans les zones rurales ou les villes. On estime que seulement 11% de la population a accès à des soins de santé standardisés. Les services médicaux gouvernementaux, qui étaient gratuits dans le passé, sont maintenant payants, à des prix élevés. Souvent, les personnes pauvres ne peuvent pas acheter les médicaments. Étant donné que les soins médicaux au Soudan ne sont pas disponibles ou sont trop chers, la majorité des personnes dépendent des remèdes traditionnels prescrits par des guérisseurs, des tradipraticiens. Les médecins traditionnels au Soudan n'ont pas de

formation officielle ni de pharmacopée et leurs connaissances sont basées sur des compétences transmises oralement et les traditions indigènes. Leur formation ne peut pas être comparée à la médecine traditionnelle chinoise ou à la médecine ayurvédique indienne et sri-lankaise, pour lesquelles une formation sérieuse est assurée (Khalid *et al*., 2012).

La famille des Solanacées est considérée comme une large et une importante famille chez les angiospermes ; les plantes de cette famille sont réparties dans les régions tropicales et tempérées du monde. Une grande diversité est observée à l'Ouest et au Sud de l'Amérique du Sud et seulement dix genres sont originaires de l'ancien et du nouveau monde. Il est à noter que *Solanum*, *Physalis* et *Lycium* sont largement distribués à la fois dans l'hémisphère oriental et occidental (D'Arcy, 1991). Cette famille est composée de 98 genres et environ 2300 espèces appartenant à 14 tribus regroupées dans trois sous-familles (D'Arcy, 1991). Au Soudan, la famille des Solanaceae est répartie dans l'Est et au centre du Soudan. Le genre *Solanum* est économiquement important, il comprend des plantes annuelles, des plantes vivaces, des sous-arbustes, des arbustes et des plantes grimpantes. Beaucoup de ces espèces sont des plantes agricoles, d'autres sont toxiques et peuvent avoir un intérêt médical. Les plantes sauvages de la famille des Solanacées sont généralement utilisées en médecine traditionnelle soudanaise par les praticiens comme agent anti-inflammatoire et en prévention du cancer, ce qui suggère un intérêt potentiel comme source de composés cytotoxiques. En outre, ils sont utilisés en médecine traditionnelle pour soigner la rhinite, le mal de dents et le cancer du sein avec des effets analgésiques et anti-inflammatoires (El Ghazali *et al*., 1986).

II. Présentation de la recherche

Le manque d'efficacité des traitements des maladies infectieuses dans les pays en développement est un problème tragique et c'est l'un des défis les plus sérieux auxquels le monde doit faire face au début de ce siècle. Pratiquement tous les décès dus à des maladies infectieuses se produisent dans les pays à faible revenu ou à revenu intermédiaire. Malgré les progrès réalisés dans la connaissance de nombreuses maladies infectieuses, beaucoup d'entre elles ont continué à causer une morbidité et une mortalité significatives. On estime que les maladies infectieuses causent plus de 90% de toutes les morbidités et les mortalités dans le monde avec plus de 90% d'entre elles dans les pays en développement (Shakya *et al.,* 2012).

Cependant, au cours des dernières décennies, de nombreux antibiotiques et autres médicaments couramment utilisés sont de moins en moins efficaces contre certaines maladies. Ce phénomène s'explique par la toxicité de beaucoup d'entre eux entrainant une mauvaise observance mais aussi en raison du développement de microorganismes pathogènes résistants aux antibiotiques. Ainsi, il est essentiel de poursuivre les recherches pour mettre au point de nouveaux médicaments entrainant moins de résistance.

En outre, le remplacement des antioxydants synthétiques par des antioxydants naturels et sains, ainsi que l'intérêt de l'industrie alimentaire et de la médecine préventive dans le développement d'antioxydants bioactifs naturels, ont favorisé la recherche sur le screening des plantes pour identifier des antioxydants intéressants (Moure *et al*., 2001).

La plupart des traitements anticancéreux ne sont pas sélectifs et affectent toutes les cellules, non seulement les cellules tumorales mais aussi les cellules normales, provoquant ainsi une toxicité systématique ou un risque accru d'autres cancers. Aussi, il est nécessaire de développer des solutions alternatives moins toxiques avec moins d'effets secondaires pour le traitement du cancer, accessibles pour le plus grand nombre de personnes.

Cependant, bien que les plantes médicinales au Soudan soient largement utilisées depuis tous les temps dans la médecine traditionnelle, il est nécessaire de faire des études systématiques et approfondies sur leurs activités biologiques et leur composition chimique en accord avec les données de la littérature. Plusieurs études ont été rapportées sur la taxonomie et sur la morphologie du pollen d'espèces végétales du Soudan appartenant aux Solanaceae (El-Ghazaly, 1990; Harley *et al*., 2004). Cependant il existe très peu d'informations concernant leur teneur en métabolites secondaires, alors que ces plantes sont largement utilisées en médecine traditionnelle au Soudan. C'est la raison pour laquelle nous avons réalisé une étude phytochimique complète sur certaines espèces de Solanaceae endogènes au Soudan.

III. Objectifs

En prenant en compte les faits rapportés ci-dessus, l'objectif principal de cette étude était d'étudier les constituants phytochimiques et les activités biologiques de certaines espèces sélectionnées (*Solanum incanum* L., *S. schimperianum* Hochst, *S. nigrum* L., *Physalis lagascae* Roem. & Schult et *Withania somnifera* (L) Dunal.) de la famille des Solanacées, endogènes au Soudan.

Les objectifs de cette étude étaient d':

1. Evaluer les extraits de feuilles des espèces sélectionnées pour leur
   a) Activité antibactérienne.
   b) Activité antiproliférative.
   c) Activité antioxidante.

2. Effectuer un screening préliminaire pour identifier la présence de métabolites appartenant aux grandes classes de métabolites secondaires en utilisant différentes techniques chromatographiques.

3. Evaluer leurs relations chimio-taxonomiques préliminaires.

4. Effectuer un fractionnement bioguidé pour l'espèce la plus active, *S. schimperianum*.

General Introduction

I. Background

The world is fertile with natural and medicinal plants. Medicinal plants are now more focused than ever because they have the capability of producing many benefits to society indeed to mankind, especially in the line of medicine and pharmacology. The medicinal power of these plants lies in phytochemical constituents that cause definite pharmacological actions on the human body. Phytochemical, natural compounds occur in plants such as medicinal plants, vegetables and fruits that work with nutrients and fibers to act against diseases or more specifically to protect against diseases (Akinmoladun *et al*., 2007).

Since very old times, herbal medications have been used for relief of symptoms of disease. Despite the great advances observed in modern medicine in recent decades, plants still make an important contribution to health care. Much interest, in medicinal plants however, emanates from their long use in folk medicines as well as their prophylactic properties, especially in developing countries (Akinmoladun *et al*., 2007).

Sudan exhibits a wide range of variation in its topography, climate, soil and hydrology. This resulted in different ecological habitats, different vegetation zones and consequently rich flora**.** Sudanese folk medicine represents a unique blend of indigenous cultures of Islamic, Arabic and African traditions. Consequently, treatments exist for a variety of diseases, both epidemic and endemic. Due to the tremendous wealth of plants in Sudan as well like many other countries, Sudan has a long tradition of folk medicine. Indigenous remedies are often the only form of therapy available to poor people living in rural areas or cities. It has been estimated that only 11% of the population has access to formal health care. The governmental medical services, which were free of charge in the past, are now available at high charges. Poor people frequently cannot afford medicines. Since medical care is not available or is too expensive, the majority of people depend on traditional remedies prescribed by unregistered traditional healers. The traditional medical practitioners in Sudan have no formal training or pharmacopoeia and their knowledge is based on acquired folklore and indigenous traditions. It cannot be compared with the Sri Lankan and Indian Ayurvedic, Yonani, or traditional Chinese medical systems, where formal training is provided (Khalid *et al*., 2012).

Solanaceae is considered as an important and large family between angiosperm and distributed throughout tropical and temperate regions of the world. Its center of diversity is

western and southern South America and only ten genera are native to new and old world, while *Solanum*, *Physalis* and *Lycium* are widely distributed across both the eastern and western hemisphere (D'Arcy, 1991). This family is composed of 98 genera and about 2300 species belonging to 14 tribes grouped in three subfamilies (Hunziker, 1979; D'Arcy, 1991). In Sudan Solanaceae family distributed in the eastern and center of Sudan. The genus *Solanum* is an economic important genus of annuals, perennials, sub-shrubs, shrubs and climbers. Many of its species are agricultural plants, poisonous and medicinally important. Solanaceae wild plants are generally used in Sudanese traditional medicine by practitioners as anti-inflammatory and chemo preventive agent suggesting their potential as a source of lead cytotoxic agent. Also, they are used in medicine for rhinitis, toothache, and breast cancer with analgesic and anti-inflammatory effects (El Ghazali *et al*., 1986).

II. Research problem

The lack of treatment of infectious diseases in developing countries is a tragic problem and one of the most serious challenges that the world must face at the beginning of this century. Virtually all deaths due to infectious diseases occur in low- and middle-income countries. Despite progress made in the basic knowledge of many infectious diseases, many of these have continued to cause significant morbidity and mortality. It is estimated that, infectious diseases cause more than 90% of all morbidities and mortalities occurring in the world with more than 90% of them occurring in developing countries (Shakya *et al.,* 2012).

However, over the past few decades, many of commonly used antibiotics and other drugs have become less and less effective against certain illnesses not, only because many of them produce toxic reactions, but also due to emergence of drug-resistant pathogenic microorganisms. Thus, it is essential to investigate newer drugs with lesser resistance.

Moreover, the need to replace synthetic antioxidants with natural and properly safe ones, together with the interest of food industry and preventive medicine in the development of bioactive naturally-occurring antioxidants, has fostered research on the screening of plants (Moure *et al*., 2001).

Most of the anticancer treatments are not selective and affect both tumor and normal cells, thereby causing systematic toxicity or increased risk of other cancers. Thus, there is a need for the development of safer alternatives for the treatment of cancer which are affordable, accessible, having less toxicity and minimum side effects.

However, despite Sudanese medicinal plants are divers and widely used in folk healing but still there is need for systematic and deep studies about their biological activity and phytoconstituent regarding to what is reported in literature. Many studies were reported on morphological taxonomy and pollen morphology of Solanaceae plant species from Sudan (El-Ghazaly, 1990; Harley *et al.,* 2004). Despite their wide application in Sudanese folk medicine, there is still a paucity of information regarding their secondary metabolites contents. This fact has spurred us to initiate a comprehensive phytochemical investigation of selected Solanaceae species endogenous to Sudan.

III.Objectives

In consideration of the above facts, the main purpose of this study was to investigate the phytochemical constituents and the biological activities of some selected species (*Solanum incanum* L*., S. schimperianum* Hochst*, S. nigrum* L*., Physalis lagascae* Roem. & Schult. and *Withania somnifera* (L) Dunal.) of Solanaceae family endogenous to Sudan.

The specific objective of this study was

I. To evaluate the leaf extracts of selected species for their:
   d) Antibacterial activity.
   e) Antiproliferative activity.
   f) Antioxidant activity.
II. To carry out preliminary screening for the presence of major classes of secondary metabolites using different chromatographic techniques.
III. To assess their preliminary chemotaxonomic relationships.
IV. To carry out bioassay guided fractionation for the most active plant species (*S. schimperianum*).

CHAPTER ONE

1. LITERATURE REVIEW

1.1 Introduction

Plants have variety of chemical constituents called 'phytochemicals' with protective and disease preventive properties. The term 'phytochemicals' are chemical substances that produced naturally in plants and demonstrate various biological activities that include antioxidant, antibacterial, antifungal, anti-inflammatory, anti-malarial and anticancer activities (Tan *et al.*, 2010). There is an increasing interest in using medicinal and aromatic plants as natural sources in pharmaceutical, food and cosmetic industries all over the world. Bioactive compounds of medicinal plants led them to be used in these industries as botanical drugs, dietary supplements, functional foods and food packaging, etc. Plants also have been used in ethnopharmacy for various diseases such as hypertension, cholesterol, eczema and diarrhea for centuries and today, their scientific validation was provided by identification and isolation of bioactive phytochemicals (Diren, 2008). These phytochemicals include phenols, alkaloids, steroids, terpenes, saponins and cardia glycosides which are clinically proven to be of valuable therapeutic importance that may help in the treatment of many diseases such as cancer, cardiovascular disease, degenerative diseases and HIV infection (Liu, 2003; Asres and Bucar, 2005). Vegetables, fruits, herbs and seeds contain phytochemicals that are rich in phenolic compounds such as flavonoids, phenolic acids, anthraquinones and coumarine (Young *et al*., 2005). Research in natural products seemed to be unbounded and limitless, and recently the interest in this area of study has revived and increased considerably especially with the latest development of technology in separation methods, spectroscopic techniques and advanced bioassays. Over the years, there have been significant findings and evidence that naturally-occurring compounds derived from higher plants have the potential to be developed as modern therapeutic drugs.

The search for bioactive compounds from plant-based medicines or organisms for the development of conventional drugs are now reviving and becoming more commercialized in the modern medicine throughout the world. Approximately, about 25% of the drugs used in the world today originate from higher plants and among them were taxol, morphine, quinine caffeine, atropine, and reserpine (Shakya *et al.,* 2012). It is undeniable that products from natural origin still continue to be valuable sources for new therapeutic agents especially with

the profound understanding of the biological significance and mechanism of actions of the active compounds. Until now, about 1% of tropical species have been studied and screened for their pharmaceutical potential against various diseases including cancer, diabetes and cardiovascular disease (Alsarhan, 2014). Plants and their extracts and derivatives still serve as a major contribution in pharmacy and medicine because of the increasing demand for medicines and foods (nutraceuticals) from the ageing population especially in Europe, Australasia and North America (Lapointe, 2008; Shahidi, 2005). As new diseases emerge and the trend for drug resistance and side-effects increases, there is the need to discover new medicinal drugs from natural origin with specific targets and least side-effects. On the other hand, synthetic drugs tend to show rapid onset of action with more side-effects in comparison to naturally methodology occurring drugs (Shakya, 2016). The various approaches to drug discovery from nature are:

1- Ethnobotanical: Ethnic and traditional medicine.

2- Random screening: Bioassay guided routes.

3- Chemotaxonomic: Screening of relative.

1.2 Natural Products

Natural products (secondary metabolites) have been the most successful source of potential drugs. Natural products continue to provide unique structural diversity in comparison to standard combinatorial chemistry, which presents opportunities for discovering mainly novel low molecular weight lead compounds. Since less than 10% of the world's biodiversity has been evaluated for potential biological activity, many more useful natural lead compounds await discovery with the challenge being how to access this natural chemical (Dias *et al*., 2012).

1.3 Natural Products from Plants

Plants have been well documented for their medicinal uses for thousands of years. They have evolved and adapted over millions of years to withstand bacteria, insects, fungi and weather conditions to produce unique, structurally diverse secondary metabolites. Their ethnopharmacological properties have been used as a primary source of medicines for early drug discovery (Mc Rae *et al*., 2007). According to the World Health Organization (WHO), 80% of people still rely on plant-based traditional medicines for primary health care. The

knowledge associated with traditional medicine (complementary or alternative herbal products) has promoted further investigations of medicinal plants as potential medicines and has led to the isolation of many natural products that have become well known pharmaceuticals (Dias *et al.,* 2012).

The use of natural products as medicines has been described throughout history in the form of traditional medicines, remedies, potions and oils with many of these bioactive natural products still being unidentified. The dominant source of knowledge of natural product uses from medicinal plants is a result of man experimenting by trial and error for hundreds of centuries through palatability trials or untimely deaths, searching for available foods for the treatment of diseases (Kinghorn *et al*., 2011).

The study of natural products has had a number of rewards. It has led to the discovery of a variety of useful drugs for the treatment of diverse ailments and contributed to the development of separation science and technology, spectroscopic methods of structure elucidation and synthetic methodologies that now make up the basics of analytical organic chemistry (Kinghorn *et al*., 2011).

The role of traditional medicine in the discovery of potent chemicals is quite crucial. Among some of the earliest successes in developing drugs from natural products, one can mention the isolation of the antimalarial agents such as the *Cinchona* tree alkaloids, pain relievers such as the morphine alkaloids as well as the development of aspirin. Quinine, originally isolated from the bark of *Cinchona* trees, *Cinchona succirubra*, was one of the principal antimalarial agents. Morphine, the major alkaloid of *Papaver somniferum* was first isolated between 1803/06. It was widely used for pain relief beginning in the 1830's, but was also recognized as addictive. The "Ebers papyrus", the Egyptian pharmaceutical record, indicates the use of willow leaves as an antipyretic agent (Viktorin and Sartorius, 1999). Following on this knowledge, chemists began to isolate compounds responsible for the remedy, and salicin, was isolated from the bark of the white willow, *Salix alba*, in 1825-26. It was subsequently converted to salicylic acid, via hydrolysis and oxidation, and proved potent as an antipyretic that was manufactured and used worldwide. To overcome the severe gastrointestinal toxicity of salicylic acid, it was converted into acetylsalicylic acid *via* acetylation and started to be marketed under the trade name aspirin in 1899. Aspirin is still the most widely used analgesic and antipyretic drug in the world (Tesso, 2005). Nitisinone, a derivative from *Callistemon citrinus* used for the treatment of hereditary tyrosinaemia type 1 (HT-1) and galantamine

hydrobromide, an Amaryllidaceae alkaloid from *Galanthus nivalis* were among the approved drugs that were developed from natural origin (Skytte *et al*., 2010). Doxorubin and paclitaxel (antitumor agents), cyclosporine A and tacrolimus (immunosuppressive agent) and lovastatin (cholesterol lowering agent) are among well-known and widely used drugs from natural product. This clearly suggests that natural products are important sources for new therapeutic agents and they have significantly contributed as lead compounds in drug development project (Taha, 2011).

1.4 Family of Solanaceae

Solanaceae is a medium-sized family with approximately 96 genera and 3000 – 4000 species occurring all over the world (D'Arcy, 1991). The name of the family comes from the Latin word *Solanum*, meaning "the nightshade plant", but the further etymology of that word is unclear; it has been suggested to originates from the Latin verb solari meaning "to soothe". This would presumably refer to alleged soothing pharmacological properties of some of the psychoactive species found in the family. It is more likely, however, that the name comes from the perceived resemblance that some of the flowers bear to the sun and its rays. The family is also informally known as the nightshade or potato family (Yasin, 1985). Members of this family are being used for medicinal purposes as early as 37 A.D. (Hill, 1952). In fact, this family is an important source of almost 300 different kinds of alkaloids (Friedman and McDonald, 1997). Solanine, scopolamine, atropine and hyoscyamine are the key of this family (Stanker *et al*., 1994).

Among angiosperm families, the Solanaceae rank as one of the most important family to human beings. Species of the family are used for food such as potato (*Solanum tuberosum* L.), tomato (*S. lycopersicum* L.), and eggplant (*S. melongena* L.,) and as drugs like *Nicotiana tabacum* L. and *N. rustica* L., *Atropa belladonna* L., *Mandragora officinarum* L. and *Duboisia* spp.

Members of the Solanaceae are extremely diverse; in terms of habit, ranging from trees to small annual herbs; in habitat, from deserts to the wettest tropical rain forests and in morphology, with astounding variation in many characters of both flowers and fruits (Knapp *et al*., 2004).

All plants including Solanaceous plants such as potato, tomato, jimson weeds, eggplants, and peppers synthesize a variety of compounds which serve as natural defenses against plant phytopathogens including fungi, viruses, bacteria, insects, and worms; reviewed in Fridman, (2004).

Solanaceae species are often rich in alkaloids that can range in their toxicity to humans and animals from mildly irritating to fatal in small quantities (Edmonds and Chweya, 1997).

The economic importance of the genus *Solanum* is also striking, including its ability to produce steroidal alkaloids many of which are of therapeutic interest (Torres *et al*., 2013). Tomato also largely contributes to dietary nutrition worldwide with beneficial effects mainly attributed to antioxidant compounds in the fruit. In addition, significant progress has been made to improve the levels of human health-promoting compounds (such as carotenoids and anthocyanins) in tomato fruits through metabolic engineering or breeding (Rigano *et al*., 2013). Beneficial effects of potato and tomato glycoalkaloids include inactivation of the herpes simplex virus protection of mice against infection by *Salmonella* Typhimurium, enhancement of general anesthetics that inhibit cholinesterase, potentiation of a malaria vaccine, lowering of plasma cholesterol in hamsters, and inhibition of growth of human colon and liver cancer cells (Friedman, 2004).

Twelve alkaloids, 35 withanolides, and several sitoindosides from *Withania somnifera* have been isolated and studied. Much of pharmacological activity of this plant has been attributed to two main withanolides, withaferin A, D and withanolide G. (Verma and Kumar, 2011). *Datura stramonium* is a rich source of tropane alkaloids (Berkov *et al*., 2006). These alkaloids are used in the chemotaxonomy of Solanaceae family. Knowledge of the complete alkaloid pattern is of interest not only phytochemically, but also in relation to aspects of alkaloid biogenesis and metabolism (El Bazaoui *et al*., 2011).

Solasodine is present in a number of *Solanum* species, such as *S. khasianum*, *S. xanthocarpum*, *S. nigrum*, *S. gracile*, *S. laciniatum*. Solasodine, a spiroketal alkaloid sapogenin with a hetorocyclic nitrogen atom, is used for the production of steroid drug in medical industry. It is also used in the preparation of contraceptive drug and have been reported to provide anticancer insecticidal, antiaccelerator cardiac activities, and antioxidant activities (Thongchai *et al*., 2010).

In Sudan, there are about 9 genera comprising 30 species. Some of them are known to be poisonous and to possess many medicinal uses in the Sudanese traditional medicine (Table 1).

Table 1: Medicinal uses of some Solanaceae plants endogenous to Sudan.

Plant name	Traditional uses in Sudan	Traditional uses in other African countries
Datura innoxia	Toothache pain (Suleiman, 2015).	Asthma, gastrointestinal problems, aches abscesses, boils, headaches tuberculosis, as antispasmodic and antiasthmatic (Bown, 1995).
D. metel	Skin disease (Kokwaro, 1976).	Skin disease, eye disease, piles, acidity, and kidney stone (Schmeltzer, 2008).
D. stramonium	Antispasmodic, hypnotic and narcotic (Broun and Massey, 1929).	Antispasmodic, hypnotic and narcotic (Broun and Massey, 1929).
Lycium periscum	Headache, abdominal pain vomiting and diarrhea (Schmeltzer, 2008).	Headache, abdominal pain, dilated puplis, vomiting, diarrhea (Schmeltzer, 2008).
Phasalysis minima	Abdominal pains (Schmeltzer, 2008).	Malaria, toothache, liver ailments, hepatitis, rheumatism, diuretic, as anti-inflammotary and disinfectant for skin disease (Schmeltzer, 2008).
Physalis lagascae	As tonic, diuretic and as poultice for swellings (Elghazali et al., 1987)	

Plant name	Traditional uses in Sudan	Traditional uses in other African countries
Solanum incanum	Anti-asmatic, snake bite, dysentery (El Gazali *et al*., 1986)	Toothache, throat and chest complains (Chevallier, 1996; Manase *et al*., 2012).
S. dubium	Kidney dysfunction (Barris *et.al*., 1983)	
S. nigrum	Fever, diarrhoea, and eye diseases (Anonym, 1982)	Headache, heat of stomach and diuretic (Boulus, 1983).
S. schimperianum	Wounds (Almoulah *et al.,* 2017).	
Withania somnifera	Rhematism, laxative abscess and diuretic (El Gazali *et al.,* 1987).	Anti-neuralgic, Alzheimer's disease, diuretic (Mirjalili, 1996).

1.5 Taxonomy of Solanaceae plants

Solanaceae plants extensively remain the subject of taxonomical studies in history because of their high diversity and beneficial properties to human beings. From the early years of the twenty centuries taxonomist from different regions paid attention to this family. Kirk (1927) studied the Solanaceae of Britain. In this area, Solanaceae was represented by twelve different genera. According to Kirk *Solanum* and *Datura* genus has narcotics properties. *Solanum nigrum* L. and *Atropa belladonna* L. are poisonous species of this area. *Datura stramonium* L. is an invasive species of the region. *Hyoscyamus niger* L. is rare species whereas in the past it was cultivated in Britain for its medicinal properties. The morphological characters he, mainly emphasized, were habit of the plant, leaf characters, corolla and fruit. Based on corolla tube length the family is divided into two major groups. One with the short corolla and other with long and bell-shaped corolla tube. He placed *Solanum* into first group whereas *Hyoscyamus, Datura* and *Atropa* are the part of the second group.

Flora of the British Isles was studied by Clapham in 1962. The morphological markers mainly used for taxonomic purposes were related to stamens, habit of plant and position of flower whether it is erected or drooping. Based on morphological markers related to androecium's characters, the family is divided into two major groups. One group consists of *Solanum* genus and this group is characterized by prominent stamens, anthers longer than filaments and opening of anthers by an apical pore. Second group possessed included stamens with anthers shorter than filament and anthers opened by a longitudinal slit. Ary and Gregory, (1972) described *Solanum nigrum*, *Datura stramonium* and *Hyoscyamus niger* in "The Oxford Book of Wild Flowers". They mentioned all of these three species as highly poisoned. However, *S. nigrum* is not poisonous. In certain regions of Pakistan, it is used as a vegetable and fruit is also edible. *Solanum* is one of the largest genus of Solanaceae in Pakistan. The genus *Solanum* was divided into two subgenus *Lycianthes* and *Solanum*. The morphological markers, given importance for the identification of species were life form, tomentum, prickles, type of inflorescence, shape and colour of flowers, shape and kind of fruits. The *Datura* and *Capsicum* are naturalized and are represented by 1-2 species only. Pojarkova (1997), described Solanaceae from USSR. He mainly emphasized on lower order taxonomy of this family, where he divided it into five tribes (Solaneae, Atropeae, Nicotianeae, Daturinae and Nicandreae*). Solanum, Capsicum, Atropa, Hyoscyamus* and *Datura* are the part of this flora. Tribe Solaneae is further divided into four sub tribes.

Solanineae, Sarachineae, Margaranthinae, and physalidinae. *Solanum* is the single genus of sub tribe Solanineae with 33 species. This large genus is divided into two subgenera; *Eusolanum* with 19 species and *Leptostemonum* with 14 species. Jennifer and James (1997) study *S. nigrum* and its related species in detail. They give description about taxonomy, phytogeny, medicinal and nutritional value of Black nightshades.

According to (Chaudhary, 2001), the key to the genera endogenous to Sudan is as follow:

1. Fruit baccate…….*Cestrum*

+Fruit a capsule…….*Petunia*

2. Calyx inflated in fruit…….*Datura*

+Calyx not inflated in fruit……*Hyoscyamus*

3. Flowers in short umbellate cymes. Anthers connivent……....*Withania*

+Flowers solarity, axillary. Anthers not connivent…… ……..….*Physalis*

4. Anthers connivent…….…….*Lycium*

+ Anthers not connivent…….…..*Nicotina*

5. Anthers dehiscing by apical pores or slits……...….*Solanum*

+Anthers dehiscing longitudinally……..….*Lycopersicon*

In another morphological Study, Andrews (1956) described the plants of the Solanaceae family in the Sudan and classified them in a simple key as below:

AA. Fruit a berry:

C. Anthers opening by slits lengthwise:

D. Calyx not enlarged in fruit or not obviously so:

c. Plant not spiny: corolla rotate, the lobes valvate……....*Capsicum*

cc. Plant spiny; corolla tubular, the lobes imbricate……..*Lycium*

DD. Calyx enlarged and inflated in fruit:

d. Flowers solitary…….....…*Physalis*

dd. Flowers in clusters……..……*Withania*

CC. Anthers opening by terminal pores……..….*Solanum*

From the above key, Andrews classified the Physalinae (*Physalis* sp.), Withaninae (*Withania* sp.) and Solaneae (*Solanum* sp.) separately including separate genera, moreover he goes below the genera to classify the *Solanum* species as below:

A. Plants without spines:

B. Inflorescences terminal and spiciform; a climber……..…….*S. welwitschii*

BB. Inflorescence terminal, axillary, extra-axillary or leaf- opposed; not climbing:

C. Leaves glaborous or scurfy, more or less undulate……..…..….*S. aethiopicum*

CC. Leaves with simple hairs…….*S. nigrum*

D. Leave with few or many stellate hairs:

a. Flower parts usually in 4, ……..*S. schimperianum*

aa. Flower parts usually in 5,s:

b. Flowers few in short racemes; branchlet and leaves sparsely covered

with stellate hairs……..*S. carense*.

bb. Flowers in short racemose 2-fid cymes; branchlets and leaves

densely covered with stellate hairs…….*S. polyanthemum*

AA. Plants with spines:

E. Leaves rarely more than1⅓ in. long:

c.Flowers solitary or a few racemose; leaves usually hastate or more

or less Pinnately lobed……..……*S. hastifolium*

cc. Flowers in peduncle cymes; leaves not as above……...….*S. albicaule*

EE. Leaves exceeding 1 ⅓ in. long:

DD. Leaves without hairs:

e. berry not more than ⅓ in in diameter……...….*S. pruinosum*

ee. Stem glaborous, branches tomentose……...….*S. hybridum*

eee. Stems with floccoase indumentum……..*S. incanum*

A phylogeny of Solanaceae is also presented based on the chloroplast DNA regions ndhF and trnLF. Chloroplast DNA (cpDNA) has proven to be an excellent source of molecular variation available for higher order (i.e., generic level and above) in phylogenetic studies of plants with 89 genera and 190 species of the family Solanaceae. Olmstead *et al.* (2008) divided Solanceae family in many different clades, of which Solanoideae (Kostel) is further divided into seven sub-clades. The most important subtribs which includes the species under this study are:

1/Physalinae (Miers) Hunz: includes *Physalis* sp.

2/Withaninae (Bohs and Olmsted): includes *Withania* sp.

3/ Solaneae (Dumort): includes all the *Solanum* sp.

In another study, Hunziker (1979) and D'Arcy (1979, 1991) divided the family Solanaceae into 2 subfamilies. The subfamily Solanoideae has curved embryos contained in flattened discoid seed and the subfamily Cestoideae with straight or slightly bent embryos in prismatic-subglobose seeds. Pollen of 5 genera viz., *Solanum, Withania, Lycium, Physalis* and *Datura* of the subfamily Solanoideae was studied. Pollen morphology of these genera showed considerable variation in exine pattern. The taxonomic significance of pollen morphology in Solanaceae is more or less obscure. Sometimes different tribes or sub tribes have similar types of pollen or vice versa. Genera referred to same tribe or sub tribe may have different type of pollen. For instance, two species of the genus Withaniai, *W. coagulans* and *W. somnifera* have quite dissimilar pollen grains and fall under different types. They have similarity with other

genera than with each other. Pollen of *W. coagulans* are very similar to grains of genus *Solanum*, while *W. somnifera* pollen are closely related to *Lycium* grains. (Perveen and Qaiser, 2007). Moreover, Perveen and Qaiser (2007) classified the Solanaceae family according to type of pollen grains, they found that, Pollen grains usually radially symmetrical, isopolar, prolate-spheroidal, or subprolate to prolate often oblate-spheroidal. Generally, tri-colporate (rarely 4-colporate), colpi with costae, colpal membrane psilate to sparsely or densely granulated, ora lalongate, sexine as thick as nexine, or slightly thicker or thinner than nexine. Tectal surface commonly scabrate or verrucate. On the basis of aperture number, exine pattern and pollen shape class, 6 distinct pollen types are recognized viz., *Datura fastuosa*-type, *Lycium dasytemum*-type, *Nicotiana plumbaginifolia*-type *Physalis divaricata*-type, *Solanum nigrum*-type and *Withaina somnifera*-type.

Moreover, according to the chemical constituents, Gemeinholzer and Wink (2001) proved that, since many secondary metabolites often show a restricted occurrence in apparently related groups of plants, it is tempting to use the distribution of secondary metabolites as a systematic marker. On considering the occurrence of the major secondary metabolite of the Solanaceae, a general pattern becomes visible. The distribution of steroidal alkaloid is a dominant character in the tribe Solaneae, whereas Withanolides represent a group of steroidal lactone typical for the tribs physalinae and Withaninae. Tetenyi (1987) recognized the Anthocercidoideae and Atropoideae as new subfamilies due to their biochemical synthesis syndromes. Similarly, Solaninae and Physalinae was accepted as separate subtribes of tribe Solaneae because of their differing and exclusive steroid synthesis. Acnistus and Dunalia were allied with Jaborosa in tribe Jaboroseae (Tetenyi, 1987).

1.6 Botanical and pharmacological aspects of plant species under the study

The five selected species of Solanaceae family investigated in this study were:

1. *Solanum incanum* L.
2. *S. nigrum* L.
3. *S. shimperianum* Hosch.
4. *Physalis minima* L.
5. *Withania somnifera* (L) Dunal.

These Solanaceae family species display varying amounts of phenotypic variation, particularly in their vegetative features such as plant habit, leaf size and form, and stem winging. A brief overview of their individual botanical aspects is given below:

1.6.1 *Solanum incanum* L.

Synonym names: *S. unguicalatum, S. duplosinuatum*

Vernacular name: El gobeen

1.6.1.1 Botanical description

Densely stellate-tomentose shrub 3-5ft. high. Leaves sinuate, ovate or ovate-elliptic, obtuse at the apex, unequal at the base, up to 7 in. long, 6 in. broad, dark-green above, paler beneath, densely stellate-hairy on both surfaces. Flowers purple or white, $_{3\backslash4}$-$1_{1\backslash2}$ in.in diameter, some unisexual. Solitary or few together, pendulous. Berry yellow, subglobose. $1_{1\backslash2}$ in, in diameter, pendulous (Andrews 1956) (Fig. 1).

1.6.1.2 Distribution in Sudan

Wide spread in Sudan but mainly found in Suakin, Gebeit road, Erkowit (Eastern Sudan).

Figure 1: *Solanum incanum*

(https://de.wikipedia.org/wiki/Solanum_incanum)

1.6.1.3 Phytochemistry

The plant is a rich source of important cytotoxic glycoalkaloids, such as solamargine and solasonine, Fukuhara and Isaokubo (1990) isolated two biologically active glycosidal alkaloids, solasonine and solamargine from fresh ripe fruit of this plant and from *S. nigrum*.

The structural difference between these two alkaloids is the carbohydrate moiety on C3 side chain of steroidal aglycone, namely solasodine (Ding *et al,* 2013). Because *S. incanum* is a potential source of compounds for steroid synthesis, quantitative estimation of solasonine and solamargine content were detected in different parts of the plant (Al Sinani *et al.* 2016).

1.6.1.4 Biological activities

Solanum incanum possess a variety of biological activities as it contains solamargine and solasonine which are economically beneficial because their chemical structures are very similar to steroidal hormones. Therefore, they have been proposed for their use as important sources in the production of medicines, such as contraceptives and steroidal anti-inflammatory drugs. These glycoalkaloids have been studied for their antidiabetic, antifungal, antiparacetic, antibiotic, antimicrobial and antiviral (Tiossi *et al*., 2012). Moreover, biological investigations of solamargine and solasonine showed significant cytotoxicity against several human cancer cell lines and skin tumours (Maurya *et al*., 2009). Also, it has been proved that solasodine glycosides are very important antitumor agents (Cui *et al.*, 2012), and both Solasonine and Solamargine possess antiproliferative activity (Li *et al*., 2016).

1.6.2 *Solanum nigrum* L.

Synonym name: *S. nodiflorum*

Vernacular name: Inab El dib, El magat

1.6.2.1 Botanical description

Herb or undershrub 1-2 ft. high; branches glabrous or pubescent. Leaves entire or sinuate-dentate, ovate, obovate or lanceolate, up to 4in. broad, slightly pubescent. Flowers white, about $_{1\backslash 2}$ in. in diameter, in few-flowerded umbellate cymes at or above the nodes, berry black, about $_{1\backslash 2}$ in. in diameter, glabrous (Andrews 1956) (Fig. 2).

Figure 2: *Solanum nigrum*

(http://easyayurveda.com/2017/02/02/black-nightshade-solanum-nigrum/**)**

1.6.2.2 Distribution in Sudan

Wide spread in Sudan but mainly found in Red Sea Hills and Erkowit (Eastern Sudan).

1.6.2.3 Phytochemistry

The main components of this plants are glycoalkaloids, solasonine and solamargine. Moh ED-in *et al.,* (2010) identified several glycoalkaloids like Solasonine, α-Solamargine, β-Solamargine and α-Solanine and their aglycones (Solasodine and Solanidine). In fact, the multiple bio-activities of *S. nigrum* are based on its various medicinal components, such as glycoalkaloids (Ding *et al.*, 2013), glycoproteins (Heo and Lim, 2005), polysaccharides (Li *et al.*, 2009), steroidal saponins (Zhou *et al.*, 2006), and polyphenolic compounds (Nawab *et al.*, 2012).

1.6.2.4 Biological activities

Pharmacological studies have indicated that the extracts and/or components from *S. nigrum* possess a variety of biological activities including antifungal, anti- malarial, hepatoprotective and antitumor effects (Sun *et al.*, 2012; Abdel-Rahim *et al.*, 2014). The antitumor activity has attracted much attention from researchers recently because of its remarkable effects (Wang *et al.*, 2010; Nawab *et al.*, 2012). Alkaloids are considered to be one of its main antitumor active components (Li *et al.*, 2008). Pharmacological experiments indicated that total steriodal alkaloids from *S. nigrum* selectively destroys sarcoma180 *in vivo* (Cham and Daunter, 1990).

1.6.3 *Solanum schimperianum* Hosch.

Synonym names: *S. carense, S. polyanthemum*

Vernacular name: Domhindib, Hantitrob

1.6.3.1 Botanical description

Shrub about 5 ft. high; stems stellately hairy when young, glabrous when old. leaves ovate acute at the apex about 1 in. long, 1\2 in. broad, covered with more or less deciduous stellate hairs. Leaves alternate, all of comparable size on same branch; petiole 1.5–3.5 cm long; blade ovate, 3.5–12.5 x 2–8 cm, entire, glabrescent to densely pubescent with stellate hairs, base usually obliquely cuneate to truncate, less frequently subcordate, margin straight, apex acute to acuminate. Inflorescence usually Flowers about 1\2 in. in diameter, in few-flowered cymes. Inflorescence usually umbel-like, 10–30-flowered, with glandular hairs; Fruit red, becoming almost black, c. 6 mm in diam. Berry globose about 1\2 in. in diameter (Andrews 1956) (Fig. 3).

1.6.3.2 Distribution in Sudan

Gebel Dambobei (North Sudan); Red Sea Hills and Erkowit (Eastern Sudan).

Figure 3: *Solanum schimperianum.*

http://www.plantdiversityofsaudiarabia.info/Biodiversity-Saudi-Arabia/Flora/Photo%20Gallery/Solanaceae/Solanaceae.htm

1.6.3.3 Phytochemistry

Ogail *et al*. (2012) isolated ten compounds from the aerial parts of *S. schimperianum,* grown in Saudia Arabia. These are; lupeol, β-sitosterol, β-sitosterol glucoside, oleanolic acid,

teferidin, teferin, ferutinin, 5-hydroxy-3,7,4'-trimethoxyflavone, retusin and kaempferol-3-O-β-d-glucopyranoside. Al-Rehaily *et al.* (2013) isolated solanopubamine (3b-amino-5a, 22aH, 25bH-solanidan-23b-ol), a steroidal alkaloid from the alkaloidal fraction of *S. schimperianum.* The steroidal alkaloid solanopubamine was observed to be present only in extracts of aerial parts of *S. schimperianum* compared with six tested aerial part of other *Solanum* species which include *S. incanum* and *S. nigrum* (Siddiqui *et al.,* 2016).

1.6.3.4 Biological activities

The hexane extract of the plant has antimicrobial activity against *Bacillus subtilus* and *Staphylococcus aureus* (Al-Ogail *et al*., 2012). The methanol extract of *S. schimperianum* was reported to have significant antitrypanosomal activity (Abdel-Sattar *et al*., 2009). Solanopubamine and semi-synthetic analogs are investigated for their *in vitro* cytotoxicity against a panel of human cancer cell lines and anti-microbial activity. Solanopubamine showed good antifungal activity only against *Candida albicans* and *C. tenuis* (Al-Rehaily *et al*., 2013).

1.6.4 *Physalis lagascae* L.

Synonym name: *P. angulat*
Vernacular name: El Hembook, El Taleb

1.6.4.1 Botanical description

Much branched herb about 1 ft. high, leaves petiolate, entire or sinuate-dentate, ovate, acuminate at the apex, sometimes sub-cordate at the base, 1-2 in. long, 1\2 in. broad. Flowers yellow, small, axillary, solitary. Anthers yellow. Berry globose.1\2in.in diameter, smooth (Andrews, 1956) (Fig. 4).

Figure 4: *Physalis lagascae.*

http://www.plantdiversityofsaudiarabia.info/Biodiversity-Saudi-Arabia/Flora/Photo%20Gallery/Solanaceae/Solanaceae.htm

1.6.4.2 Distribution in Sudan

Khartoum (Central Sudan); Erkowit (Eastern Sudan).

1.6.4.3 Phytochemistry

Physalins are commonly isolated compounds from *Physalis* species. They are well recognized as the taxonomic maker for the genus (Pérez-Castorena *et al*., 2004).

1.6.4.4 Biological activities

Physalins were reported to possess a wide array of bioactivities including, antileishmanial (physalins B and F) (Guimaraes *et al*., 2009), immunomodulatory (physalins B, F and G) (Soares *et al*., 2003), immunosuppressive (physalin H) (Yu *et al*., 2010), antiinflammatory (physalins A and O) (Ji *et al.,* 2012), antimalaria (physalins B, D, F and G) (Sá *et al*., 2011), antibacterial (physalin D) (Helvacı *et al*., 2010), antitumor (physalin F) (Chiang *et al*., 1992) and bacteriostatic (physalin A derivatives) (Li *et al*., 2012) activities.

Physalin F, a seco-steroid from *Physalis angulata* L., has immunosuppressive activity in peripheral blood mononuclear cells from patients with HTLV1-associated myelopathy (Lorena *et al*., 2016).

1.6.5. *Withania somnifera* (L) Dunal

Synonym name: *Physalis somnifera*

Vernacular name: Sekeran, Sammal Farakh, Faqaish

1.6.5.1 Botanical description

Much branched under shrub 2-7 ft. high; stems stellate-tomentose. Leaves entire or sinuate, elliptic to broadly ovate-lanceolate, acute to rounded at the apex, abruptly acute to long decurrent at the base, $1_{1\backslash 2}$ -4in. long, $_{3\backslash 4}$-3 in. broad. Flowers colourless or pale-green, $_{1\backslash 4}$ in.in diameter.2-6 together in axillary cluster. Calyx campanulate, divided to about the middle into 5 acute triangular lobes, inflated in fruit, $_{2\backslash 5\text{-}3\backslash 4}$in. long. Berry red when mature, globose, enclosed by brown papery inflated calyx (Andrews 1956) (Fig. 5).

Figure 5: *Withania somnifera.*

http://www.plantdiversityofsaudiarabia.info/Biodiversity-Saudi-Arabia/Flora/Photo%20Gallery/Solanaceae/Solanaceae.htm

1.6.5.2 Distribution in Sudan

Rashad (Western Sudan); Erkowit (Eastern Sudan).

1.6.5.3 Phytochemistry

Phytochemical studies on the aerial parts of *W. somnifera*, led to the isolation of a chlorinated steroidal lactone (27-acetoxy-4b,6 a-dihydroxy-5b-chloro-1-oxowitha-2,24-dienolide), adiepoxy withanolide (5b,6b,14a,15a-diepoxy-4b,27-dihydroxy-1-oxowitha-2,24-dienolide), and withaferin A (Choudhary *et al*., 2010). Patel *et al.* (2013) isolated the withanolide from the hybrid species of *W. somnifera*.

1.6.5.4 Biological activities

Chlorinated steroidal lactone, adiepoxy withanolide and withaferin A exhibited a growth inhibition and cytotoxic activity against human lung cancer cell line (NCI-H460), while the withaferin A being the most potent (Choudhary *et al*., 2010). Six withanolides isolated from *W. somnifera* were tested for anti-diabetic activity based on glucose uptake in skeletal myotubes. Withaferin A was found to increase glucose uptake, with 10 M producing a 54% increase compared with control (Jonathan *et al*., 2015). L-asparaginase isolated from fruits of *W. somnifera* also exhibited a growth inhibitory effect against lymphoblastic leukemia (Oza *et al*., 2010). Withaferin A has been scientifically validated for other different pharmacological activities including adaptogenic, anti-stress, anti-convulsant, immunomodulatory, neurological, anti-inflammatory, antitumor, cardioprotective, and neuroprotective activities (Patel *et al.,* 2013).

1.7 Secondary metabolites in Solanaceae family

Secondary metabolites are generally not essential for the growth, development or reproduction of an organism and are produced either as a result of the organism adapting to its surrounding environment or are produced to act as a possible defense mechanism against predators to assist in the survival of the organism. The biosynthesis of secondary metabolites is derived from the fundamental processes of photosynthesis, glycolysis and the Krebs cycle to afford biosynthetic intermediates which, ultimately, results in the formation of secondary metabolites also known as natural products (Dias, 2012).

In order to understand the uses of medicinal plants and their mode of action; it is necessary to knew something about their active constituents and their effectiveness. Some important groups of the active constituents present in Solanaceae plants and their mode of action are given below:

1.7.1 Alkaloids

A precise definition of the term 'alkaloid' (alkali-like) is somewhat difficult because there is no clear-cut boundary between alkaloids and naturally occurring complex amines. Typical alkaloids are derived from plant sources, they are basic, they contain one or more nitrogen atoms and they usually have a marked physiological action on man or other animals (Evans, 2002). In true alkaloids the basic units of biogenesis are amino acids. The non-nitrogen containing rings or side chains are derived from terpene units and/or acetate, while methionine is responsible for the addition of methyl groups to nitrogen atoms. Alkaloids are basic and form water-soluble salts. Most alkaloids are well-defined crystalline substances that react with acids to form salts. In plants, they may exist in the free state, as salts or as N-oxides. The different criteria currently used for the classification of alkaloids are biogenesis, structural relationship, biological origin and spectroscopic/spectrometric properties (chromophores in UV spectroscopy, ring systems in mass spectrometry) (Evans, 2010). Based on amino acid precursor, alkaloids can be further subdivided. The principal precursors arornithine, lysine, nicotinic acid, tyrosine, tryptophan, anthranilic acid and histidine. Ornithine gives rise to pyrrolidine and trypane alkaloids, lysine to piperidine, quinolizidine and indolizidine alkaloids and nicotinic acid to pyridine alkaloids. Tyrosine produces phenylethylamines, tetrahydroisoquinoline, benzyltetrahydroisoquinoline, phenethylisoquinoline, terpenoid tetrahydroisoquinoline and Amaryllidaceae alkaloids.

Tryptophan gives rise to β-carboline, terpenoid indole, quinoline, pyrroloindole and ergot alkaloids. Anthranilic acid acts as a precursor to quinazoline, quinoline and acridine alkaloids, while histidine gives imidazole derivates (Mohy-UD-Din, 2010).

Atropine, hyoscyamine and hyoscine are alkaloids obtained from Solanaceae plants. They are also found in the plant families like Erythroxylaceae, Convolvulaceae, Proteaceae, Orchidaceae, Euphorbiaceae, Brassicaceae, Rhizophoraceae (Evans, 1979) and in the fungus *Amanita muscaria*. Their common structural element is the azabicyclo octane system, and over 150 tropane alkaloids have been isolated. Tropane alkaloids, possess anticholinergic and spasmolytic properties; they are commonly used as an anaesthetic and spasmolytic and in eye surgery. These are a series of secondary metabolites that have been mainly described in the Solanaceae family and initially in the genus *Atropa* that act as feeding deterrents and antimicrobial defenses for the plants (Ashtiania and Sefidkonb, 2011).

1.7.2 Glycoalkaloids

Glycoalkaloids, a class of nitrogen-containing steroidal glycosides, are naturally occurring secondary metabolites commonly found in the Solanaceae family which includes many significant agricultural plants, such as tomato, potato, eggplant, pepper, nightshade, thorn apple, and capiscum. For example, solasodine, has been found in about 200 *Solanum* species. Glycoalkaloids are generally found in all plant organs, with the highest concentrations occurring in flowers, sprouts, unripe berries, young leaves or shoots (metabolically active parts). They are regarded as defensive allelochemicals against a number of pathogens and predators including fungi, viruses, bacteria, insects, and worms. The types of steroidal glycoalkaloids produced by Solanaceous plants differ from species to species. The differences can be manifested as a presence or absence of a C-C double bond, variety of functional groups (e.g., hydroxyl, acetyl) and sugar groups, as well as in the stereochemistry of these functional groups (Tek, 2006). Other examples of steroidal glycoalkaloids are solanidine, solanine, chaconine, veratramine, muldamine; samandarin.

Steroidal alkaloids are characterized by the presence of an intact or modified steroid skeleton with nitrogen. Since nitrogen is inserted into a non-amino acid residue these compounds belong to a subgroup of pseudoalkaloids (or isoprenoid alkaloids) (Laurila, 2004). Structural variation in the family of plant steroidal glycoalkaloids is limited to two main groups, based on the skeletal type of the aglycone. One is the spirosolan type, similar to spirostan, but with nitrogen in place of the oxygen in ring F (forming a tetrahydrofuran and piperidine spiro-

linked bicyclic system). Second is the solanidane type, where N connects spirostan rings E and F rings. All types can contain double bonds and hydroxyls in various positions. At least 90 structurally different steroidal alkaloids have been found in over 350 *Solanum* species (Laurila, 2004). The majority of plants utilize glycoalkaloids as the main aglycone is solasodine in the form of water soluble triglycosides solasonine (SN) and solamargine (SM), which occurs in approximately 200 species of *Solanum* plants. These two compounds bear the same aglycone, solasodine, and differ from each other only in the nature of the trioses involved, namely, solatriose for solasonine and chacotriose for solamargine (Al Sinani *et al.* 2015) (Fig. 6).

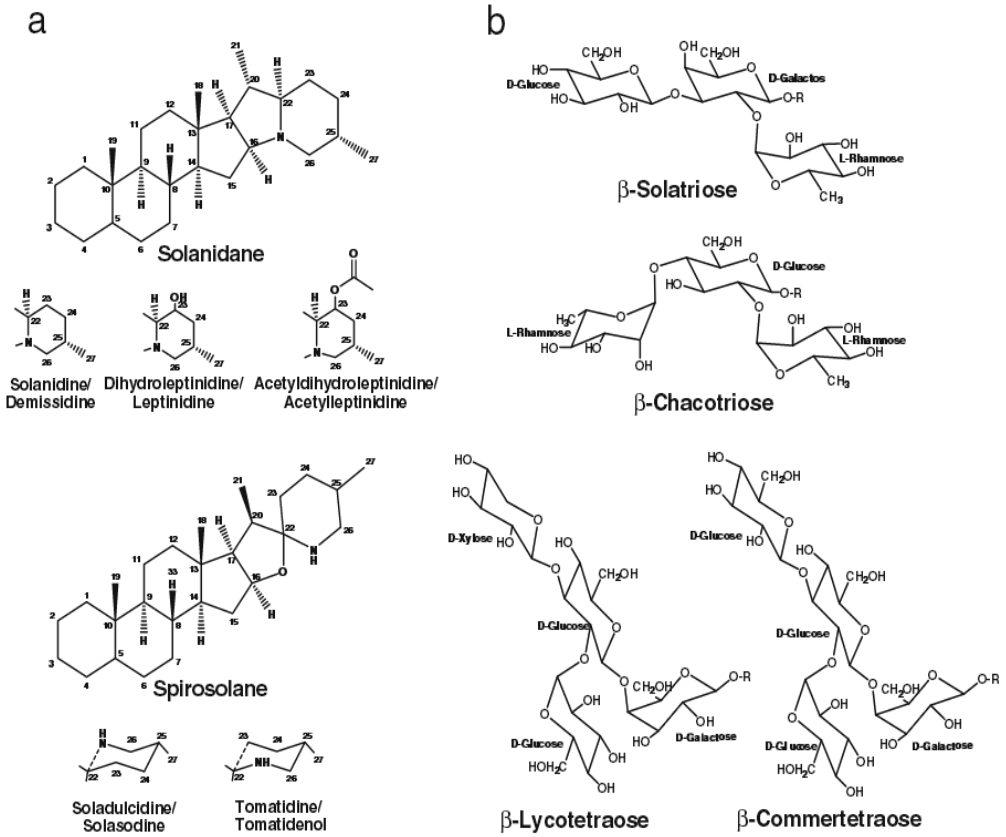


Figure 6: Structures of common Solanum steroidal glycoalkaloid aglycones (a) and glycoside residues (b). a; Complete structural formulas for the two major heterocyclic ring classes, solanidane and spirosolane, are shown with structural features of individual aglycones shown below. The numbers reflect conventional numbering of carbons in steroidal structures. b; Structural formulas for common glycoside residues attached to C3 of the aglycones (R) (Ginzberg *et al.,* 2009).

1.7.2.1 Toxicity of Glycoalkaloids

Glycoalkaloids are potentially toxic compounds. Typically, potato tubers contain about 20-60 mg of total glycoalkaloid (TGA) per 100 g of freeze-dried matter, equivalent to 4-12 mg of TGA per 100 g fresh weight (fwt). At these concentrations glycoalkaloids enhance potato flavor. However, at concentrations greater than 20 mg per 100 g fwt they impart a bitter taste and can cause gastroenteritic symptoms, coma, and even death. The toxic dose is considered to be approximately 2-5 mg kg^{-1}body weight (bw) whereas the lethal dose is probably 3-6 mg kg^{-1}. Due to human toxicity, 200 mg TGA/kg fwt potatoes is accepted as the upper safety limit. The major toxic properties of glycoalkaloids are due to i) the ability of glycoalkaloids to bind with membrane 3β-hydroxy sterols and to disrupt membrane function and ii) the ability to inhibit acetylcholinesterase. The toxicity of glycoalkaloids has largely been overestimated; chacotriose-based glycoalkaloids are highly active whereas solatriose-based glycoalkaloids have exhibited lower or no activity with regard to membrane-disruptive activity (Laurria, 2004).

1.7.3 Saponins

Saponins are one of the biggest classes of compounds in natural products chemistry. The classical definition of saponins is based on their surface activity. Many saponins have detergent properties. 'Saponin' comes from the Latin word sapo meaning soap. *Saponaria officinalis* (common name soapwort) is an example of a saponin containing plant that has been employed for hundreds of years, as a natural soap. Saponins occur in nature as glycosides; that is, they contain a sugar moiety linked to an aglycone. The aglycone or non-saccharide portion of the saponin molecule is called the genin or sapogenin (Hostettmann and Marston, 1995; Oakenfull and Sidhu, 1986). Saponins are an important group of secondary metabolites in plants that on the basis of the aglycon nature are divided in two main groups: triterpenoid saponins (based on C30 aglycon) and the steroid saponins (based on C27 aglycon). The steroid saponins from plants are in turn divided in three groups: cholestane, furostane and spirostane (Sadeghi *et al*., 2013). Saponins are widely distributed in the plant and marine animal kingdoms. A large number of saponins are biologically active. Despite their diverse chemistry, saponins have some common characteristic properties. These include:

1. Bitter taste.

2. Formation of stable foams in aqueous solution.

3. Hemolysis of red blood cells.

4. Toxicity to cold-blooded animals such as fish, snails, insects, etc.

5. An ability to interact with bile acids, cholesterol, or other 3β- hydroxysteroids in aqueous of alcoholic solution to form mixed micelles orcoprecipitates (Hostettmann, 1991).

Recent publications have described the importance of spirostanes and furostanes and their glycosides not only as economically important raw materials convertible into various steroid hormonal drugs, but also as biologically active materials having independent value. Anticancer, cytotoxicity, antitumor, anti-inflammatory, antioxidant, antiviral, antifungal, antimicrobial, molluscicidal and antihypercholesteremic activities and as plant growth stimulant have been reported for steroidal glycosides (Hostettmann, 1991).

1.7.3.1 Biological activity of *Solanum* glycoalkaloids and steroidal saponins

The *Solanum* contains steroidal alkaloids that showed biological effects like antifungal, and antiviral ones, but the most important feature is that it also shows considerable anticancer effects. For example, solamargine causes the human hepatoma cells death (Hep3B) by apoptosis; α-solasonine from *S. crinitum* and *S. jabrense* has a cytotoxic effect on the leukemia cells; chaconine, solanine, tomatine, and their derivatives inhibit the human colon (HT29) and liver (HepG2) cancer cells to grow; β-2-solamargine from *S. nigrum* has a toxic effect on the cell lines: HT-29 (colon), HCT-15 (colon), LN C aP (prostate), PC-3 (prostate), T47D (breast and MDA-MB-231 (breast) (Sammani *et al* ., 2013). Previous studies have showed that α-solanine and α-chaconin can induce gastro-intestinal and systemic effects *in vivo* and *in vitro*, by disruption of cell membranes and inhibition of acetylcholinesterase activity (Langkilde *et al*., 2008). In another study, the antimicrobial activity of steroidal saponins of *S. xanthocarpum* showed high antimicrobial activity against *Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli* and strong antifungal activity as well (Kajaria *et al*., 2012).

1.7.4 Flavonoids

Flavonoids are present in most plant tissues and often in vacuoles. The basic structures of flavonoid molecules are composed of three rings with various substitutions, including glycosylation, hydrogenation, hydroxylaltion, malonylation, methylation and sulfation. Flavonoids are divided into classes according to their substitutes and oxidation level on the

middle ring. The main subclasses and their respective food sources are anthocyanidins (red, purple and blue berries), flavanols (teas, red grapes and red wines), flavones (green leafy species), flavonols (ubiquitous in foods), flavanones (citrus), and isoflavones (soybeans). In nature, they are present principally as glycosylated, esterified, and polymerized derivatives. Sugar moieties attached to flavonoids increases polarity of the molecules for their storage in plant cell vacuoles (Yu Yang *et al*., 2008).

The function of flavonoids in flowers is to provide colours attractive to plant pollinators. In leaves, these compounds are increasingly believed to promote physiological survival of the plant, protecting it from, for example, fungal pathogens and UV-B radiation (Harborne and Williams, 2000). In addition, flavonoids are involved in photosensitisation, energy transfer, the actions of plant growth hormones and growth regulators, control of respiration and photosynthesis, morphogenesis and sex determination.

The basic structural feature of flavonoid compounds is the 2-phenyl-benzo pyrane or flavane nucleus, which consists of two benzene rings (A and B) linked through a heterocyclic pyrane ring (C). Flavonoids can be classified according to biosynthetic origin. Some classes, for example chalcones, flavanones, flavan-3-ols and flavan-3, 4-diols, are both intermediates in biosynthesis as well as end products that can accumulate in plant tissues. Other classes are only known as end products of biosynthesis, for example anthocyanidins, proanthocyanidins, flavones and flavonols. Two additional classes of flavonoid are those in which the 2-phenyl side chain of flavanone isomerises to position 3, giving rise to isoflavones and related isoflavonoids. The neoflavonoid is formed through further isomerisation to position 4 (Tim and Lamb, 2005).

Many polyphenolic and glycosides of quercetin, kaempferol and myricetin had been reported from various species of *Solanaceae* family (Fig. 7). 10 flavonoids from leaf extract of 11 species belonging to the section *Solanum*; including coumarins (such as scopoletin) were isolated. Flavonols and anthocyanidins for *S. scabrum* in Nigeria, and the anthocyanin pigments were also found in European samples of this species. Quercetin is the most commonly occurring flavonol aglycone detected in *S. nigrum* Complex. It forms many glycosides like quercitrin, isoquercitrin and rutin together with rhamnose and glucose as sugar moieties attached in different patterns (Mohy-UD-Din , 2010).

Flavonoids have been reported to possess many useful properties, including anti-inflammatory activity, oestrogenic activity, enzyme inhibition, antimicrobial activity

antiallergic activity, antioxidant activity, vascular activity and cytotoxic antitumor activity (Harborne and Williams, 2000). For a group of compounds of relatively homogeneous structure, the flavonoids inhibit a perplexing number and variety of eukaryotic enzymes and have a tremendously wide range of activities. In the case of enzyme inhibition, this has been postulated to be due to the interaction of enzymes with different parts of the flavonoid molecule, e.g. carbohydrate, phenyl ring, phenol and benzopyrone ring. It has been suggested that because flavonoids are widely distributed in edible plants and beverages and have previously been used in traditional medicine, they are likely to have minimal toxicity. However, this family of compounds has a diverse range of activities in mammalian cells (Middleton *et al.,* 2000). Numerous research groups have sought to elucidate the antibacterial mechanisms of action of selected flavonoids. The activity of quercetin, for example, has been at least partially attributed to inhibition of DNA gyrase. It has also been proposed that sophoraflavone G and (−) epigallocatechin, gallate inhibit cytoplasmic membrane function, and that licochalcones A and C inhibit energy metabolism (Tim and Lamb, 2005).

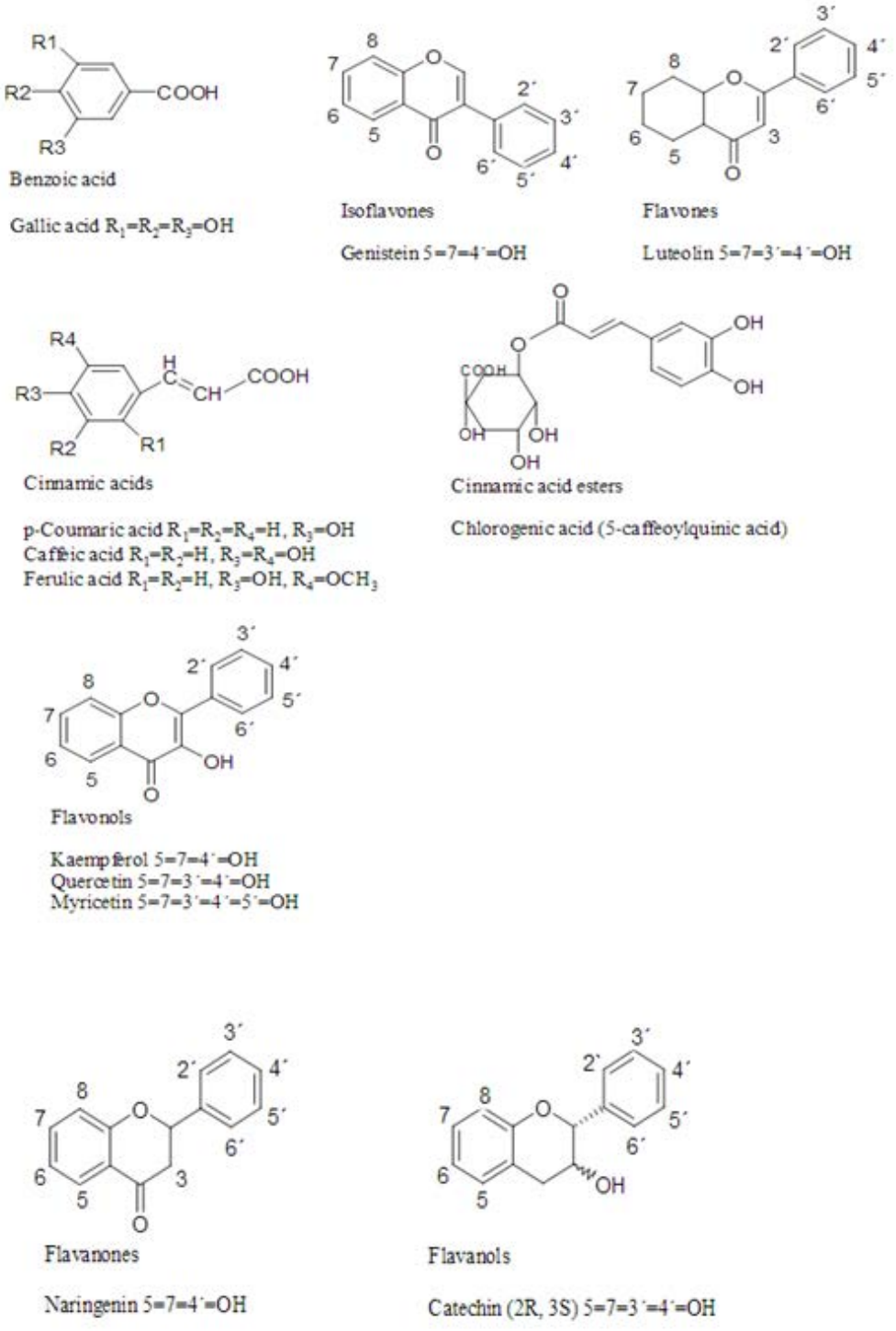


Figure 7: Structure of polyphenolic compounds found in plants of the Solanaceae family.

1.8 Biological activity of phytochemicals

Plant secondary metabolites are sought after because they are known to exhibit numerous biological activities that promote positive health effects. These activities include antioxidant, antibacterial and anticancer.

1.8.1 Antioxidant activity

Oxidation is essential to many living organisms for the production of energy to fuel biological processes. The oxidative stress, defined as ‘‘the imbalance between oxidants and antioxidants in favor of the oxidants potentially. Almost all organisms are well protected against free radical damage by enzymes such as superoxide dismutase and catalase, or compounds such as ascorbic acid, tocopherols and glutathione (Niki *et al.,* 1994). However, the uncontrolled production of oxygen derived free radicals is involved in the onset of many diseases such as cancer, rheumatoid arthritis, and atherosclerosis as well as in degenerative processes associated with aging (Halliwell and Gutteridge, 1984). In modern western medicine, the balance between antioxidation and oxidation is believed to be a critical concept maintaining a healthy biological system (Davies, 2000). The similar concept of balance called yin yang has existed in traditional Chinese medicine for more than 2000 year (Prior and Cao, 2000). Reactive oxygen (ROS) and nitrogen (RNS) species are products of normal cellular metabolism. However, at high concentrations, these species may be important mediators of damage to cellular structures, such as nucleic acids, lipids and proteins. The oxidation of any of these substrates, if uncontrolled, can contribute to the development of chronic diseases such as cancer, hypertension, diabetes mellitus, cardiovascular and neurodegenerative diseases (Valko *et al*., 2007). In this sense, there is great interest in finding natural antioxidants from plant materials, and various extracts and isolated compounds have been investigated for their antioxidant activity, using different methods, (Zadra *et al*., 2012).

The health promoting benefits of antioxidants of plants are thought to be resulted from their potential effects against the reactive oxygen/nitrogen species. Restriction on the use of synthetic antioxidants due to their possible undesirable effects on human health has led to a growing interest in natural antioxidants of plant origin in recent years. Hence, the development of antioxidants from natural origin has attracted considerable attention and is thought to be a desirable development. A multitude of natural antioxidants have been isolated from different kinds of plant materials such as oil seeds, cereal crops, vegetables, fruits, leaves, roots, spices and herbs. Moreover, several studies have indicated that medicinal plants contain a wide variety of natural antioxidant such as phenolic acids, flavonoids and tannins, which possess antioxidant activity (Muruhan . *et al*., 2013).

Natural antioxidants either in the form of raw extracts or their chemical constituents are very effective to prevent the destructive processes caused by oxidative stress (Zengin *et al*., 2011).

Natural antioxidants may function (a) as reducing agents, (b) as free radical scavengers, (c) as complexers of prooxidant metals, and (d) as quenchers of the formation of singlet oxygen. Examples of polyphenol natural antioxidants derived from plant sources include vitamin E, flavonoids, cinnamic acid derivatives, curcumin, caffeine, catechins, gallic acid derivatives, salicylic acid derivatives, chlorogenic acid, resveratrol, folate, anthocyanins and tannins. Many plants have been identified as having potential antioxidant activities and their consumption recommended. Bioactive phenols, especially bioflavonoids, are very interesting as antioxidants because of their natural origin and the ability to act as efficient free radical scavengers. In last two decades the number of publications on the potential health benefits of polyphenols, has increased enormously. Tea (black and green tea) is one of the most commonly consumed beverages in the world and is rich in polyphenolic compounds collectively known as the tea flavonoids (Lie and Xie, 2000; Katalinic *et al*., 2006).

Many Solanaceae species were reported as oxidative substances. For example, *S. fastigiatum* exhibited strong antioxidant activity in the DPPH assay (Sabir, 2008). Muthuvel *et al*. (2014) Study the effect of *S. nigrum* aqueous extract as antioxidant agent. Jimoh *et al.* (2010) compared the effect of antioxidant activity of acetone, methanol, water and butanol extract of *S. nigrum* leaves and should that all extracts displayed strong ABTS radical scavenging inhibition.

1.8.2 Antimicrobial activity

Commensally micro-organisms constitute the normal flora of the healthy body. They live in skin and on the mucous membranes of the upper respiratory tract, intestines and vagina, and obtain nourishment from the secretions and food residues. They are generally harmless, but under certain circumstances, they may invade the tissues and cause disease, thus acting as opportunist pathogens. True pathogens are the micro-organisms that are adapted to overcoming the normal defenses of the body and invading the tissues; their growth in the tissues, or their production of the poisonous substances (toxins), damages the tissue and causes the manifestations of the disease. The process of the microbial invasion of the body is called infection. Those infective diseases that are ready communicable from person to person are called infectious or contagious (Shakya *et al*., 2012). Infectious diseases are the leading causes of death throughout the world that accounts for nearly one half of all death in the tropical countries, which are also becoming a serious problem in developed countries. Antibiotics are sometime associated with adverse effects including hypersensitivity, immuno

suppressant and allergic reactions. Because of the resistance that pathogenic build against antibiotics, there is a great interest in the search for new antimicrobial drugs also from nature. Natural crude drug extracts and biologically active compounds isolated from plant species used in traditional medicine can be prolific resources for such new drugs, (Al-Fatimi *et al.,* 2007; Hussain *et al.,* 2011). Among the potential sources of new agents, plants have long been investigated. They contain many bioactive compounds that can be of interest in therapeutic (Djeussi *et al*., 2013).

Solanum species are one of the most potent plants known to have antimicrobial activity against pathogenic microorganisms. For example, *S. torvum* (leaf, stem and roots) showed antibacterial and antifungal activity (Parameswari *et al*., 2012). The methanol and aqueous extracts of leaves of five different medicinal plants; *S. nigrum, S. torvum, S. trilobatum*, *S. surattense* and *S. melongena* showed variable activity against *Xanthomonas campestris* and *Aeromonas hydrophila* (Kumar *et al.,* 2016).

1.8.3 Antitumor activity

Cancer is rapidly becoming a global pandemic, with incidence and death rates rising in low- and middle-income countries. It is a multistep disease which interacts with surrounding factors; physical, environmental, metabolic, chemical, and genetic factors, and induce directly and/or indirectly incidence of cancers. A report for The International Agency for Research on Cancer (IARC) - specialized cancer agency of the World Health Organization estimated that about 14.9 million cancer cases around the world in 2013, of these 7.7 million cases were in men and 6.9 million in women and further this number is expected to increase to 24 million by 2035 (Bray *et al*., 2013).

Over half a century after beginning chemotherapy for tumor treatment, phytochemicals have become an important part of antineoplastic agents. About 70% of anticancer drugs approved between 1940 and 2002 are either natural products or developed based on knowledge gained from natural products. Important examples for the success of anticancer drugs originally obtained from plants are the Vinca alkaloids from *Catharanthus roseus*, camptothecin from *Camptotheca acuminata*, paclitaxel from *Taxus baccata*, and podophyllotoxin isolated from *Podophyllum peltatum*. Anthocyanins from *S. tuberosum* L. var. *vitelotte* were found to cause inhibition of proliferation and apoptosis in different cancer cell models (Bontempo *et al.,* 2013). Water extract, ethanol extract and n-butanol extract of *S. nigrum* was found to be inhibit the growth of human gastric cancer MGC-803 cells (Ding *et al*., 2013).

Ethanol extract from ripe fruits of *S. nigrum* had growth-inhibitory effect on MCF-7 human breast cancer cells. Results from proliferation assay using tritium uptake showed that the proliferative capacity of MCF-7 cells was strongly suppressed in the presence of *S. nigrum* ethanol extract. This was further confirmed through MTT assay and trypan blue exclusion experiments, which showed a very close correlation between the *S. nigrum* extract concentration and the surviving cell numbers (Son *et al*., 2003). Three new sesquiterpoinds from *S. lyratum,* named solajiangxin D) and vetispirane-type (2–3, named solajiangxins E and 2-hydroxysolajiangxin E) were isolated and were found to show significant cytotoxicity against three human cancer lines (P-388, HONE-1 and HT-29) and gave ED_{50} values in the range 2.1–3.7 mg/ml (Yao *et al*., 2013). The roots of *W. somnifera* also possess nootropic activity. It has been reported that it also inhibits DMBA-induced carcinogenesis in mice and transplantable mouse tumour, sarcoma180 (Christina *et al*., 2004).

1.9 Computational chemistry and molecular modeling

Computational chemistry is a branch of chemistry that uses principles of computer science to assist in solving chemical problems. It uses the results of theoretical chemistry, incorporated them into efficient computer programs to calculate the structures and properties of molecules and solids. Its necessity arises from the well-known fact that apart from relatively recent results concerning the hydrogen molecular ion, the quantum n-body problem cannot be solved analytically and much less in closed form. While its results normally complement the information obtained by chemical experiments, it can in some cases predict hitherto unobserved chemical phenomena. It is widely used in the design of new drugs and materials. The methods employed cover both static and dynamic situations. In all cases the computer time and other resources (such as memory and disk space) increase rapidly with the size of the system being studied. The system can be a single molecule or a group of molecules. Computational chemistry methods range from highly accurate to very approximate; highly accurate methods are typically feasible only for small systems (Smith, 1997).

1.9.1 Molecular modeling (docking)

Structure-based drug design is a powerful method, especially when used as a tool within an armamentarium for discovering new drug leads against important targets. After a target and a structure of that target are chosen, new leads can be designed from chemical principles or

chosen from a subset of small molecules that scored well when docked in silico against the target after a preliminary assessment of bioavailability.

Each year, new targets are being identified, structures of those targets are being determined at an amazing rate and our capability to capture a quantitative picture of the interactions between macromolecules and ligands is accelerating. To understand the design concepts of the various types of binding enzyme inhibitors, a basic knowledge of the binding forces between an enzyme's active site and its inhibitors is required. The forces involved between a substrate or an inhibitor binding and an enzyme's active site are the same forces that are experienced by all interacting organic molecules. These include ionic (electrostatic) interactions, ion-dipole and dipole-dipole interactions, hydrogen bonding, hydrophobic interactions, and van der Waals interactions (Klebe, 2006).

CHAPTER TWO

2. Materials and methods

2.1 Plant materials

Leaves of the studied plant materials were collected from eastern Sudan, Erkowit region, in April 2012. Botanical identification and authentication were performed and voucher specimens (No. 2012/4SN for *S. incanum,* No. 2012/4SI for *S. nigrum*; No. 2012/4SS for *S. schimperianum;* No. 2012/4PL for *P. lagascae* and No. 2012/4WS for *W. somnifera*) have been deposited in Botany Department Herbarium, Faculty of Science, University of Khartoum, Sudan.

2.2 Preparation of plant extracts

2.2.1 Methanolic extract

Twenty grams of the dry powder from each plant material were macerated in methanol (200 mL), at room temperature for 72 h. The extracts were evaporated under vacuum to dryness to obtain 1.9 g (*S. incanum*), 4.3 g (*S. schimperianum*), 4.8 g (*S. nigrum*), 4.4 g (*P. lagascae*) and 4.1 g (*W. somnifera*).

2.2.2 Steroidal glycoalkaloids fractions (SGAFs)

Extraction of SGA was carried out according to the method described by Mohy-Ud-Din (2010). Twenty grams of each powdered plant material were extracted with 5% aqueous acetic acid at room temperature for 30 min. Samples were then vacuum filtered through a Whatman n^{o} 4 filter paper. The polar fraction was then basified with NH_4OH (pH = 11.0) and extracted with water saturated n-butanol. Finally, the solvent was evaporated with a rotavapor to dryness to obtain 2.1 g (*S. incanum*), 2.5 g (*S. schimperianum*), 2.9 g (*S. nigrum*), 2.4 g (*P. lagascae*) and 2.0 g (*W. somnifera*).

2.3 Biological activity

2.3.1 Antibacterial activity assay

2.3.1.1 Microorganisms

Standard strains of microorganism, obtained from Medicinal and Aromatic Institute of Research, National Research Center, Khartoum, were used in this study. The bacterial species used were the Gram-negative *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC27853), and Gram positive *Bacillus subtilis* (ATCC 6633) and *Staphylococcus aureus* (ATCC 25923).

2.3.1.2 Minimal inhibitory concentration (MIC) assay

The two-fold serial microdilution method described by Eloff (1998) was used to determine the MIC values for each extract against bacteria growth. All dilutions were prepared under aseptic conditions. A volume of 100 µL of the extracts (1 mg/mL) dissolved in DMSO (5%, v/v) in duplicate was serially diluted two-fold with sterile distilled water and 100 µL of bacterial culture in MH Broth, corresponding to 10^6 CFU/mL, was added to each well. Gentamicin and amoxicillin were used as positive controls and DMSO as negative control. Plates were incubated overnight at 37 °C. Afterwards, 40 µL of 0.2 mg/mL of p-iodonitrotetrazolium violet (INT) was added to each well to indicate microbial growth. The colourless salt of tetrazolium acts as an electron acceptor and is reduced to a red coloured formazan product by biologically active organisms. The solution in wells remains clear or shows a marked decrease in intensity of colour after incubation with INT at the concentration where bacterial growth is inhibited. Plates were further incubated at 37 °C for 2 h and the MIC was determined as the lowest concentration inhibiting microbial growth, indicated by a decrease in the intensity of the red colour of the formazan. The experiment was performed in triplicate.

2.3.2 Cell viability assay

2.3.2.1 Cell culture

Anti-proliferative activities of each extract were evaluated with four cell lines established from human breast carcinoma samples (MCF7 and MDA-MB-231) and from human colon adenocarcinoma samples (HT29 and HCT116). HCT116 and HT29 cells were cultivated in Dulbecco's minimum essential medium (DMEM, Eurobio, Courtaboeuf, France)

supplemented with 10% (v/v) fetal calf serum (Eurobio), 1% Penicillin/streptomycin (Eurobio) and 2 mM L-glutamine (Eurobio). MCF7 and MDA-MB-231 cells were grown in RPMI medium with the same additives. Cells were routinely seeded at 100 000 cells /mL and maintained weekly in a humidified atmosphere of 5% CO_2 at 37 °C.

2.3.2.2 MTT procedure

Cell viability assay was performed using the thiazolyl blue tetrazolium bromide (MTT) procedure as described by Mosman (1983). In brief, cancer cells were seeded in 96-well plate at 10 000 cells/well for HT29, MCF-7 and MDA-MB231 cells, at 5 000 cells/well for HCT116 cells (Greiner-Bio-One GmbH, Friekenhanusen, Germany). Twenty four hours after seeding, 100 µL of medium containing increasing concentrations of each extract (final concentration range from 0.5 to 400.0 µg/mL) were added to each well for 72 h at 37 °C. Dried extracts were firstly diluted with DMSO to a final concentration at 50 mg (w/v)/mL or 200 mg (w/v)/mL. After incubation, the medium was discarded and 100 µL/well of MTT solution (0.5 mg/mL diluted in DMEM or RPMI medium) were added and incubated for 2 h. Water-insoluble formazan blue crystals were finally dissolved in DMSO. Each plate was read at 570 nm. IC_{50} was calculated using GraphPad Prism (GraphPad Software, La Jolla, CA, USA). Data are expressed as IC_{50} ± SD obtained from quadruplicate determinations of two independent experiments (n = 8). As a control, we tested usually fenofibrate, a member of fibrate family, on HT29 and HCT116 cells, in order to confirm a moderate effect on HT29 cell viability (IC_{50} = 28.3 ± 0.9 µM) whereas IC_{50} is > 50 µM when HCT116 cells are used.

2.3.3 Antioxidant activity studies

2.3.3.1 DPPH radical-scavenging test

Antioxidant activity of extracts/compounds was estimated using *in vitro* 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging radical method (Yagi *et al.,* 2013). Test samples were dissolved separately in methanol to get test solution of 1 mg/mL. Series of extract solutions of different concentrations (1, 5, 10, 20, 40, 60, 80 and 100 µg/mL) were prepared by diluting with methanol. Assays were performed in 96-well, microtiter plates. 140 µL of $0.6.10^{-6}$ mol/L DPPH were added to each well containing 70 µL of sample. The mixture was shaken gently and left to stand for 30 min in dark at room temperature. The absorbance was measured spectrophotometrically at 517 nm using a microtiter plate reader (Synergy HT Biotek, logiciel GEN5). Blank was done in the same way using methanol and sample without DPPH and

control was done in the same way but using DPPH and methanol without sample. Ascorbic acid was used as reference antioxidant compound. Every analysis was done in triplicate.

The ability to scavenge DPPH radical was calculated by the following equation:

DPPH radical scavenging activity (%) = 1- [(Abs_{sample} – Abs_{blank})]/ ($Abs_{control}$)] x 100

Where;

Abs_{sample} is the absorbance of DPPH radical + sample;

Abs_{blank} is the absorbance of sample+ methanol;

$Abs_{control}$ is the absorbance of DPPH radical + methanol.

The IC_{50} value was calculated from the linear regression of plots of concentration of the test sample against the mean percentage of the antioxidant activity. Results were expressed as mean ± SEM and the IC_{50} values obtained from the regression plots (Sigma PlotsR 2001, SPSS Science) had a good coefficient of correlation, ($R^2 = 0.998$).

2.3.3.2 ABTS radical-scavenging test

A second *in vitro* method was performed to estimate antioxidant potential of the extracts: 2,2'-azino-bis 3-ethylbenzthiazoline- 6-sulphonic acid (ABTS) scavenging radical assay, based on the method of Re *et al*. (1999). Test samples were dissolved separately in methanol to get test solution of 1 mg/mL. Series of extract solutions of different concentrations (1, 5, 10, 20, 40, 60, 80 and 100 µg/mL) were prepared by diluting with methanol. The ABTS radical cation ($ABTS^{*+}$) was produced by reacting 7 mM stock solution of ABTS with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12 h before use. The obtained ABTS radical solution was diluted with methanol to an absorbance of 0.700 ± 0.02 at 734 nm. 190 µL of ABTS radical solution were added to each well containing 10 µL of sample. The mixture was shaken gently and left to stand for 15 min in dark at room temperature. The absorbance was measured spectrophotometrically at 734 nm using a microtiter plate reader (Synergy HT Biotek®, logiciel GEN5). The $ABTS^{*+}$ scavenging capacity of the extract was compared with that of ascorbic acid and the percentage inhibition calculated as:

ABTS radical scavenging activity (%) = [($Abs_{control}$ – Abs_{sample})]/ ($Abs_{control}$)] x 100

Where;

$Abs_{control}$ is the absorbance of ABTS radical (= 0.700 ± 0.02);

Abs_{sample} is the absorbance of sample + ABTS radical.

The IC_{50} value was calculated from the linear regression of plots of concentration of the test sample against the mean percentage of the antioxidant activity obtained from three replicate assays. Results were expressed as mean ± SEM and the IC_{50} values obtained from the regression plots (Sigma PlotsR 2001, SPSS Science) had a good coefficient of correlation, (R^2 = 0.9926).

2.3.4 Tyrosinase inhibition assay

Mushroom tyrosinase inhibitory assay was performed using the DOPA-chrome method with some modifications (Moet *et al.,* 2008). Briefly, the extracts were dissolved in 10% DMSO in distilled water at a concentration of 5% w/v. Four test tubes (A, B, C, D) were used for each extract. One mL of 2.5 m ML-DOPA and 1.8 mL of 0.1 M phosphate buffer (pH 6.8) were added to each tube and subsequently incubated at room temperature for 10 minutes. After incubation, reagents were added as follows; Tube A (0.1 mL 10% DMSO, 0.1 mL tyrosinase enzyme at a concentration of 605 unit/mL), tube B (0.1 mL water, 0.1 mL of 10% DMSO), tube C (0.1 mL tyrosinase enzyme, 0.1 mL herbal extract), tube D (0.1 mL water, 0.1 mL herbal extract). The final concentration of each extract in reaction tubes was 1.67 mg/mL. After incubation at room temperature for 25 minutes, the absorbance of each tube was measured at 492 nm to monitor the formation of the DOPA-chrome. Each reaction tubes were prepared in 3 replications. Percentage of inhibition of tyrosinase activity was calculated as follows.

$$\% \text{ Tyrosinase inhibition} = 100 \times [(A-B)-(C-D)]/ (A-B)$$

Where; A, B, C, D were the absorbance of mixture of tube A, B, C, D, respectively. Kojic acid at a concentration of 1% was also determined as a positive control. After addition to the reaction tube, final concentration was 0.33 mg/mL.

2.4 Phytochemistry

2.4.1 Qualitatative tests for secondary metabolites

- **Test for Alkaloids**

The various extract/ fractions were basified with ammonia and extracted with chloroform. The chloroform solution was acidified with dilute hydrochloric acid. The acid layer was used for testing the alkaloids. The acid layer was treated with few drops of Dragendroff's (Potassium Bismuth Iodide) reagent. Formation of reddish brown precipitate indicates the presence of alkaloids.

- **Test for Flavonoids**

Lead acetate test: To the extract, few drops of aqueous basic lead acetate solution were added. Formation of yellow precipitate indicates presence of flavonoids.

- **Test for Sterols/ Triterpenes**

Liebermann-Burchard test for Sterols: To the chloroform solution, few drops of acetic anhydride were added and mixed well. One ml of concentrated sulphuric acid was added from the sides of the test tube, appearance of reddish brown ring indicates the presence of sterols. Appearance of deep red colour indicates the presence of triterpenes.

- **Test for Tannins**

Ferric chloride test: To extracts a few drops of 1% neutral ferric chloride solution were added, formation of blackish blue colour indicates the presence of tannins.

- **Test for Saponins**

Foam test: Small amount of extract was shaken with little quantity of water, if foam produced persists for 10 minutes, it indicates the presence of saponins.

2.4.2 Determination of total phenol

Total phenols contents in the methanol extract were recorded using modified Folin Ciocalteu method (Wolfe *et al*., 2003). An aliquot of the extract was mixed with 5 mL Folin Ciocalteu reagent (previously diluted with water at 1:10 v/v) and 4 mL (75 g/L) of sodium carbonate. The tubes were vortexes for 15 sec and allowed to stand for 30 min at 40 °C for color development. Absorbance was then measured at 765 nm using the SHIMADZU UV-2550 UV-VS spectrophotometer. Sample extracts were evaluated at a final concentration of 0.1 mg/mL. Total phenolic contents were expressed as gallic acid (ROTH) equivalents in milligram per gram sample (mg/g).

2.4.3 Preliminary screening with thin layer chromatography (TLC)

Chromatographic separations were carried out using precoated silica gel GF245 TLC plates. Chromatograms were developed by the ascending method at room temperature. After dryness, TLC plates were viewed under UV light at 254 and 366 nm for fluorescence or quenching spots and then plates were sprayed with different spray reagents.

The most commonly used solvent systems in the present work were:

1. Petroleum ether: acetone (7:3 v/v).
2. n-Buotanol: acetic acid: water (4:1:5 v/v).
3. Petrolum ether: ethyl acetate (8:2 v/v).
4. Ethyl acetate: methanol: water (20:2.7:2 v/v).
5. Toluene : ethyl acetate (93:7 v/v).
6. Toluene: ethyl acetate: formic acid (5:4:1 v/v).
7. Ethyl acetate: acetic acid: formic acid: distilled water (100: 11: 11: 20, v/v/v/v).

2.4.3.1 Preparation of spray reagents

- **H_2SO_4 solution (20%)**

About 20 mL of concentrated sulphuric acid was added to 80 mL methanol.

- **Dragendroff's reagent**

(A) Bismitintrate (0.6 g) was dissolved in 2 mL concentrated HCl. 10 mL of water was added. (B) Potassium iodide (6 g) was dissolved in 10 mL water. Solution (A) mixed with (B). 7 mL concentrated HCl was added to the mixure, then diluted with 400 mL water.

- **Natural Products (NP) reagent**

One gram of diphenylboric acid aminoethyl ester is dissolved in 200 mL of ethyl acetate. Polyethylene Glycol (PEG) 400 reagent: 10 g of PEG 400 is dissolved in 200 mL of dichloromethane.

- **Calculation of R_f value**

R_f values were calculated as follows:

$$R_f \text{ value} = \frac{\text{Distance moved by the solute}}{\text{Distance moved by the solvent}}$$

2.4.4 Column Chromatography (CC)

Column chromatography was performed on open glass columns packed with silica gel of particle size (40-63μm) (Merck, type 60). Each extract was chromatographed after being dissolved in a small amount of appropriate solvent and mixed with a few amounts of silica gel and dried with air, then added into the top of the column and covered by a thin layer of silica gel.

2.4.5 Isolation and purification of compounds from *S. schimperianum* methanolic leaf extracts

The methanolic crude extract of *S. schimperianum* leaf (10 g) was subjected to CC (43 X 3cm) on silica gel (120g), using a gradient of hexane to acetone as eluent and final washing with pure methanol. Fractions of 10 ml portions were collected in 133 test tubes. Finally, 9 fractions were obtained on combining of elutes according to their similarity in behavior on TLC. Fractions 8 and 9 (1070 mg) were combined and subjected repeatedly to silica gel CC (21.5 X 2.5 cm) and eluted with hexane: acetone mixture of increasing polarity and sephadex LH20 (18.5 X1.5 cm) column eluted with chloroform and methanol (2:1 and 1:1 v/v) to afford semipure compound (200 mg) which was further subjected to Cobi flash column eluted with

acetone: ethyl acetate mixture of increasing polarity to afford pure crystals of compound **N-1** (35mg) eluted by acetone: ethyl acetate (6:4 and 5:5, v/v).

Fractions 5 (F-5) (1806 mg) and 6 (F-6) (1004 mg) were subjected separately to silica gel CC (19.5 X 2.5 cm), eluted with hexane: acetone mixture of increasing polarity and fractions of 10 mL were collected and combined on the basis of their TLC profile. F-5-7 (553 mg) obtained from F-5 and F-6- 8 (189 mg) obtained from F-6 were subjected separately to reversed phase silica gel column eluted with methanol: water (2:1, v/v) to give two pure compounds with the same R_f value in 5 different solvent systems and therefore were combined together to give compound **N-2** (30 mg).

Fraction 2 (550 mg) was subjected to Combi flash column, eluted with hexane: ethyl acetate mixture of increasing polarity to obtain compound **N-3** (28 mg) eluted by hexane: ethyl acetate (8:2, v/v).

2.4.6 Analytical Techniques

2.4.6.1 Gas Chromatography\Mass Spectroscopy analysis (GC\MS)

Gas chromatography coupled to mass spectrometry analyses were performed using QP2010-Shimadzu equipment (Shimadzu, Kyoto, Japan) operating in the EI mode at 70 eV. An SLB5 column (30 m x 0.25 mm x 0.25 μm) was employed with a 20-min temperature program of 190-325°C at 5°C/min. The injector temperature was 280° C, the flow rate of the carrier gas (helium) was 0.8 mL/min, and the split ratio was 1:50. Identification of components present in extracts was based by computer matching with the Wiley 229, Nist 107, Nist 21 Library, as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature.

2.4.6.2 NMR spectroscopy

All NMR experiments were performed with a Bruker Avance DRX-400 instrument (Bruker Spectrospin, Rheinstetten, Germany) operating at a proton frequency of 400.13 MHz. This spectrometer was equipped with a 5 mm broadband inverse detection z-gradient probe tuned to 13C (100.61 MHz). For all 1D and 2D NMR experiments pulse sequences provided by the spectrometer manufacturer were used. The spectra were measured in 0.6 ml of deuteriochoroform($CDCl_3$) or deuterioacetone (CD_3OCD_3) or deuteriomethanol (CD_3OD) at 300 K. Chemical shifts are expressed in ppm and the spectra were calibrated to the residual

solvent signals. Samples were run either in deuteriochlorochorm ($CDCl_3$), deuteriomethanol (CD_3OD) and deuterioacetone (CD_3OCD_3). The chemical shifts were in ppm relative to residual solvent signal. The coupling constants were measured in Hz and the following abbreviations are adopted: s (singlet); d (doublet); t (triplet) m multiplet); dd (doublet of doublet); dt (doublet of triplets).

2.4.6.3 Mass Spectra (MS)

Electron impact mass spectra (EI-MS) were recorded on Finnegan MAT 311 mass spectrometer with a MASPECO Data System. Peak matching and field desorption (FAB-MS) experiments were performed on a Finnegan MAT 312 mass spectrometer.

Hight resolution mass spectra (HR-MS) were recorded in ESI with a Bruker microTOFQ

2.5 Cluster analysis

Similarity between the five plants was analyzed on the basis of their GC/MS profile obtained from their methanolic extracts. Data were scored as an individual compound present (1) or absent (0) and the scores were used to create a similarity matrix using the PAST 3.01 software package. A dendrogram and Principal Component Analysis (PCA) were conducted through similarity matrix based on Jaccard's similarity coefficient (Jaccard, 1908).

2.6 Molecular docking

The docking study was performed with the docking module of molecular operating environment (MOE) software. The 3D structures of the compounds were downloaded from the web-based database Pubchem, then were transferred and saved into MOE database, the energy of each compound was minimized by MMFF94x force field. Protein 3D structure used in the current study (PDB code:4X1F) was retrieved from the protein data bank (http://www.rcsb.org/). All water molecules were removed, the 3D structure was protonated and energy minimized using MMFF94x force field. Finally, the 3D structure of the prepared protein was saved as PDB file. The prepared protein crystal structure was retrieved to be used as the receptor for docking. The receptor was verified as Receptor + Solvent and the site as Ligand Atoms. The Placement method used was Triangle Matcher. The first scoring function was set to London dG & the refinement to force field. The docking process was then started by retaining 10 poses. The final refined poses were ranked by the MM/GBVI binding free energy estimation.

CHAPTER THREE

3. Results and Discussion

3.1 Part One

3.1.1 Extractive yields and physical characteristic

Extractable matter percentage is one of the parameters used for the characterization of botanical drugs (Gami and Parabia, 2010). With the exception of *S. incanum*, methanol extracts of the investigated plants were in the range of 21-24% where higher extractable matter content was obtained from *S. nigrum. S. incanum* displayed the least extractable matter content (10%). Extractable matter content of the SGAFs range between 10 to 15% where the highest yield obtained from *S. nigrum* (15%) followed by *S. schimperianum* (13%), *P. lagascae* (12%), *S. incanum* (11%) and *W. somnifera* (10%) respectively.

Many researchers found that higher yields of extracts could be achieved by using more polar solvent (Harborne, 1973; Anwar *et al.,* 2003; Siddhuraju and Becker, 2003).

The colour and texture of both types of extracts were also reported (Table 2). All methanol extracts of studied species were dark green in colour and their texture generally appeared to be sticky. The SGAFs extracts varied in colour from brown (*S. incanum* and *S. schimperianum*), to yellow (*P. lagascae*) to reddish-black (*S. nigrum* and *W. somnifera*) and the texture generally appeared to be either powdered or sticky.

This variation in color and texture could be attributed to the kinds of chemical component of each extract and the polarity of the solvent.

Previous studies showed that variation in color, texture and the number of bioactive phytochemicals produced by plants depends on the age of the plant, nature of the soil and processing of plant material (Siddhuraju and Becker, 2003).

Table 2: Yield and physical characteristic of methanol and Steroidal glycoalkaloids fractions of leaf extracts of the five Solanaceae plants.

Plant species	Yield (%)	Colour	Texture
Methanol extracts			
S. incanum	10	Dark green	Sticky
S. schimperianum	22	Dark green	Sticky
S. nigrum	24	Dark green	Sticky
P. lagascae	22	Dark green	Sticky
W. somnifera	21	Dark green	Sticky
Steroidal glycoalkaloids fractions			
S. incanum	11	Brown	Powder
S. schimperianum	13	Dark brown	Powder
S. nigrum	15	Reddish-black	Powder
P. lagascae	12	Yellow	Viscous
W. somnifera	10	Reddish-black	Viscous

3.1.2 Biological activity of leaf extracts of selected Solanaceae species

The methanol extracts and SGAFs of *S. incanum, S. schimperianum, S. nigrum, P. lagascae* and *W. somnifera* leaf were evaluated for their *in vitro* antibacterial, anti-proliferative and antioxidant activities.

3.1.2.1 Antibacterial activity

Methanolic extracts and SGAFs of *S. incanum, S. schimperianum, S. nigrum, P. lagascae* and *W. somnifera* leaf were evaluated for their *in vitro* antibacterial activity against Gram positive bacteria; *S. aureus* and *B. subtilis*, and Gram negative bacteria; *E. coli*, and *P. aeruginosa* and results are presented in Table 3.

The sensitivity of tested Gram-positive and Gram-negative bacteria to different extracts was variable. The antibacterial activity of the SGAFs of *S. incanum, S. schimperianum* and *P. lagascae* against *B. subtillis* exceeded the effect of their corresponding methanolic extract by 7.8-fold, 3.9-fold and > 2-fold respectively while that of *S. nigrum, P. lagascae* and *W. somnifera* SGAFs against *S. aureus* was higher by 33.3-fold for the first and > 66.7-fold for the two last than their respective methanolic extracts. SGAFs of *S. nigrum* and *W. somnifera* against *E. coli* increased by > 66.7-fold and *S. incanum* and *P. lagascae* against *P. aeruginosa* increased by > 4-fold and >32.3-fold than their respective methanolic extracts. In contrary, the antibacterial activity of SGAF of *S. incanum* against *S. aureus* and *E. coli* reduced by 8.4-fold and 2.1-fold respectively compared to their respective methanolic extracts. The same observation was obtained for *P. lagascae* (2-fold) against *E. coli.* However, the antibacterial activity of *S. schimperianum* against *S. aureus, E. coli* and *P. aeruginosa* was comparable in the two types of extracts, the same was true for the antibacterial activity of *S. nigrum* and *W. somnifera* against *P. aeruginosa.* These results supported the previously reported antibacterial activity for crude extracts of *S. nigrum* (Jimoh *et al*., 2010), *S. incanum* (Britto and Senthinkumar, 2001; Taye *et al*., 2011), *S. schimperianum* (Al-Oqaila *et al*., 2012) and *W. somnifera* (Alam *et al.,* 2012).

Table 3: Antibacterial activity of leaf extracts of five Solanaceae plants.

Plant species	*B. subtilis*	*S. aureus*	*E. coli*	*P. aeurginosa*
	Minimum inhibitory concentration (µg/mL)			
	Methanol extract			
S. incanum	250	15	15	> 1000
S. schimperianum	126	> 1000	> 1000	> 1000
S. nigrum	> 1000	500	> 1000	63
P. lagascae	> 1000	> 1000	32	> 1000
W. somnifera	> 1000	> 1000	> 1000	15
	Steroidal glycoalkaloids fraction (SGAF)			
S. incanum	32	126	32	250
S. schimperianum	32	> 1000	> 1000	> 1000
S. nigrum	> 1000	15	15	63
P. lagascae	500	15	63	31
W. somnifera	> 1000	15	15	15
	Antibiotics (+ve control)			
Amoxicillin	4	4	-	-
Gentamicin	-	-	4	4

3.1.2.2 Anti-proliferative activity

Methanolic extracts and SGAFs of *S. incanum, S. schimperianum, S. nigrum, P. lagascae* and *W. somnifera* leaf were tested, *in vitro*, for their potential anti-proliferative activity against HT29, HCT116, MCF7 and MDA-MB231 cell lines (Table 4). Results of the methanolic extracts showed that only *S. schimperianum* leaf demonstrated interesting anti-proliferative activity against the four cell lines with IC_{50} values in the range of 2.69 to 19.83 µg/mL. The highest anti-proliferative activity was obtained against HT29 (2.69± 0.56 µg/mL) followed by HCT116 (5.70 ± 0.51µg/mL), MDA-MB231 (7.01 ± 0.25 µg/mL) and MCF7 (19.83± 3.83 µg/mL) respectively. However, all other methanolic extracts showed anti-proliferative activity against the four cell lines with IC_{50} values >50 µg/mL.

The anti-proliferative activity of SGAFs was variable. Potent anti-proliferative activity was observed for the SGAF of *W. somnifera* leaf where the highest activity was obtained against

HCT116 (1.29 ± 0.06 µg/mL) followed by MCF7 (2.34 ± 0.68 µg/mL), HT29 (4.23 ± 0.25 µg/mL) and MDA-MB231 (5.00 ± 0.75 µg/mL) respectively. Ichikawa *et al*. (2006) reported that, withanolides (bioactive compounds of *W. somnifera*) inhibit cyclooxygenase enzymes, lipid peroxidation, and proliferation of tumor cells through the suppression of nuclear factor-κB (NF-κB) and NF-κB-regulated gene products. *P. lagascae* leaf SGAF displayed the second highest anti-proliferative activity where the highest anti-proliferative activity was obtained against HCT116 (4.79 ± 0.77 µg/mL) followed by MCF7 (5.09 ± 0.24 µg/mL), MDA-MB231 (6.90 ± 0.30 µg/mL) and HT29 (7.45 ± 0.59 µg/mL) respectively. This is the first time to illustrate the antiproliferative activity of *P. lagascae,* previous study on *P. crassifolia* demonstrated its potent and selective cytotoxicity against prostate cancer cells (Ya-ming *et al*., 2016). In contrast, to the methanolic extract, the SGAF of *S. schimperianum* leaf exhibited anti-proliferative activity with IC_{50} values (9.13 - 23.21 µg/mL) higher than those obtained for the methanolic extract (2.69 - 19.83 µg/mL). *S. incanum* and *S. incanum* leaf SGAFs were less active showing anti-proliferative activity against the four cell lines with IC_{50} values >50 µg/mL. Ding *et al*. (2013) found that SGAs exhibited antitumor activity and induced apoptosis on human gastric cancer MGC-803 cells. They further stated that the number and type of sugar and the substitution of a hydroxyl on steroidal alkaloid backbone play an important role in the anti-proliferative activity.

Table 4: Anti-proliferative activity of leaf extracts of five Solanaceae plants.

Plant species	HT29	HCT116	MCF7	MDA-MB231
	Methanol extract			
S.incanum	>50	>50	>50	>50
S. schimperianum	2.69 ± 0.56	5.70 ± 0.51	19.83± 3.83	7.01 ± 0.25
S.nigrum	>50	>50	>50	>50
P. lagascae	>50	>50	>50	>50
W. somnifera	>50	>50	>50	>50
	Steroidal glycoalkaloids fraction (SGAF)			
S.incanum	>50	>50	>50	>50
S.schimperianum	9.13 ± 0.67	13.39 ± 0.61	23.21 ± 3.08	17.70 ± 2.00
S.nigrum	>50	>50	>50	>50
P. lagascae	7.45 ± 0.59	4.79 ± 0.77	5.09 ± 0.24	6.90 ± 0.30
W. somnifera	4.23 ± 0.25	1.29 ± 0.06	2.34 ± 0.68	5.00 ± 0.75

3.1.2.3 Antioxidant activity

3.1.2.3.1 DPPH free radical scavenging activity

Percentage inhibition of DPPH free radical scavenging activity of methanol extracts of studied species are presented in Figure 8. DPPH radical scavenging activity of the tested extracts was concentration dependent where the maximum antioxidant effect was observed in highest concentration (1000 mg/L) of all tested samples. All extract showed strong scavenging activity and extracts of *P. lagascae* (210.39 %) and *S. schimperianum* (91%) revealed percentage inhibition higher than that obtained by ascorbic acid. At concentration 100 mg/L, all extracts showed also strong scavenging activity comparable to that of ascorbic acid. However, all extracts gave very weak scavenging activity at low concentrations (2 and 0.02 mg/L). The IC_{50} value was calculated and lower value of IC_{50} indicates a higher antioxidant activity. The ranking order of the investigated plants methanol extracts on the basis of their DPPH scavenging capacity was *S. schimperianum* (IC_{50} 156.1 µg/mL) > *W. somnifera* (IC_{50} 168.9 µg/mL) > *S. incanum* (IC_{50} 177.1 µg/mL) > *S. nigrum* (IC_{50} 179.1 µg/mL) > *P. lagascae* (IC_{50} 199 µg/mL).

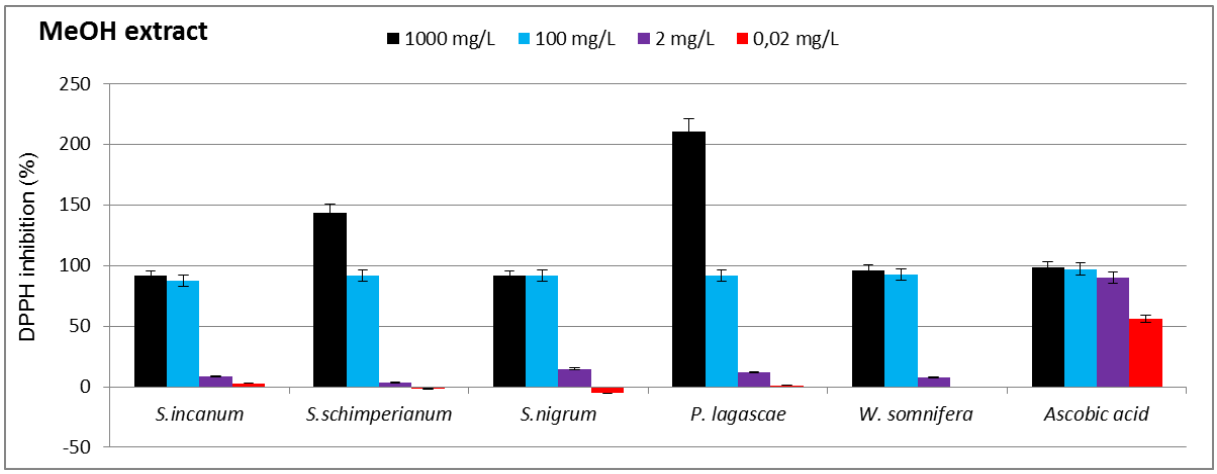


Figure 8: DPPH free radical scavenging activity of leaf methanolic extracts of selected Solanaceae species.

Percentage inhibition of DPPH free radical scavenging activity of SGAFs of studied species are presented in Figure 9. At concentration 1000 mg/L, *S. schimperianum* (148.88%) and *W. somnifera* (126.23%) displayed high scavenging activity higher than that obtained by ascorbic acid (93.44%). For other species only the SGAF of *S. nigrum* (86.88%) showed high

scavenging activity. Interestingly, at concentration 100 mg/L, all species including *S. incanum* and *P. lagascae* showed high scavenging activity and at concentration 2 mg/L, only the SGAF of *S. nigrum* (90.16%) displayed high scavenging activity comparable to that of ascorbic acid while all other SGAFs gave very weak scavenging activity at this lowest concentration. Antioxidant capacity of the SGAFs were also expressed in terms of IC_{50} value and the ranking order of their DPPH scavenging capacity was *S. schimperianum* (IC_{50} 3.5 µg/mL) > *P. lagascae* (IC_{50} 81.9 µg/mL) > *W. somnifera* (IC_{50} 87.1 µg/mL) > *S. nigrum* (IC_{50} 111.9 µg/mL) > *S. incanum* (IC_{50} 160.4 µg/mL) (Table 5). Generally, it was clear that the SGAFs of all species demonstrated higher scavenging activity than their corresponding methanol extracts.

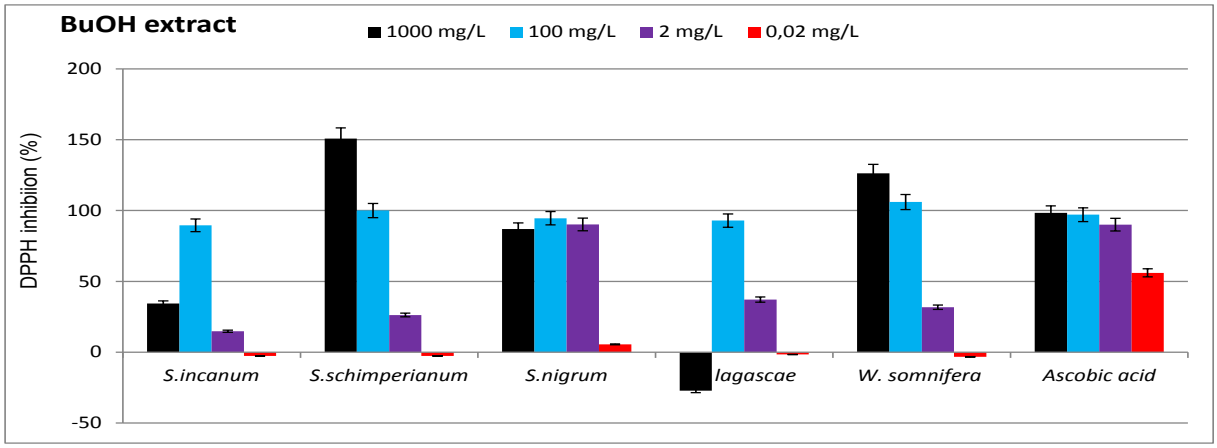


Figure 9: DPPH free radical scavenging activity of leaf BuOH extract of selected Solanaceae species.

Table 5: IC_{50} values of DPPH free radical scavenging activity of leaf extracts of selected Solanaceae species.

Plant species	IC_{50} (µg/mL)	
	MeOH extract	SGAFs
S. incanum	177.9 ± 5	160.4 ± 7
S. schimperianum	156.1 ± 3	3.5 ± 0.2
S. nigrum	179.1 ±1	111.9 ± 3
P. lagascae	199.0 ± 1	81.9 ± 3
W. somnifera	168.9 ± 7	87.1 ± 0.5
Ascorbic acid	1.5 ± 0.1	

3.1.2.3.2 ABTS free radical scavenging activity

Results of ABTS free radical-scavenging properties of the methanol extracts and SGAFs of studied species are presented in Figure 10. Generally, the highest percentage inhibition of methanol extracts of studied species (except *S. schimperianum*) was observed at concentration 1000 mg/L. *S. nigrum* (91.42%) displayed highest activity followed by *W. somnifera* (90.54%) and *P. lagascae* (86.95%) respectively while, *S. schimperianum* (23.53) showed very weak scavenging activity. Interestingly, at concentration 100 mg/L, *S. incanum* (90.54%) gave high scavenging activity similar to that displayed by *W. somnifera* at concentration 1000 mg/L whereas, at concentrations 2 mg/L it exhibited moderate (58.28%) activity. All other species showed weak scavenging activity at concentrations 100, 2 and 0.02 mg/L. The ranking order of the investigated plants methanol extracts on the basis of their ATBS scavenging capacity (in terms of IC_{50} value) was *S. nigrum* (IC_{50} 250 µg/mL) > *S. schimperianum* (IC_{50} 521.3 µg/mL) > *S. incanum* (IC_{50} 616.4 µg/mL) > *P. lagascae* (IC_{50} 782.3 µg/mL) > *W. somnifera* (IC_{50} 1674.1 µg/mL).

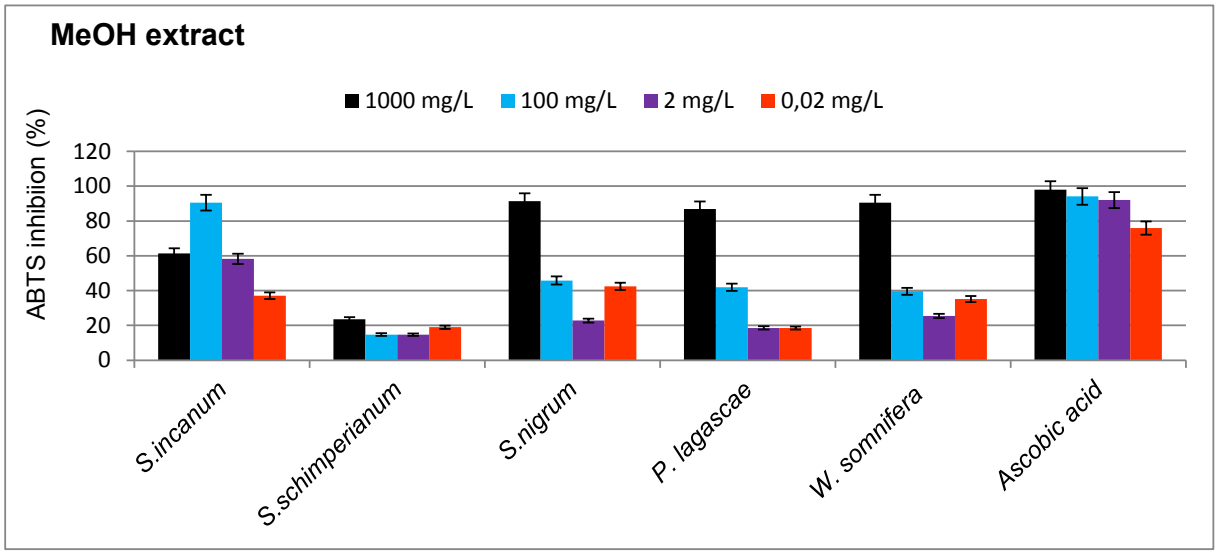


Figure 10: ABTS free radical scavenging activity of leaf methanolic extracts of selected Solanaceae species.

Percentage inhibition of ABTS free radical scavenging activity of SGAFs of studied species are presented in Figure 11. Generally, high ABTS radical scavenging activity of the tested extracts was obtained at concentrations 1000 and 100 mg/L. At concentration 1000 mg/L, *W. somnifera* (91.5%) displayed the highest activity followed by *S. nigrum* (91.06%), *S.*

schimperianum (86.73%), *P. lagascae* (81.82%) and *S. incanum* (61.29%) respectively. Again, at concentration 100 mg/L, *W. somnifera* (91.2%) displayed the highest activity. Although, *S. incanum* exhibited moderate (61.29%) activity at concentration 1000 mg/L, it gave high activity (90.54%) at concentration 100 mg/L more or less similar to that displayed by *S. nigrum* (90.62%). *P. lagascae* (85.19%) and *S. nigrum* (71.11%) showed also good activity. Interestingly, at concentrations 2 and 0.02 mg/L, *S. nigrum* displayed highest activity with percentage inhibition of 90.69 and 78.59 respectively while, other species showed either moderate or weak scavenging activity. The ranking order of the investigated plants SGAFs on the basis of their ATBS scavenging capacity (in terms of IC_{50} value) was *S. schimperianum* (IC_{50} 3.5 µg/mL) > *S. incanum* (IC_{50} 45.7 µg/mL) > *W. somnifera* (IC_{50} 49.3 µg/mL) > *P. lagascae* (IC_{50} 95 µg/mL) > *S. nigrum* (IC_{50} 144.9 µg/m L) (Table 6).

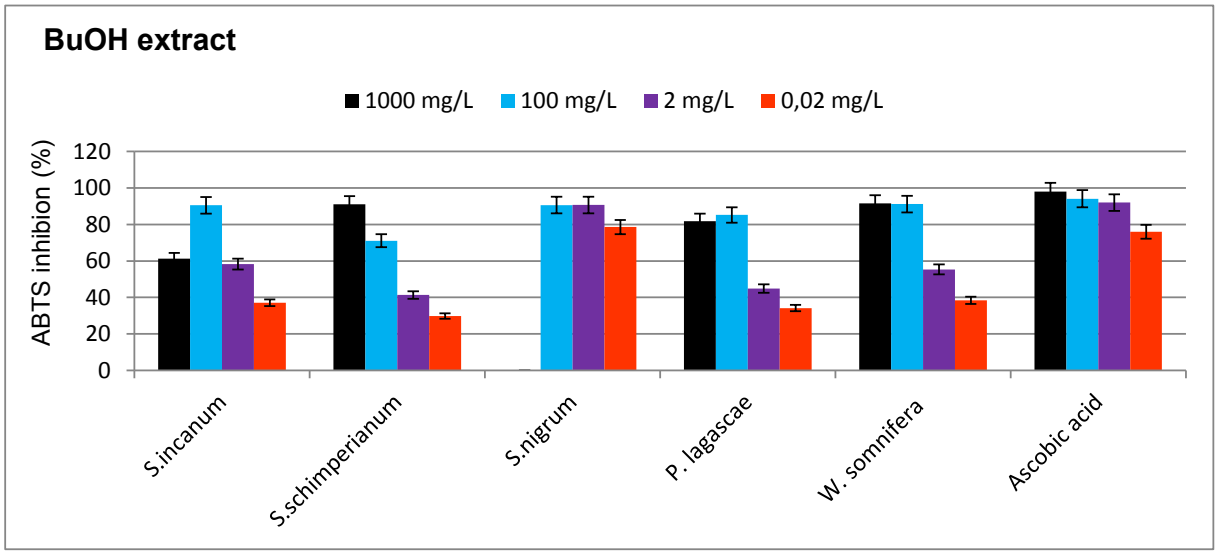


Figure 11: ATBS free radical scavenging activity of leaf SGAFs of selected Solanaceae species.

Table 6: IC_{50} values of ATBS free radical scavenging activity of leaf extracts of selected Solanaceae species.

Plant species	IC_{50} (µg/mL)	
	MeOH extract	SGAFs
S. incanum	616.4 ± 4	45.7± 2
S. schimperianum	521.3 ± 3	3.5 ±0.3
S. nigrum	250 ± 3	144.9 ± 2
P. lagascae	782.3 ± 5	95 ± 3
W. somnifera	1674.1 ± 8	49.3 ± 1
Ascorbic acid	1.2 ± 0.2	

As in DPPH assay, it was clear that the SGAFs of all species demonstrated higher scavenging activity than their respective methanol extracts. The SGAFs increased the DPPH and ABTS scavenging capacity respectively by 1.1- and 13.5-fold for *S. incanum,* 1.6- and 1.7-fold for *S. nigrum,* 2.4- and 8.2-fold for *P. lagascae* and 1.9- and 34-fold for *W. somnifera*. Furthermore, *S. schimperianum* displayed the strongest antioxidant activity where a sharp increase by 45- and 140-fold was observed for SGAFs.

Moreover, the scavenging of the ABTS radical was found to be generally different than that of DPPH radical and IC_{50} values obtained from the methanol extracts in both assays were higher than those obtained in SGAFs. Several studies have reported variations in the biological activities of extracts prepared using different extraction techniques (Dhanani *et al.,* 2013). Zheng and Wang (2001) and Jimoh *et al.* (2010) reported that factors like stereoselectivity of the radicals or the solubility of the extract in different testing systems affect the capacity of extracts to react and quench different radicals. Wang *et al*. (1998) found that some compounds which have ABTS scavenging activity did not show DPPH scavenging activity.

3.2 Part two

3.2.1 Phytochemistry

3.2.1.1 Qualitative preliminary phytochemical analysis

Qualitative preliminary phytochemical analysis was performed initially with different chemical reagents to detect the nature of phytoconstituents and their presence in the investigated plant species. Results revealed the presence of secondary metabolites like alkaloids, flavonoids, tannins, sterols/triterpenoids, and saponins and they all devoid of anthraquinones and cardiac glycosides (Table 7).

Table 7: Qualitative preliminary phytochemical analysis of the five Solanaceae plants.

	S. incanum	*S. schimperianum*	*S. nigrum*	*P. lagascae*	*W. somnifera*
Alkaloid	+++	+++	+++	+++	+++
Anthraquinones	ND	ND	ND	ND	ND
Flavonoid	++	++	++	++	++
Sterols/triterpenoids	+++	+++	+++	+++	+++
Saponins	+	+	+	+	+
Tannins	+	+	+	+	+
Cardiac glycosides	ND	ND	ND	ND	ND

+++, high content; ++, moderate; + low content; ND, not detected.

3.2.1.2 Total phenolic content of leaf methanol extracts of the selected Solanaceae species

Phenolic compounds have widely been reported to be largely responsible for the antioxidant activity of plant extracts (Guo *et al.,* 1997; Awika *et al*., 2003; Kuti and Konuru, 2004). For this reason, the total polyphenolic content of leaf methanolic extracts of the selected Solanaceae species was determined. Total polyphenolic content was expressed as mg gallic acid equivalent (GAE) per 100 g sample on dry basis and results are listed in Table 8. The amount of total polyphenolics, varied in the studied species and ranged from 0.199 to 0.477 mg GAE/100 g). *P. lagascae* had the highest phenolic content followed by *S. schimperianum* and *W. somnifera* (0.396 mg GAE/100 g) while *S. nigrum* (0.257 mg GAE/100 g) and *S. incanum* (0.190 mg GAE/100 g) had the lowest content.

Table 8: Total polyphenolic content of leaf methanol extracts of the selected Solanaceae species.

Plant species	Concentration (mg GAE/100 g)
S. incanum	0.199 ± 0.036
S. schimperianum	0.396 ± 0.04
S. nigrum	0.257 ± 0.024
P. lagascae	0.477 ± 0.038
W. somnifera	0.396 ± 0.029

Previous studies demonstrated that the antioxidant activity of leaf of *S. incanum* (Konaté *et al*., 2011), *S. nigrum* leaf (Jimoh *et al*., 2010; Loganayaki *et al*., 2010), *W. somnifera* leaf (Fernando *et al*., 2013), was mainly attributed to the phenolic content of extracts. In this study, the correlation coefficient (R^2) between the antioxidant capacities and the total polyphenols of the methanol extracts was also determined using Pearson's correlation coefficients (Fig. 12 and 13). The R^2 between the antioxidant capacities obtained from DPPH and ABTS assays were 0.3727 and 0.1757 respectively. Thus, it was clear that the antioxidant capacity of these plants did not correlate with their polyphenolic content, which indicated that the phenolic compounds could not be the main contributor to the antioxidant capacities of leaf of these plants. This was in agreement with previous studies on other plant species which showed low correlations between phenolic compounds and antioxidant activity, an indication that phenolic compounds may not be the only compounds responsible for antioxidant activity (Fombang *et al.,* 2005).

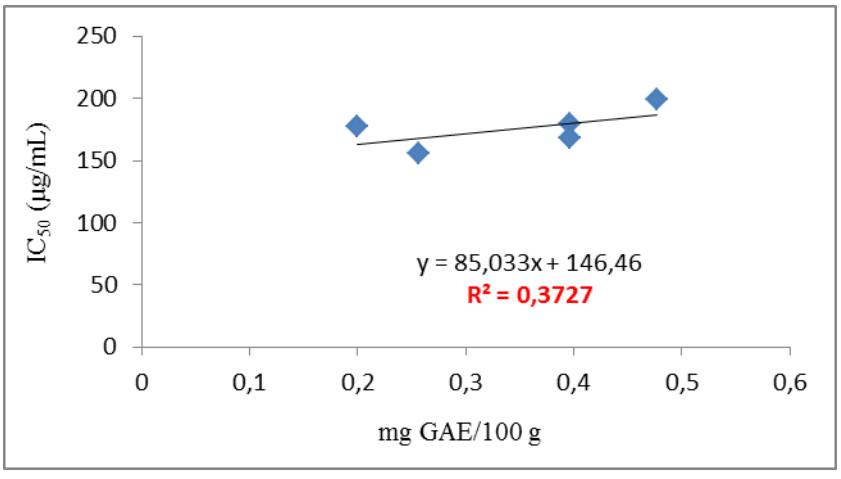


Figure 12: Correlation coefficient (R^2) between the antioxidant capacities (DPPH) and the total polyphenols of the methanol extracts of the selected Solanaceae species.

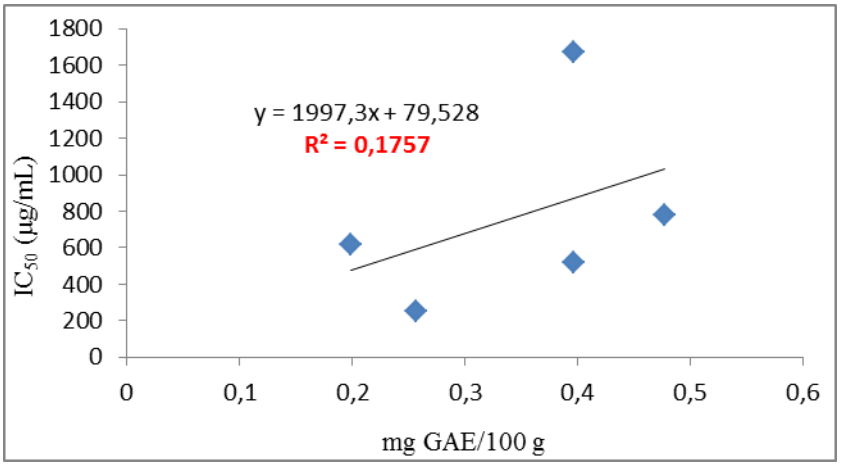


Figure 13: Correlation coefficient (R^2) between the antioxidant capacities (ATBS) and the total polyphenols of the methanol extracts of the selected Solanaceae species.

3.2.1.3 GC-MS analysis of the methanol extracts and SGAFs of the selected Solanaceae species leaf

In the present study, a GC-MS procedure was applied for the identification of chemical profile of the methanol and SGAFs extracts of *S. incanum, S. schimperianum, S. nigrum, P. lagascae* and *Withania somnifera* leaf. The relative retention times (Rt) and mass spectra of the extract components were compared with the standard mass spectra in the library and with data from the literature.

Many compounds belonging to different classes of secondary were identified from the methanol extracts and SGAFs of species under study (Fig. 14- 19 and Tables 9 and 10):

Steroidal saponins

The steroidal saponins, which exhibiting a characteristic base peak fragment ion of m/z 139, were identified in the two types of extracts as:

Diosgenin (25(R)-spirost-5-en-3β-ol) has M^+ at *m/z* 414 corresponds to molecular formula $C_{27}H_{42}0_3$, exhibited molecular ion peak at *m/z* 139 and some major fragment ions at *m/z* 271, 282, 300, 342 and 414 which are characteristic for spirostanol sapogenin (Taylor *et al.,* 1997).

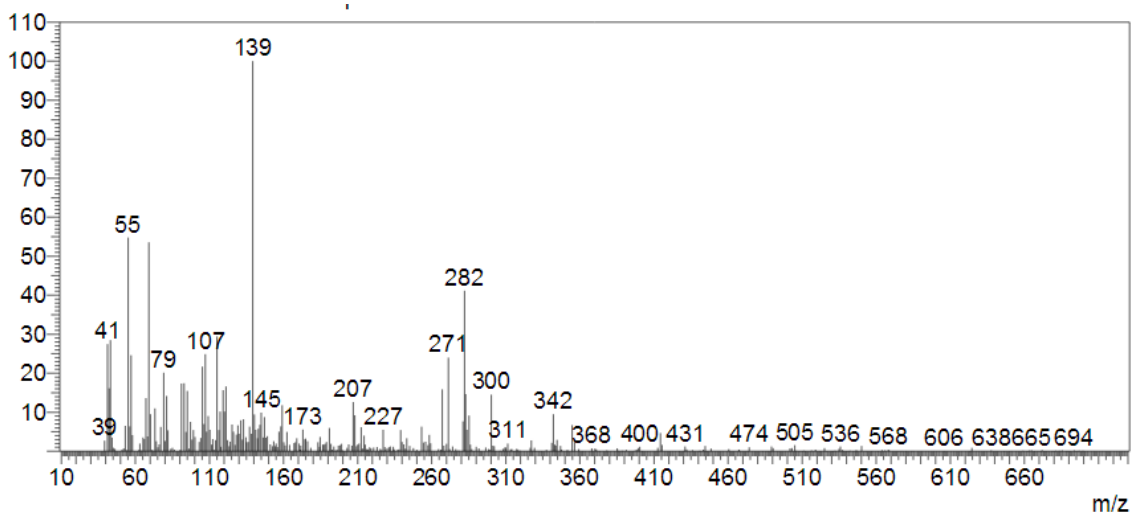


Figure 14 : Mass spectrum of Diosgenin.

Neotigogenin ((25S)-5R-spirostan-3-ol) has M^+ at *m/z* 416 corresponds to molecular formula $C_{27}H_{44}O_3$, showed characteristic fragment ions at *m/z* 416, 287, 273 and 139 similar to the fragment ions obtained by Santos and Branco (2014).

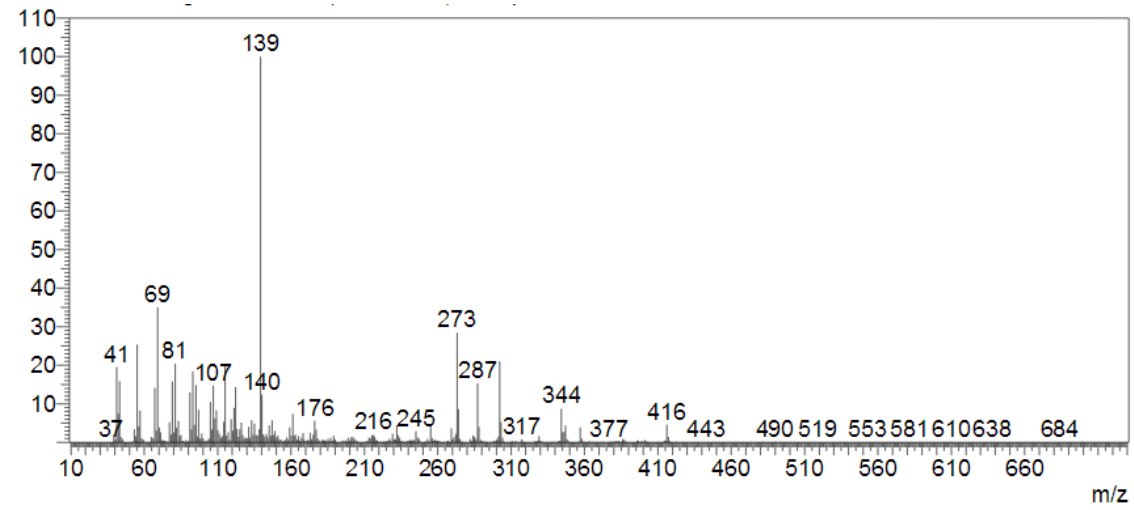


Figure 15: Mass spectrum of Neotigogenin.

Sarsasapogenin ((3,5,25S)-Spirostan-3-ol) has M^+ at *m/z* 416 corresponds to molecular formula $C_{27}H_{44}O_3$, exhibited molecular ion peaks at *m/z* 139, 273, 287, 344 characteristics for spirostan structure, with its ketone spiro acetal and differ from diosgenin as it contains a double bond in the steroid nucleus.

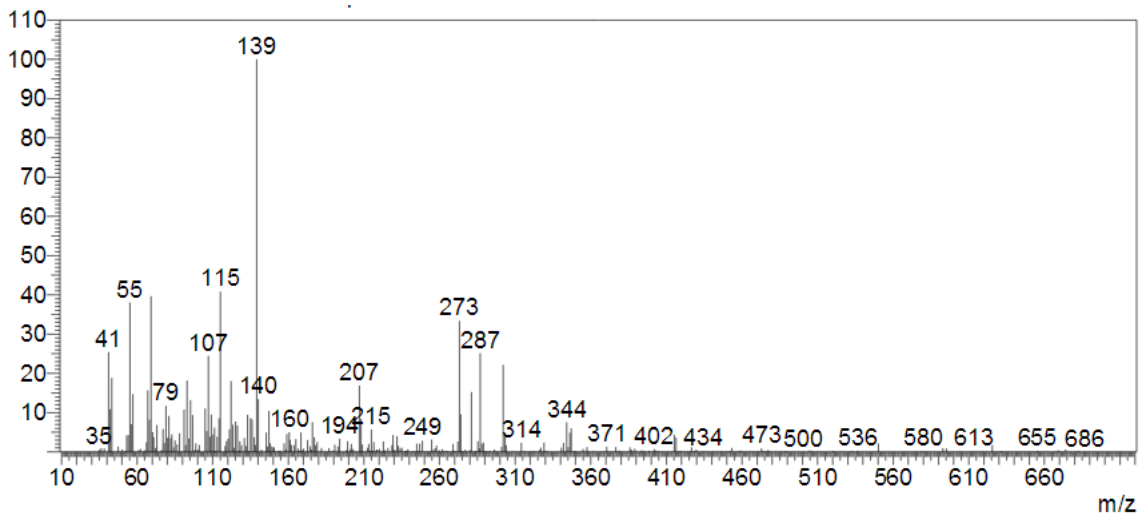


Figure 16: Mass spectrum of Sarsasapogenin.

Steroidal alkaloids

The steroidal alkaloids identified in the two types of extracts were:

Solasodine has M^+ at *m/z* 413 corresponds to molecular formula $C_{27}H_{43}NO_2$, showed characteristic fragment ions at *m/z* 413, base peak at 114 *m/z* and the intense fragment peak at 138 m/z similar to that obtained by Chauhan *et al.* (2011).

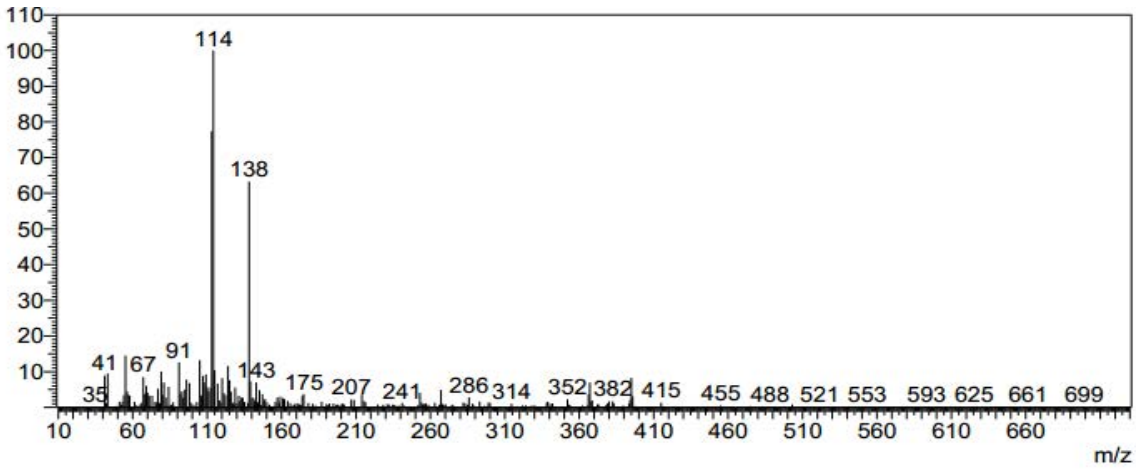


Figure 17: Mass spectrum of Solasodine

Tomatidine exhibited M^+ at *m/z* 415 corresponds to molecular formula $C_{27}H_{45}NO_2$ and intense fragments at *m/z* 138 and 114 which are characteristic for the spirosolane-type alkaloids (Kuronen *et al*., 1999).

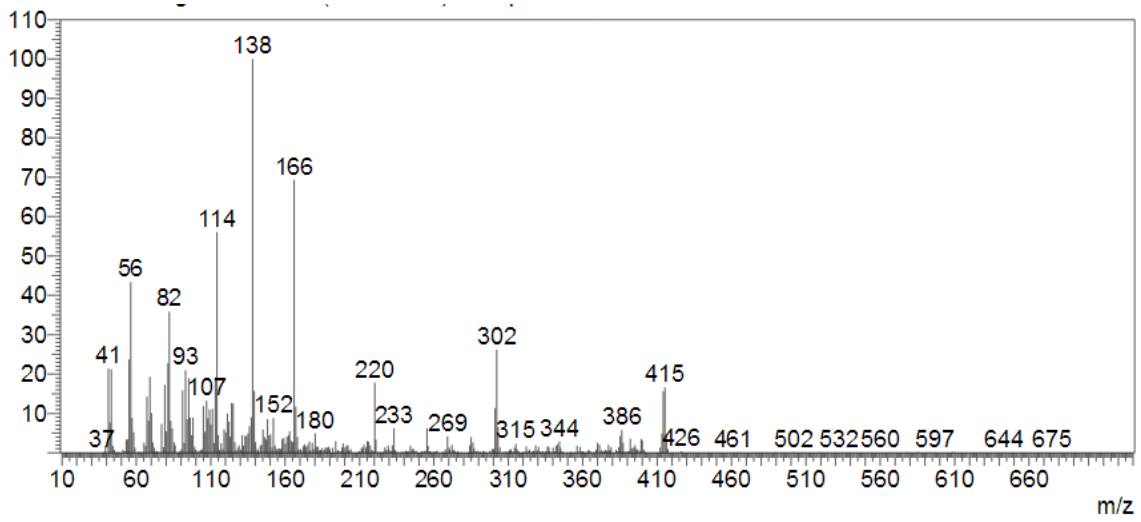


Figure 18: Mass spectrum of Tomatidine.

Solanocapsine gave M^+ at *m/z* 430 corresponds to molecular formula $C_{27}H_{46}NO_2$.The other minor fragment ions at *m/z* 111, 412 are identical with the published mass spectrum by Aij F. *et al.* (1976). The most striking feature in these spectra are the base peaks at *m/z* 111, because there were no corresponding peaks in the spectra of the unmethylated compounds.

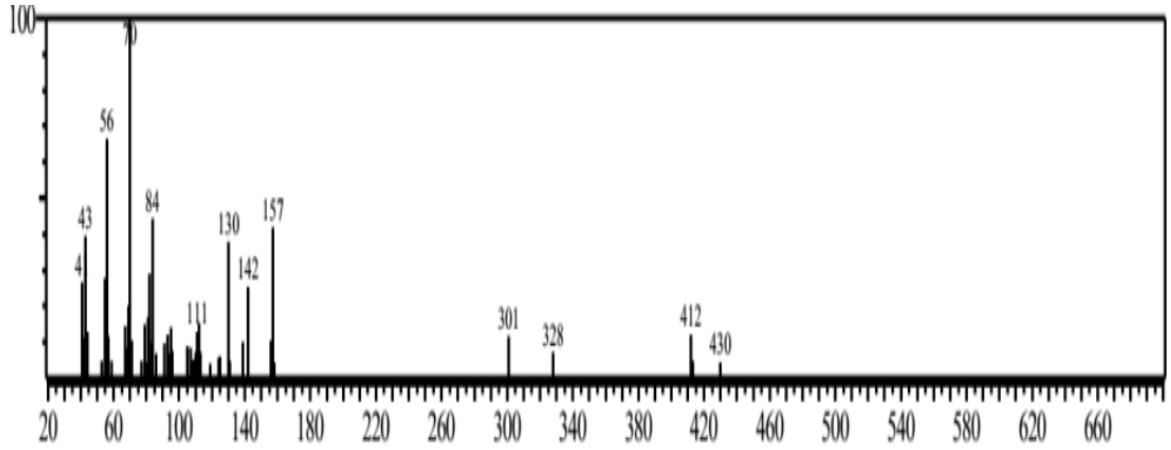


Figure 19: Mass spectrum of Solanocapsine.

Other compounds

Compounds belong to other class of natural products like sterols, phenols, terpenes, alkanes, organosilicon, carbohydrates and vitamins were identified by comparison of their mass fragmentation patterns with mass spectra of available database from NIST and WILEY8 library (Table 9-10).

Most of these detected compounds were known for their significant biological activity; for example; literature survey reveals that solasodine has wide range of pharmacological activity such as diuretic, anticancer, antifungal, hepatoprotective, cardiotonic, antispermatogenetic, antiandrogenic, immunomodulatory, antishock, antipyretic and central nervous system related activity (Desai *et al.,* 2011; Patel *et al.,* 2013). Solanocapsine which was also found previously in many *Solanum* species like *S. pseudocapsicum, S. argentinum* and *S. tucumanense* was found to cause the acetyl cholinesterase inhibition. Steroidal sapogenin is considered as a basic compound in the hemisyntheis of steroid drugs such as cortisone and sexual hormones (Brenac *et al.*, 1996). Steroidal saponins were known for their hypolipidaemic activity and known to reduce the glucose level in diabetic animals (Gupta *et al.,* 2009). Diosgenin, which was also found in *S. incanum* and *S. xanthocarpum,* has been used for the treatment of various types of disorder such as leukemia, inflammation, hypercholesterolemia and cancer. It has been shown to have anticancer activity in mice, breast cancer, and colon cancer (Bertrand *et al*, 2004; Raju *et al*., 2006). It induced apoptosis in human rheumatoid arthritis, human osteosarcoma 1547 cell line, HT-29 human colon cancer cells (Raju *et al*, 2004). It is also able to prevent bone loss to the same extent as that of oestrogen. It is a typical initial intermediate for synthesis of steroidal compounds, oral contraceptives and sex hormones (Patel *et al.,* 2012). The cytotoxicity against Vero cells and antiviral effect against human herpes virus type 1 (HHV-1), murine encephalomyocarditis virus (EMCv), and vaccinia virus strain Western Reserve (VACV-WR) of neotigogenin and tigogenin (isolated previously from *S. paniculatum*) were also reported (Valadarose *et al*., 2009). Besides, neotigogenin has also antifungal activity (Yang *et al*., 2006)

Steroids are biologically active molecules. The identification of a number of steroidal compounds in *Solanum* species might have some ecological significance (Nazifi *et al*., 2008). Steroidal glycosides are included in a protective molecules found in plants named 'phytoanticipins' or 'phytoprotectants' as they are considered to be a part of plants' defence systems (Morrissey and Osbourn, 1999; Gus-Mayer *et al*., 1994). Moreover, Stigmasterol and

its derivatives have been shown to possess antidiabetic, antifeedant, antitumor, antimutagenic and antioxidant activity (Kaur *et al.,* 2011).

As previously noted that the phenols were not the main contributors to the antioxidant activity of the Solanaceae plants under the study. However, the GC/MS results identified the presence of other compounds which were known to possess significant antioxidant activity. Gupta *et al.* (2009) demonstrated that spirosta-steroidal saponins (diosgenin, tigogenin and sarsapogenin) possessed *in vivo* beneficial antioxidant effect and consequently hepatoprotective effects better than those of the total crude extracts of both *Solanum xanthocarpum* and *S. nigrum*. Moreover, Gong *et al* (2010) suggested that diosgenin is a very useful compound to control hyperlipidemia by both improving the lipid profile and modulating oxidative stress and prevent H(2)O(2)-induced apoptosis of HUVECs, in partly through regulating mitochondrial dysfunction pathway. Tomatidine and solasodine which were isolated from *S. aculeastrum* berries were found to reveal strong synergistic antioxidant activity (Koduru, 2007). Stigmasterol and its derivatives have been shown to possess antioxidant activity (Kaur *et al.,* 2011).

Table 9: GC-MS profile of methanol leaf extracts of the selected Solanaceae species.

Class of metabolites /Name of compound	RT	Formula	MW	*S.incanum*	*S.schimperianum*	*S.nigrum*	*P. lagascae*	*W. somnifera*
Steroidal saponin								
Diosgenin	28.83	$C_{27}H_{42}0_3$	414	P	P	P		
Neotigogenin	28.94	$C_{27}H_{44}O_3$	416		P			
Sarsasapogenin	28.96	$C_{27}H_{44}O_3$	416		P	P		
Pseudodiosgenin	30.41	$C_{27}H_{40}O_3$	414	P	P			
Steroid								
Beta –Sitosterol	29.02	$C_{29}H_{50}O$	414	P	P	P		
Stigmasterol	28.63	$C_{29}H_{48}O$	412	P		P		
Lupeol	29.63	$C_{30}H_{50}O$	426	P				
Stigmasta-5,22-dien-3-ol, acetate	27.59	$C_{31}H_{50}O_2$	454		P			
5-Cholestene-3-ol, methyl	28.47	$C_{30}H_{50}O$	426	P	P			
Campesterol	28.48	$C_{28}H_{48}O$	400	P	P			
Diterpene								
Phytol	19.98	$C_{20}H_{40}O$	296		P			
Flavonoid								
4H-1Benzopyran-4-one, 5-hyd	27.30	$C_{31}H_{20}O_{10}$			P			
Essential oil								
2-Cyclohexene-1	15.30	$C_{13}H_{20}O_2$	208	P	P		P	
Vitamin E	29.78	$C_{29}H_{50}O_2$	430					
α Tocopherol	27.82	$C_{29}H_{50}O_2$	430	P	P	P		
Monosacharid								
β-D- Glucopyranose	13.37	$C_6H_{10}O_5$	162	P	P		P	P
Alkane								
Octadecane	28.88	$C_{18}H_{37}$	288	P		P		

P, present.

Table 10: GC-MS profile of SGAFs of the selected Solanaceae species leaf.

Class of metablites /Name of compound	RT	Formula	MW	*S.incanum*	*S.schimperianum*	*S.nigrum*	*P. lagascae*	*W. somnifera*
Steroidal saponin								
Diosgenin	28.84	$C_{27}H_{42}0_3$	414	P				
Sarsasapogenin	28.95	$C_{27}H_{44}O_3$	416			P		
Neotigogenin	29.00	$C_{27}H_{44}O_3$	416			P		
Steroidal alkaloid								
Solasodine	28.35	$C_{27}H_{41}NO$	413	P				
Tomatidine	29.76	$C_{27}H_{45}NO_2$	415		P			
Solanocapsin	30.53	$C_{27}H_{46}NO_2$	430		P			
25-Dedhydrotigogenin	28.13	$C_{27}H_{42}O_3$	414			P		
Steroid								
Cholesterol	20.97	$C_{27}H_{46}O$	386			P		
Campesterol	28.48	$C_{28}H_{48}O$	400			P		
Stigmasterol	28.64	$C_{29}H_{48}O$	412			P		
Beta-Sitosterol	29.03	$C_{29}H_{50}O$	414			P		
Stigmasta-5,22-dien-3-ol acetate	27.45	$C_{31}H_{50}O_2$	454			P		
Benzene ring								
2-(4-Hydroxy-3-methoxphenyl	26.71	$C_{18}H_{16}O_6$	328		P			
1H-Indole-3-carboxaldehyde	16.96	C_9H_7NO	145	P		P		
17a-Methyl-3-beta-meth	23.29	$C_{21}H_{33}NO_2$	133	P				
Indolin-2-one, 1-methyl-3	14.79	$C_{13}H_{17}NO$	203			P		
Cholestan-16-one						P		
Alkane								
Heptadecane, 2,6,10,14-tetramethyl	8.61	$C_{21}H_{44}$	296				P	

P, present.

3.2.2 Distribution of detected compounds in the studied species and their chemotaxonomical interpretation

The three *Solanum* species contained the steroidal saponin Diosgenin. Sarsasapogenin was detected in the methanol extract of *S. schimperianum* and *S. nigrum* leaf while Pseudodiosgenin was found in *S. incanum* and *S. schimperianum.* Neotigogenin was only detected in *S. schimperianum.* Steroids were also identified where the three *Solnum* species contained Beta –Sitosterol and 5-Cholestene-3-o1, methyl and Cholest-5-en-3-ol, 24 methyl were detected in *S. incanum* and *S. schimperianum* and *Stigmasterol* was found in *S. incanum* and *S. nigrum.* Lupeol and Stigmasta-5, 22-dien-3-ol, aceta was found in *S. incanum* and *S. schimperianum* respectively. Phenol, 3,5-bis (1,1-dimethylethyl and 4-((1E)-3-Hydroxy-1-pr were found in *S. incanum* leaf. Other compounds belong to diterpene (Phytol), flavonoid (4H-1Benzopyran-4-one, 5-hyd) were identified in *S. schimperianum*, (2-Cyclohexene-1) in *S. incanum, S. schimperianum* and *P. lagascae*, vitamin E in the three *Solnum* species, monosaccharide (β-D- Glucopyranose) in all species except *S. nigrum* and alkane (Octadecane) in *S. incanum* and *S. nigrum*.

SGAFs of *S. incanum* leaf revealed the presence of steroidal saponin Diosgenin while that of *S. nigrum* leaf showed the presence of Sarsapogenin and Neotigogenin. Steroidal alkaloids; Solasodine was the only steroidal alkaloid detected in SGAFs of *S. incanum* leaf. Tomatidine and Solanocapsin, were only detected in SGAFs of *S. schimperianum* leaf while SGAFs of *S. nigrum* leaf showed the presence of 25-Dedhydrotigogenin. Steroids namely; Cholesterol, Campesterol, Stigmasterol, β-Sitosterol and Stigmasta-5, 22-dien-3-ol acetate were only detected on SGAFs of *S. nigrum* leaf.

However, the chemical profile by GC-MS technique of *P. lagascae* and *W. somnifera* leaf did not demonstrate the presence of all the above-mentioned compounds.

Although GC/MS technique is not considered as best method to determine the chemical profile of methanol extracts and SGAFs, the data obtained in this study suggested that steroidal alkaloid, steroidal sapogenins and steroids might also contribute to the chemical taxonomy of the genus *Solanum* and could be considered as chemotaxonomic markers.

The combined dataset obtained by GC-MS analysis of methanolic extracts and SGAFs were used to determine the similarity between the different plant species. Based on Jaccard similarity indices, the five plants are grouped into two main clusters (Fig. 20). Cluster I contains *P. lagascae* and *W. somnifera* with the highest similarity percentage of 64. The three *Solanum* species, *S. incanum*, *S. nigrum* and *S. schimperianum*, were grouped together in Cluster II. The species in this cluster have similarity percentages of 16 (*S. nigrum* vs. *S. schimperianum*), 25 (*S. incanum* vs. *S. nigrum*) and 31 (*S. incanum* vs. *S. schimperianum*). Similarity between members of the two clusters was in the range of zero (no similarity) to 8% indicating good separation between the different plants species.

Principle component analysis (PCA) indicated that the first and second components accounts for 44.75 and 31.30% of the variation between the plant species. As shown in Fig. 21, two groups which are similar to those in the dendrogram, are formed. Group 1 indicates the close similarity of *S. incanum* to *S. schimperianum* compared to its relation to *S. nigrum*. On the other hand, *P. lagascae* and *W. somnifera* are shown to be very close to each other in group 2. These data support the morphological classification of Solanaceae plants from Sudan proposed by Andrews (1956) in separating the *Solanum* species in one group and *P. lagascae* and *W. somnifera* in another group. However; although morphologically, *S. schimperianum* is closer to *S. nigrum* than to *S. incanum* (Table 11), chemical dataset in this study suggested a close relationship between *S. schimperianum* and *S. incanum* than to *S. nigrum*. Therefore, detailed infrageneric taxonomic information joined to chemical and molecular data are needed for the classification and evolution of the Solanaceae plants.

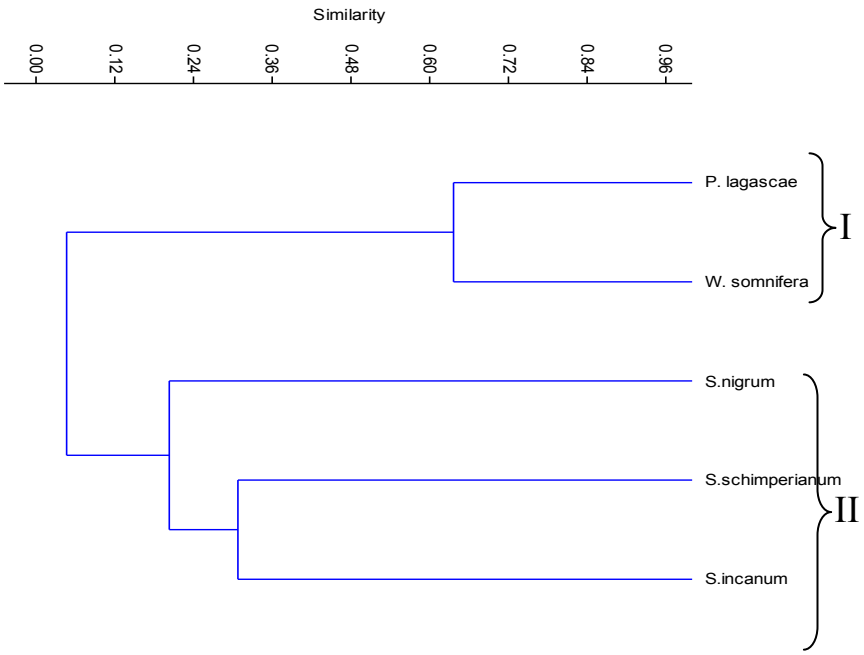


Figure 20: Dendrogram showing the relative similarity between the GC/MS profiles of leaf extracts of the selected Solanaceae species.

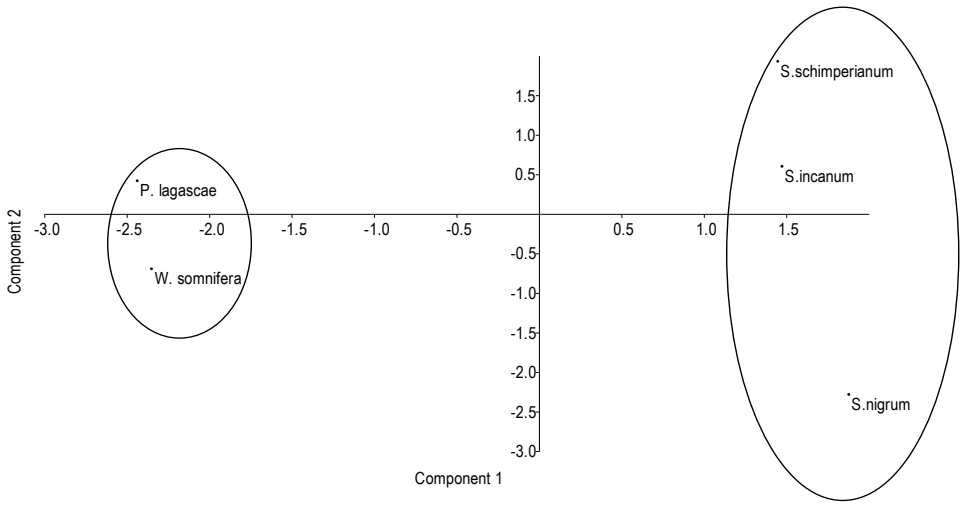


Figure 21: Two-dimensional view of Principle Components Analysis (PCA) based on the combined data of GC-MS analysis for the plant species under the study.

Table 11: Morphological characters of the investigated Solanaceae plants.

Morphological character	*S. incanum*	*S. schimperianum*	*S. nigrum*	*P. lagascae*	*W. somnifera*
Habit	under shrub	under shrub	under shrub	Herb	under shrub
Leaves	elliptic	ovate	ovate or lanceolte	ovate	ovate lanceolte
Leaves margine	dentate	entire	entire	entire or dentate	entire
Leaf apex	obtuse	acute	acute-acuminate	acuminate	acute to rounded
Presence of hair	hairy	hairy	hairy	not hairy	hairy
Stem	with spines	no spines	no spines	no spines	no spines
Flowers	solitary or in clusteres	racemes	in clusteres	solitary	axillary clusters
Flower colour	purple	purple	white	yellow	pale green
Berry	subglobose	globose	globose	globose	globose
Berry colour	yellow	red	black	yellow	red

3.3 Part three

3.3.1 Phytochemical studies on *Solanum schimperianum* leaf

According to the results obtained from biological activity, *S. schimperianum* leaf was subjected to detailed phytochemical study. Moreover, there are few studies in this plant.

3.3.1.1 LC–MS analyses

Methanolic extract of *S. schimperianum* was analyzed using liquid chromatography coupled to an Orbitrap mass spectrometer with an electrospray ionization source (LC-MS). Twelve known hydroxycinnamic acid amides (HCAAs) were detected and *N*-caffeoyl agmatine appeared with the highest intensity (Table 12, Fig. 22). Furthermore, the positive HR-ESI-MS Full-scan mass spectrum revealed the presence of several isobaric, high intensity quasi-molecular ions at *m/z* 207.17998, 208.18777, and 216.18523, appearing predominantly as doubly charged $[M+2H]^{2+}$ ions (Fig. 1). The mass errors between the theoretically calculated and measured masses ranged from 0.50 to 5.00 ppm. Based on high-resolution accurate mass measurements and analysis of their isotopic patterns the elemental composition of the three above mentioned ions were determined as $C_{27}H_{44}ON_2$, $C_{27}H_{46}ON_2$ and $C_{27}H_{46}O_2N_2$, respectively. In the MS^2 of all three $[M+H]^+$ parent ions at *m/z* 413.35229, 415.36771 and 431.36389, a subsequent loss of ammonia ($[M+H-NH_3]^+$) and water ($[M+H-(H_2O+NH_3)]^+$) was observed. The elemental composition, together with the MS^2 data permitted us to assign the above mentioned chemical compositions to 3-amino steroid alkaloids (Fig. 22).

There have been several reports in the literature describing isolated 3-amino steroid alkaloids from the genus *Solanum*, having an elemental composition of $C_{27}H_{46}ON_2$, as Solacallinidine (Bird *et al.*, 1979a) and Soladunalinidine (Bird *et al.*, 1979b) (Fig. 23). Moreover, another isobaric compound, the 3β-amino steroid alkaloid Solanopubamine, was recently isolated by Al-Rehaily *et al* (2013) in high quantity from the aerial parts of *S. schimperianum.* This data allowed us to tentatively assign one of the discovered ions having an elemental composition $C_{27}H_{46}ON_2$ as Solanopubamine (Fig. 23). The ions with elemental composition $C_{27}H_{44}ON_2$ can be putatively described as dehydro derivatives of the 3-amino steroid alkaloids mentioned above. The ions having elemental composition $C_{27}H_{46}O_2N_2$ can be assigned tentatively as Solanocapsine, found previously in *S. pseudocapsicum* (Aliero *et al.*, 2006).

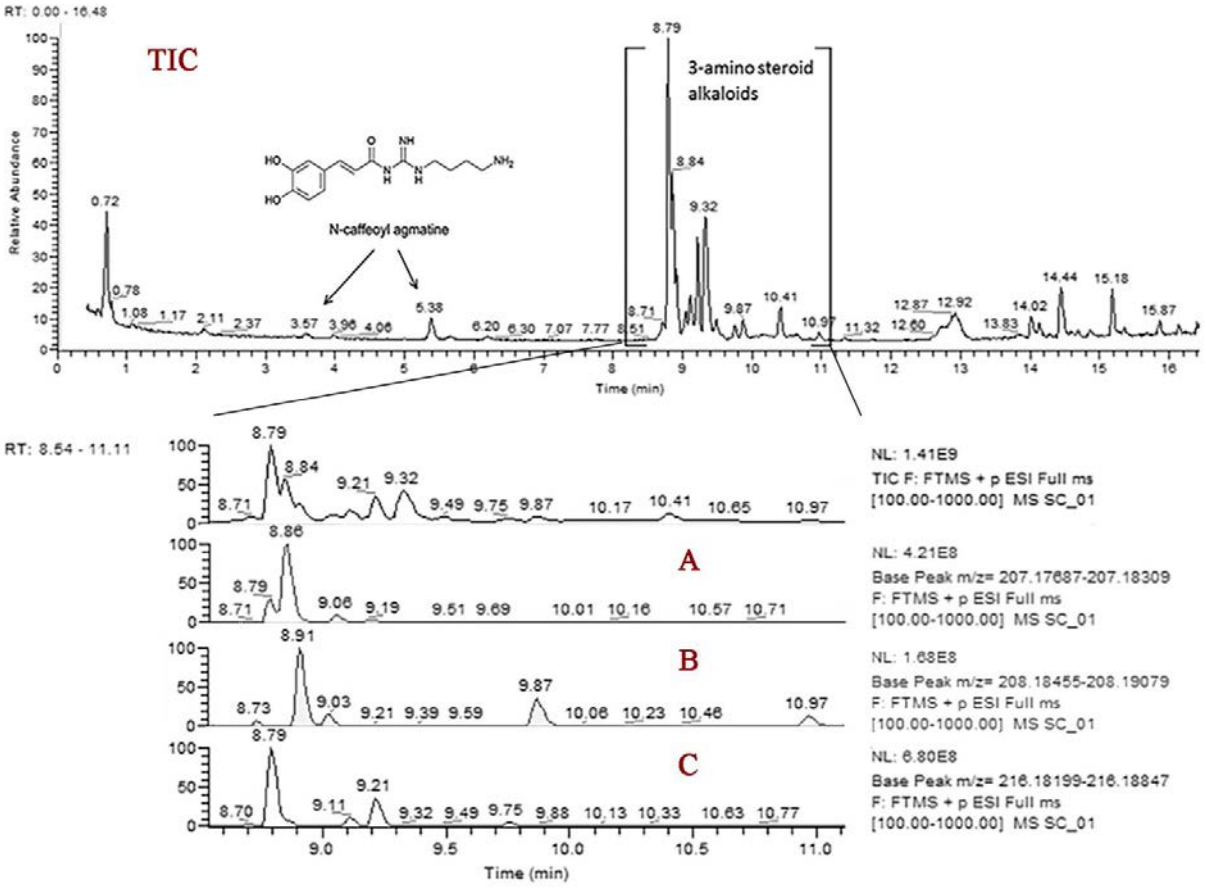
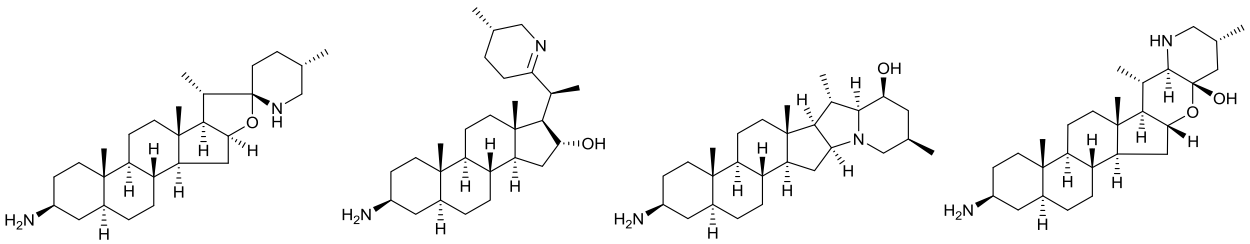


Figure 22: Total ion chromatogram (TIC) and extracted ion chromatograms for steroid alkaloids.

(A) Extracted ion chromatogram for base peak at *m/z* 207. (B) Extracted ion chromatogram for base peak at *m/z* 208. (C) Extracted ion chromatogram for base peak with *m/z* 216.

Table 12: Detected HCAAs in leaf extract of *Solanum schimperianum*.

№	Name	t_R (min)	Elemental composition	$[M+H]^+$	MS^2
1.	*N*-caffeoyl putrescine	2.13, 3.39	$C_{13}H_{18}N_2O_3$	251.13901	251.14, 234.11, 163.04, 115.09, 89.11, 72.08
2.	*N*-feruloyl putrescine	4.33, 5.74	$C_{14}H_{20}N_2O_3$	265.15466	260.14, 177.05, 145.03, 115.09, 89.11, 72.08
3.	*N*-coumaroyl agmatine	4.99, 6.72	$C_{14}H_{20}N_4O_2$	277.1659	260.14, 147.04, 131.13, 114.10, 89.11, 72.08
4.	*N*-coumaroyl tyramine	10.13, 10.53	$C_{17}H_{17}NO_3$	284.12811	164.07, 147.04, 121.065
5.	*N*-caffeoyl agmatine	3.57, 5.38	$C_{14}H_{20}N_4O_3$	293.16081	276.13, 163.04, 131.13, 114.10, 89.11, 72.08
6.	*N*-sinapoyl putrescine	5.09	$C_{15}H_{22}N_2O_4$	295.16523	278.14, 207.065, 175.04, 115.09, 89.11, 72.08
7.	*N*-caffeoyl tyramine	9.77	$C_{17}H_{17}NO_4$	300.12303	163.04, 147.04, 138.09, 121.065
8.	*N*-feruloyl agmatine	5.88, 7.76	$C_{15}H_{22}N_4O_3$	307.17647	290.15, 177.05, 157.11, 131.13, 114.10
9.	*N*-feruloyl tyramine	10.40, 10.82	$C_{18}H_{19}NO_4$	314.13868	194.08, 177.05, 164.07, 145.03, 121.065
10.	N^{ε}-feruloyl-lysine	5.04, 6.46	$C_{16}H_{22}N_2O_5$	323.16015	177.05, 147.11, 145.03, 84.08
11.	*N*-sinapoyl agmatine	6.18, 8.56	$C_{16}H_{24}N_4O_4$	337.18703	320.16, 207.065, 131.13, 114.10
12.	*N*-feruloyl-3-methoxytyramine	10.63, 11.09	$C_{19}H_{21}NO_5$	344.14924	194.08, 177.05, 151.07, 145.03



Soladunalinidine Solacallinidine Solanopubamine Solanocapsine

Figure 23: Some 3-amino steroid alkaloids found in Solanaceae plants.

(Fadl Almoulah *et al*.2017).

A variety of HCAAs have been found throughout the genus *Solanum* and were found to represent the main phenylpropanoid constituents in 12 *Solanum* species (Sun *et al*., 2015a & b). However, the HCAAs reported in this study were also detected previously from the roots where, N^{ε}-feruloyllysine, as well as HCAAs of agmatine, cadaverine and sinapoyl putrescine were reported in genus *Solanum* for the first time (Voynikov *et al.,* 2016). HCAAs have been reported to possess good activity against wide range of microbial pathogens (Guzman, 2014). Feruloyl dopamine, feruloyl tyramine and feruloyl tryptamine were found effective against *S. aureus* 209 and *S. pyogenes* with MIC values between 190 and 372 µM (Georgiev *et al.*, 2012). N^{ε}-feruloyl lysine exhibited MIC of 349 µM against *S. aureus* 3359 and ATCC 6538 P (Voynikov *et al.*, 2016). Solanopubamine was found to exhibit good antifungal activity against *Candida albicans* and *C. tenuis* with MIC of 12.5 µg/mL (Al-Rehaily *et al*., 2013).

However, the steroidal alkaloid detected in this study might not be the main contributor to the antiproliferative activity of this species. Previous study showed that solanopubamine was found inactive against several cancer (SK-MEL, KB, BT-549, SK-OV-3, HL-60) cell lines (Al-Rehaily *et al*., 2013). A derivative of solanocapsine, O-methylsolanocapsine, isolated from *S. pseudocapsicum* leaf was found to possess cytotoxic properties against HeLa cell lines, with IC_{50} values of 39.90 ± 0.03 and 34.65 ± 0.06 by MTT and SRB assays, respectively (Dongre *et al*., 2007). On the other hand, several data in the literature are reporting the antiproliferative activity and mechanism of action of hydroxycinnamic acids and derivatives against several cancer cell lines (Rocha *et al.,* 2012; Eun-Ok *et al.,* 2014; Tavares-da-Silva *et al.,* 2016).

3.3.1.2 Bioassay guided fractionation of the methanol extract

Bioassay-guided fractionation method is commonly employed in drug discovery research, due to its effectiveness to directly linked the analyzed extract and targeted compounds using fractionation procedure that followed with certain biological activity.

Accoding to the results obtained from biological activities screening of the five investigated plants and beside the few published data as well, *S. schimperianum* was selected for bioassay guided fractionation using the antioxidant activity as the tested biological assay.

The methanolic extract of the leaf was subjected to CC as described in method section and the 9 major fractions obtained were screened for their antioxidant property using the DPPH and ABTS assays (Fig.24). Results showed that from the 9 fractions, 5 gave antioxidant potentiality in both assays and with IC_{50} ranged between 22 to 8 µg/mL for the DPPH assay and 10 -25 µg/mL for the ABTS assay. Highest antioxidant activity was displayed by fraction 8 for the DPPH assay and fractions 5 and 6 for the ABTS assay. Moreover, the 9 fractions were developed in TLC plate and was sprayed with DPPH solution to determine antioxidant spots (Fig. 25). Results showed that fractions 2,5, 6, 8 and 9 displayed yellow spots confirming their antioxidant property.

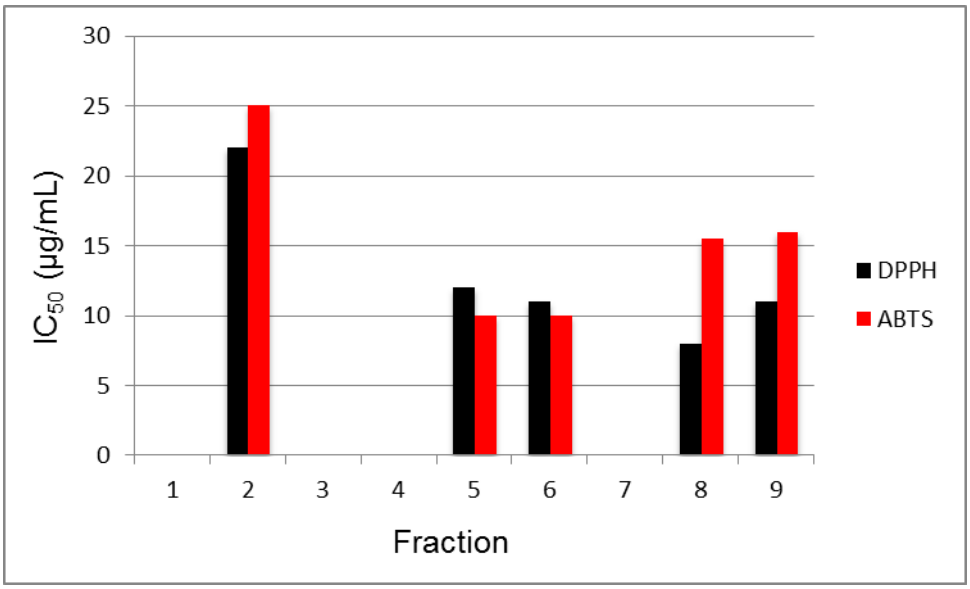


Figure 24: Antioxidant activity of major fractions obtained from CC of methanolic extract of *S. schimperianum* leaf.

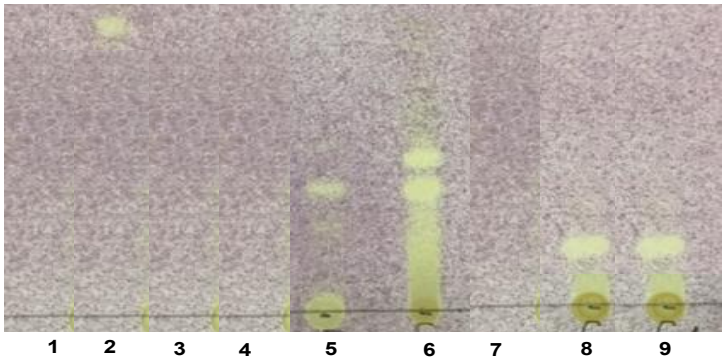


Figure 25: Chromatogram of major fractions obtained from CC of methanolic extract of *S. schimperianum* leaf.

Solvent system: $CHCl_3$: MeOH (3:2, v/v)

Spray reagent: DPPH solution.

3.3.1.3 Isolation and identification of pure compounds

Fractions 8 and 9 were combined and subjected to repeated coulum chromatography to get compound **N-1** (35 mg), as yellow crystals, R_f value of 0.22 in $CHCl_3$: MeOH (3:2) and gave brown colour after spraying with 10% H_2SO_4 (Fig. 26) and yellow colour after spraying with Neo reagent.

Figure 26: TLC of compound N-1.

Solvent system: $CHCl_3$: MeOH (3:2, v/v)

Spray reagent: 10% H_2SO_4

Fractions 5 and 6 were subjected separately to repeated colum chromatography to obtain pure compound and the compound obtained from each fraction was proven to be identical as they revealed the same Rf value in TLC using different solvent system. Thus fractions 5 and 6 gave compound N-2 (30 mg), as a greenish-yellow powder, R_f value of 0.78 in Toluene: Ethyl acetate: Formic acid (5:4:1) and brown colour after spraying with 10% H_2SO_4 (Fig. 27).

Figure 27: TLC of compound N-2.

Solvent system: Toluene: Ethyl acetate: Formic acid (5:4:1, v/v/v)

Spray reagent: 10% H_2SO_4

Fraction 2 was also purified by repeated colum chromatography to obtain pure compound N-3 (28 mg), as white colour powder, R_f value of 0.76 in hexane: acetone (9:1) and grey colour after spraying with 10% H_2SO_4 (Fig. 28).

Figure 28: TLC of compound N-3.

Solvent system: Hexane: Acetone (9:1, v/v)

Spray reagent: 10% H_2SO_4

3.3.1.3.1 Characterization of compound N-1

Compound N-1 has the following characteristics:

- LC-ESI-MS, negative ion mode, *m/z*: 447.0927 $[M-H]^-$, corresponding to the formula $C_{21}H_{20}0_{12}$.

The NMR data obtained from compound N-1 include ^1H NMR and ^{13}C NMR spectra, in addition to proton – proton correlated spectroscopy (HH-COSY), heteronuclear single quantum coherence (HSQC), heteronuclear multiple bond coherence (HMBC) (Fig. 29, 30, 31, 32 and 33) showed signals assignable to:

- The ^1H NMR spectrum indicated the presence of four aromatic protons at 8.05 (d, *J*= 10 Hz, 1H), 6.88 (d, *J*= 10Hz, 1H), 6.38 (d, *J*= 2.4Hz, 1H), 6.19 (d, *J*= 2.4Hz, 1H).

- The *meta* coupling (*J* = 2.4Hz) between the proton 6.19 and 6.38 ppm are tipically of H-6 and H-8 at of ring A of flavonoid compound.

- The chemical shifts of the signals attributable to the B cycle of the genin indicate the presence of an aromatic quaternary carbon (122.7 ppm), an aromatic quaternary carbon bearing a hydroxyl group (161.5 ppm), four aromatic CH two by two at 132.3 ppm and 116.0 ppm. The equivalence of the signals confirms that the hydroxyl group is in the 4' position.

- In the ring B we can observe the presence of system 2H AA' and 2H XX'. This is tipically of kaempferol aglycone.

- In the sugar region, only one anomeric proton is present with a constant coupling of 7.2 Hz. This confirme the presence of *β*-glucose.

- The ^{13}C NMR spectrum displayed 19 signals.

- The coupling visible in the HBMC, between H1" and C-3, confirme that the kaemfperol has a *O*- *β* glucosilated in position 3.

The comparison of these data with those reported in the literature suggested the identification of compound N-1 as kaempferol-3-glucoside or astragalin.

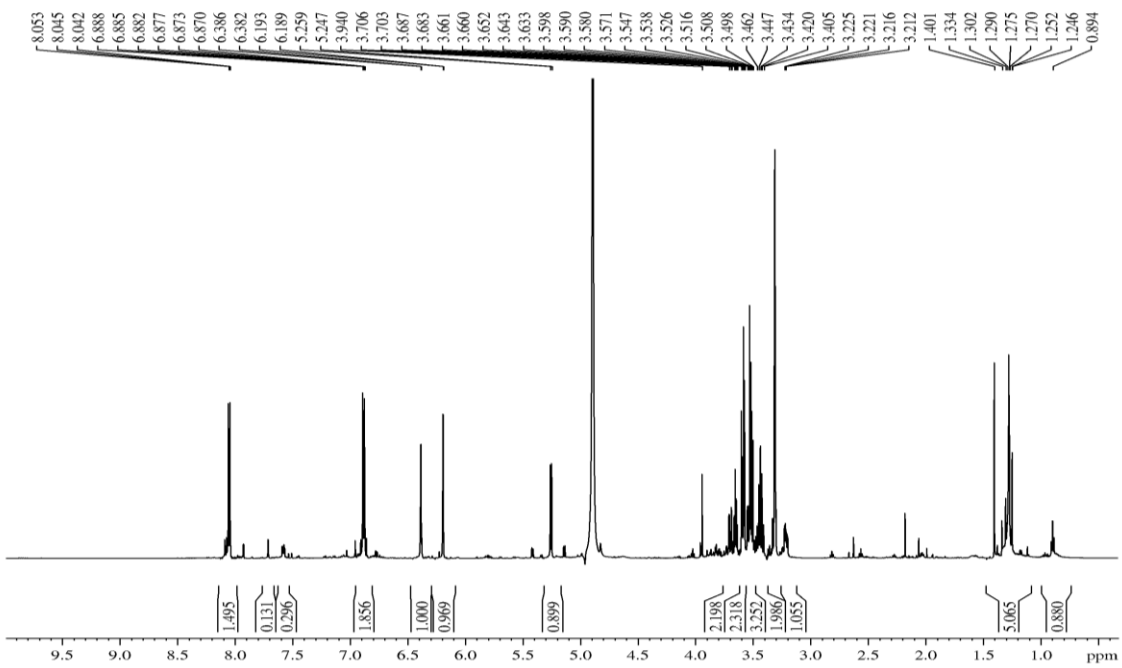


Figure 29: a) ^{1}H spectrum of compound N-1 in CD_3OD at 600 MHz

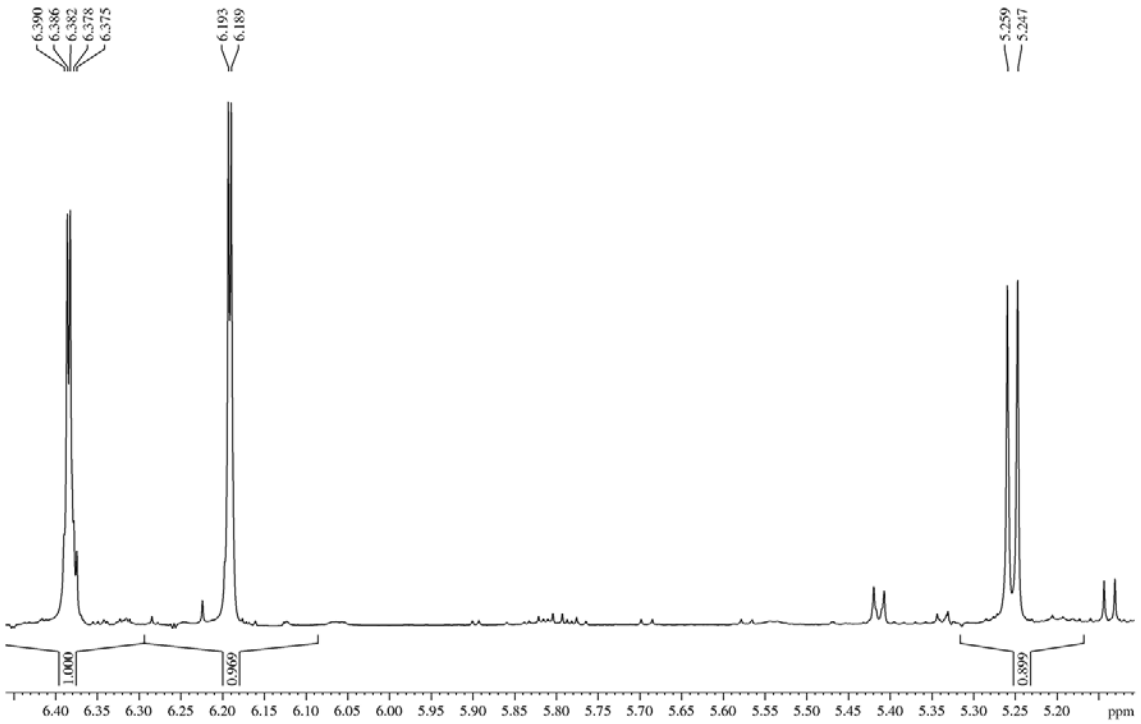


Figure 30: b) Zoom of ^{1}H spectrum of compound N-1 in CD_3OD at 600 MHz

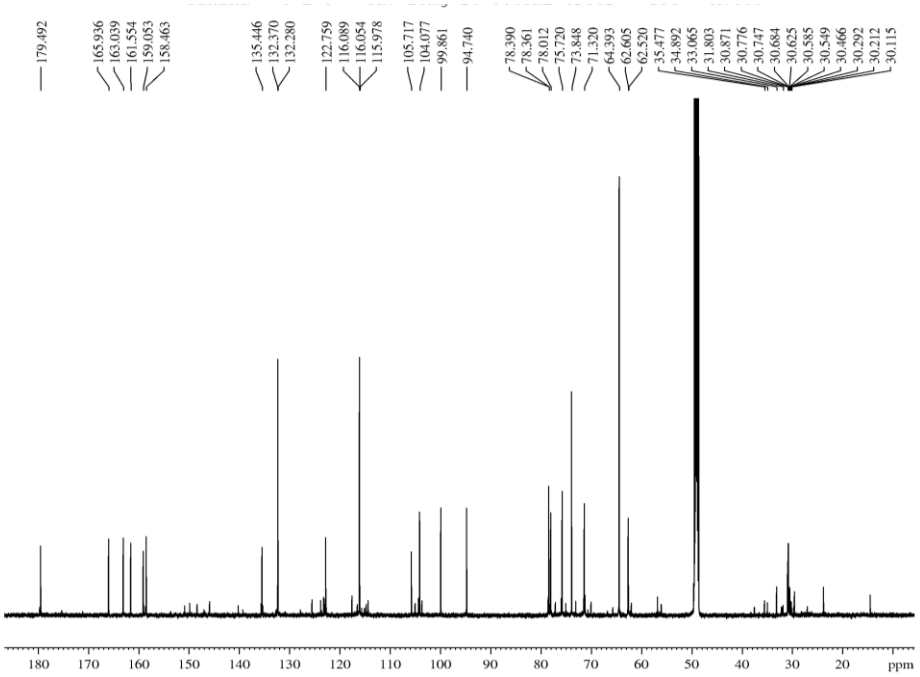


Figure 31: ^{13}C spectrum of compound N-1 in CD_3OD at 150 MHz.

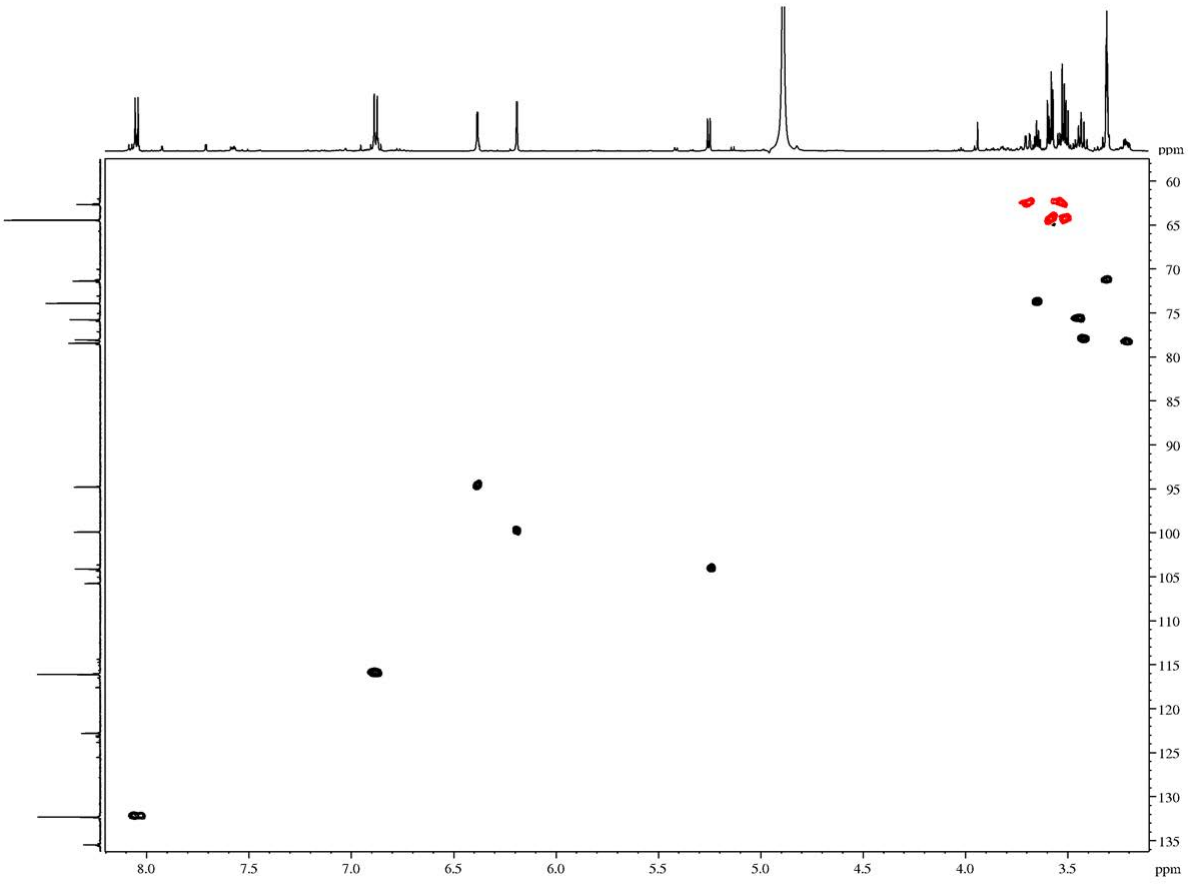


Figure 31: HSQC spectrum of compound N-1 in CD_3OD at 600 MHz.

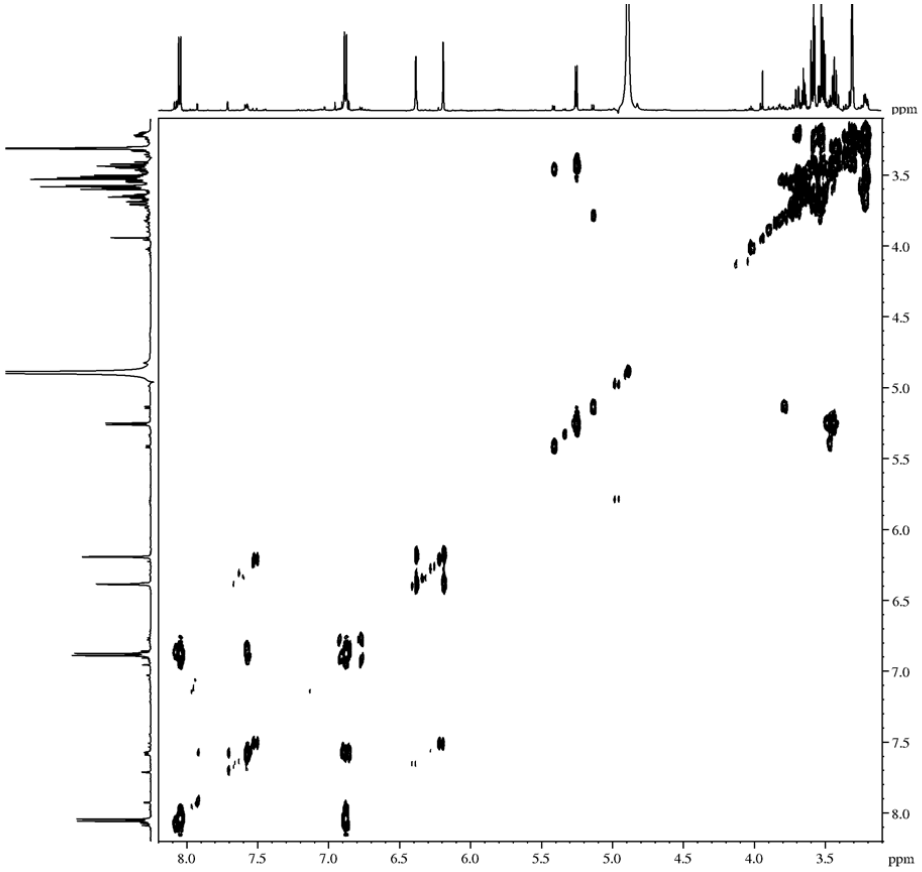


Figure 32: COSY spectrum of compound N-1 in CD_3OD at 600 MHz.

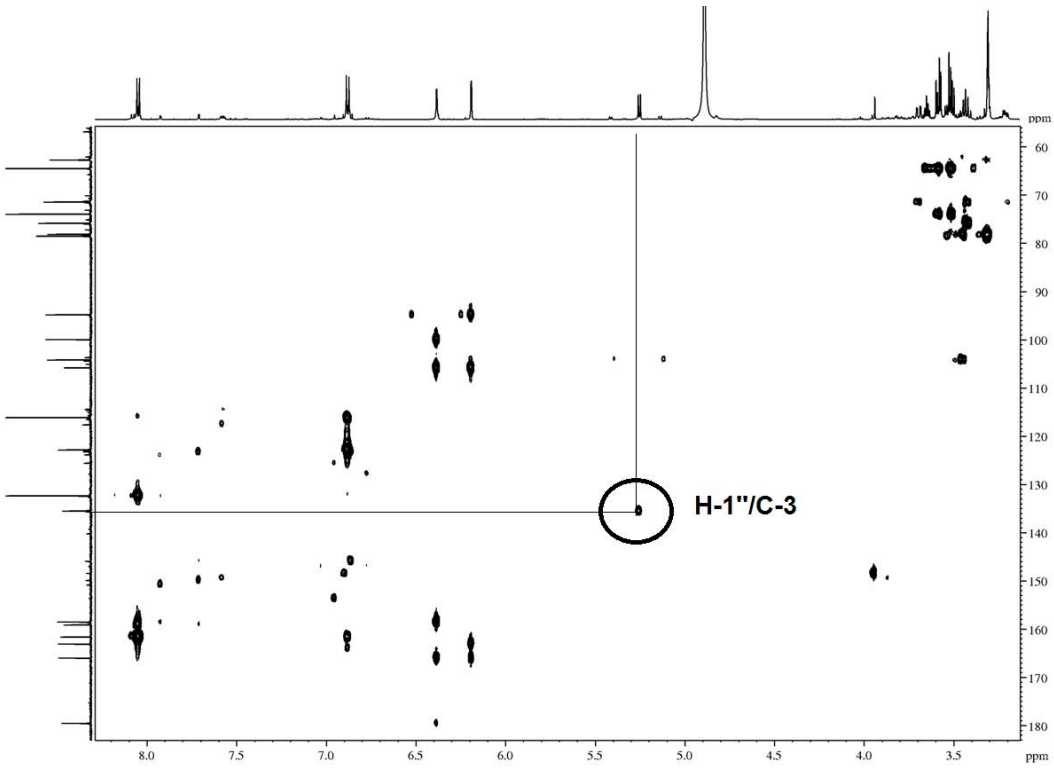


Figure 33: HMBC spectrum of compound N-1 in CD_3OD at 600 MHz.

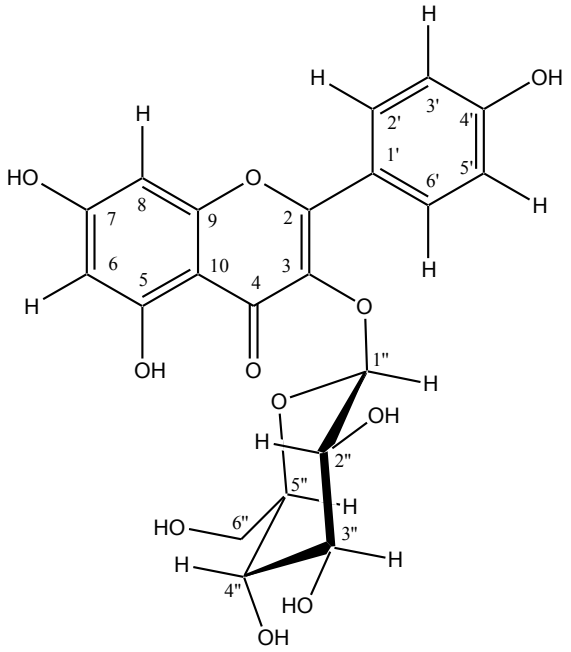


Figure 32: Structure of kaempferol-3-glucoside or astragalin.

Table 13: ^{13}C NMR and ^{1}H NMR (600 MHz, acetone-d_6) spectra of compound N-1.

Position	**^{1}H ppm**	**^{13}C ppm**	**Selected HMBC correlation**
C-2		159.0	H-2'/H-6'
C-3		135.5	H-1"
C-4		179.5	H-8
C-5		163.0	H-6
C-6	6.19 (d, *J*= 2.4Hz, 1H)	99.9	H-8/H-1"/H-5
C-7		165.9	H-8/H-6
C-8	6.38 (d, *J*= 2.4Hz, 1H)	94.7	
C-9		158.5	
C-10		104.1	
C-1'		122.7	
C-2'	8.05 (d, *J*= 10 Hz, 1H)	132.3	
C-3'	6.88 (d, *J*= 10Hz, 1H)	116.0	
C-4'		161.5	H-2'/H-6'/H-3'/H-5'
C-5'	6.88 (d, *J*= 10 Hz, 1H)	116.0	
C-6'	8.05 (d, *J*= 10 Hz, 1H)	132.3	
C-1"	5.22 (d, *J*= 7.4, 1H)	105.7	
C-2"	3.44 (m, 1H)	75.7	
C-3"	3.44 (m, 1H)	78.0	
C-4"	3.65 (m, 1H)	71.3	
C-5"	3.22 (m, 1H)	78.4	
C-6"	3.59 (m, 1H) et 3.51 (m, 1H)	64.4	

3.3.1.3.2 Characterization of compound N-2

Compound N-2 has the following characteristics:

- LC-ESI-MS, negative ion mode, *m/z*: 301.0348 $[M-H]^-$, corresponding to the formula $C_{15}H_{10}O_7$.

The NMR data obtained from compound N-1 include ^1H NMR and ^{13}C NMR spectra, in addition to proton – proton correlated spectroscopy (HH-COSY), heteronuclear single quantum coherence (HSQC), heteronuclear multiple bond coherence (HMBC) (Fig. 35, 36, 37, 38 and 39) showed signals assignable to:

- In the proton spectrum, we can observe two doublets at 6.30 and 6.53 ppm with respectively constant coupling of J = 2.0Hz. This is typically of *meta* coupling in the aromatic system. The two signals are characteristic of flavonoids ring A.

- We can also observe three aromatic protons at 7.00 (d, J= 8.4Hz, 1H), 7.70 (dd, J=2.2, 8.4Hz, 1H) and 7.83 (d, J=8.4Hz, 1H). The coupling constants are tipically of the 1', 3', 4' trisubstituted aromatic system. In our case the B ring of quercetin.

- The ^{13}C NMR spectrum displayed 15 signals.

- No anomeric protons are visible.

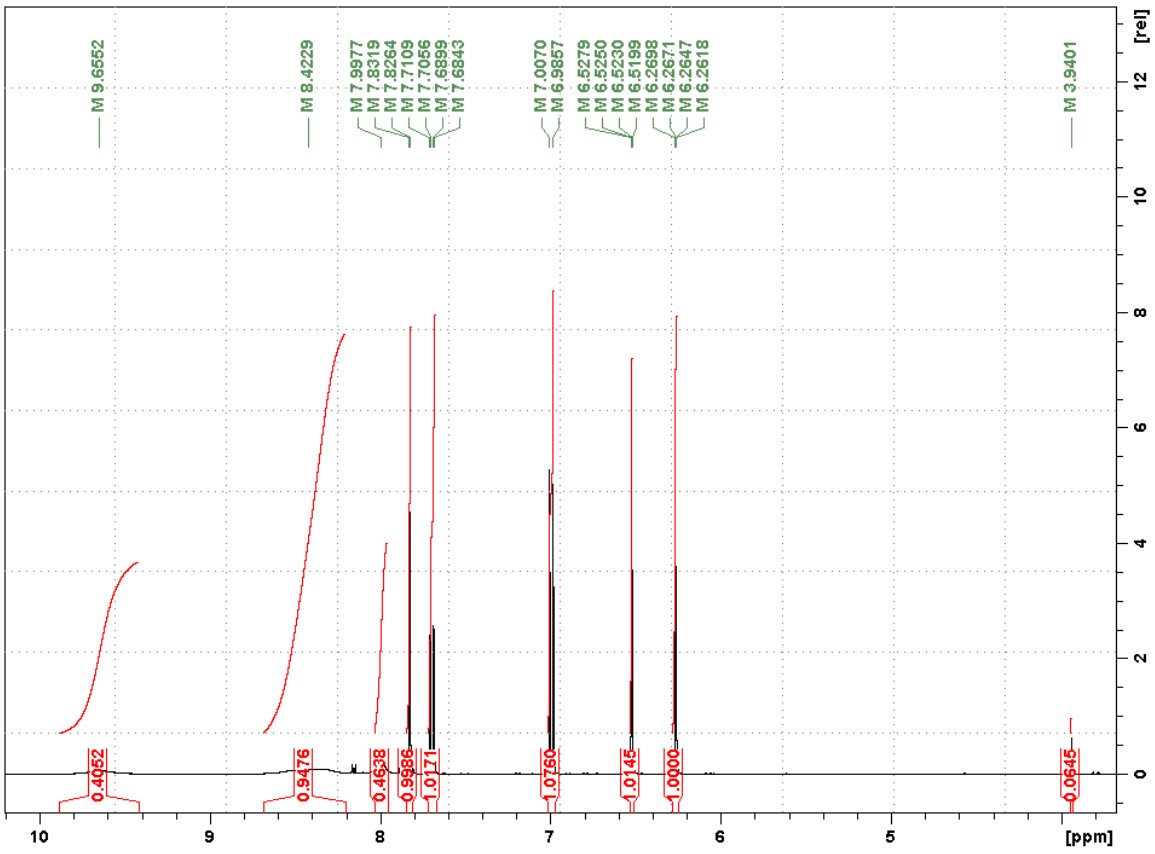


Figure 33: ^{1}H spectrum of compound N-2 in acetone d_6 at 400 MHz.

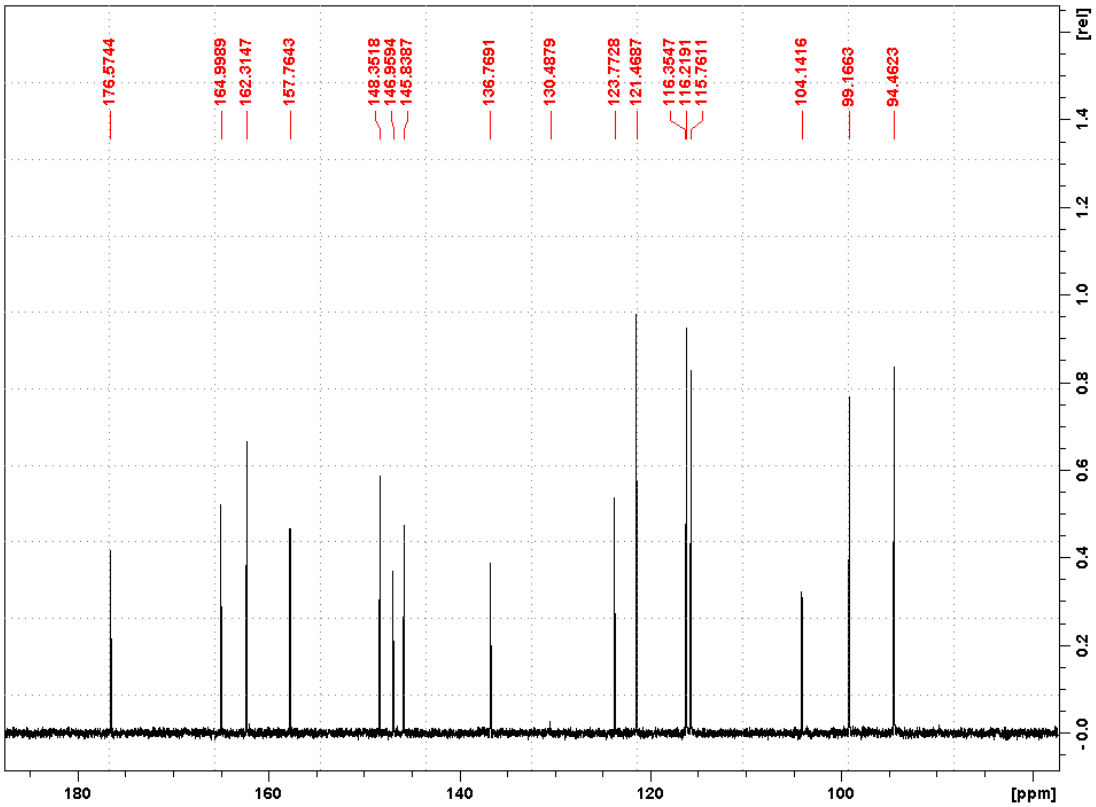


Figure 34: ^{13}C spectrum of compound N-2 in acetone d_6 at 100.6 MHz.

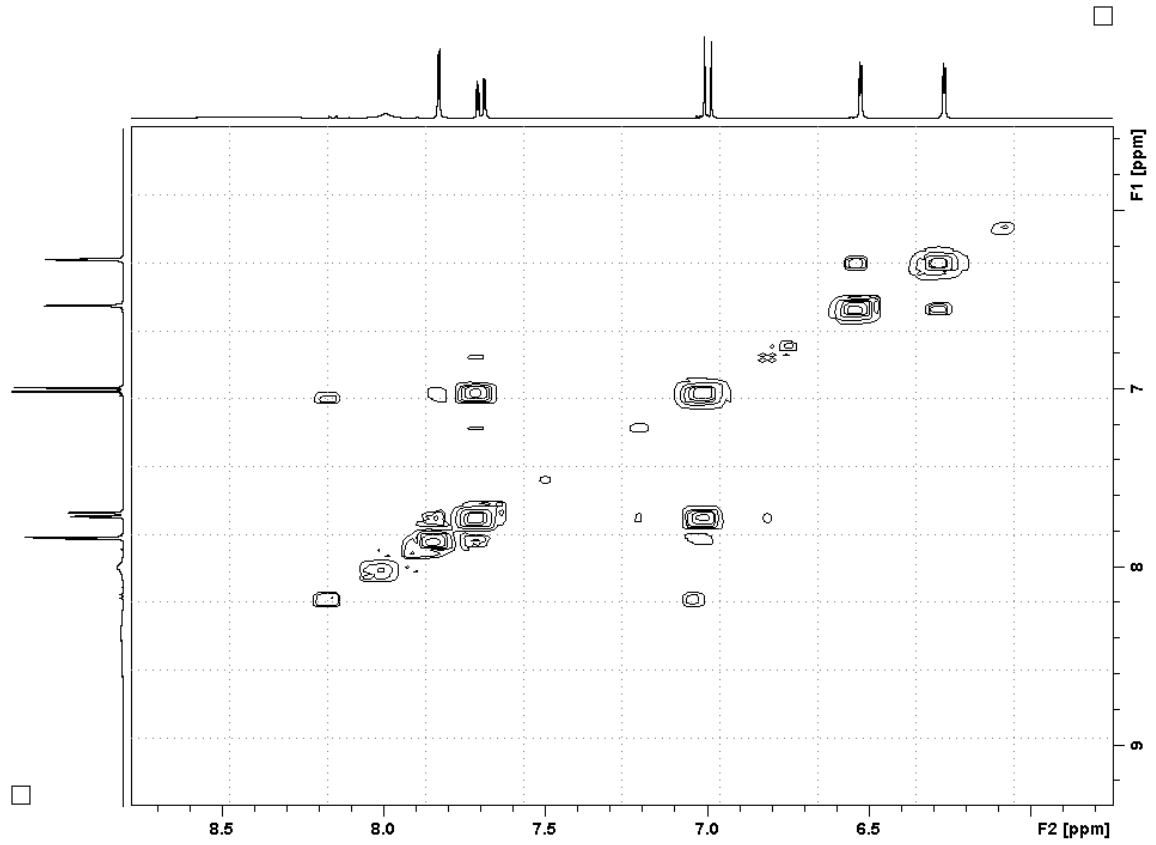


Figure 35: COSY spectrum of compound N-2 in acetone d_6 at 400 MHz.

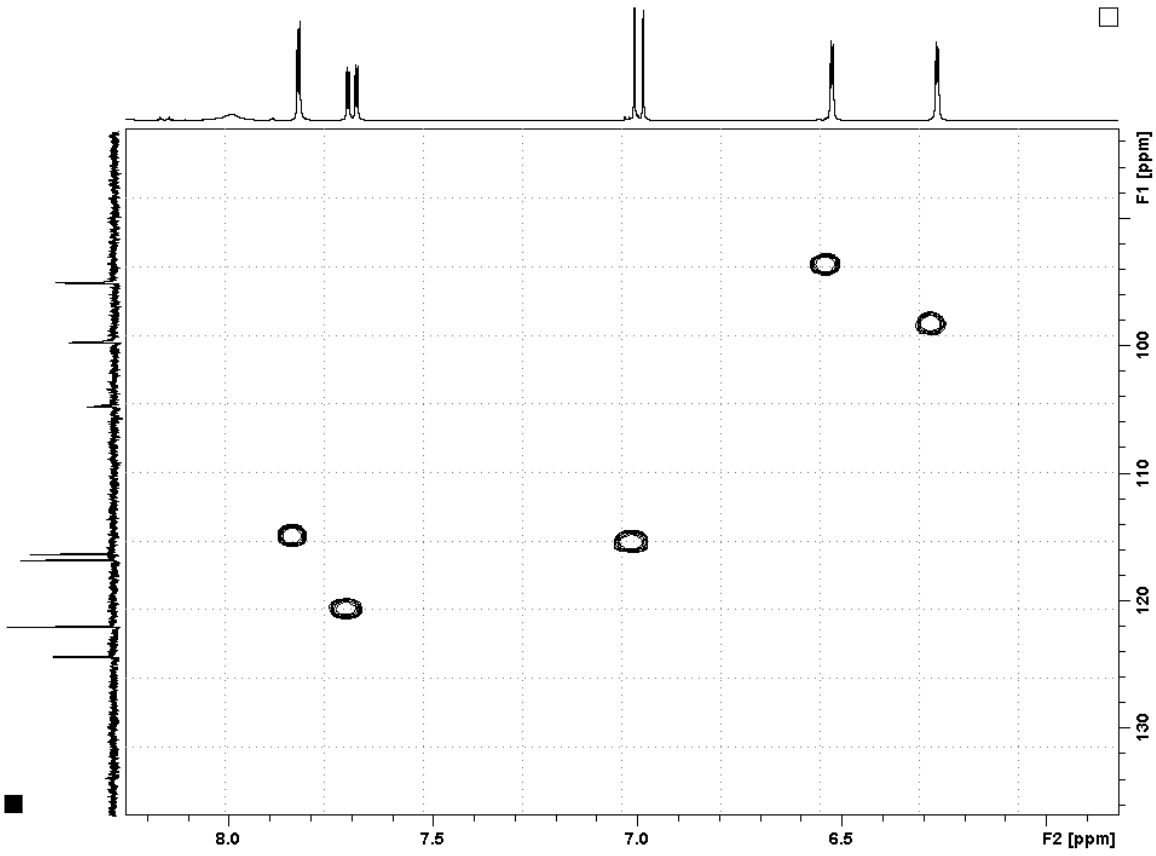


Figure 36: HSQC spectrum of compound N-2 in acetone d_6 at 400 MHz.

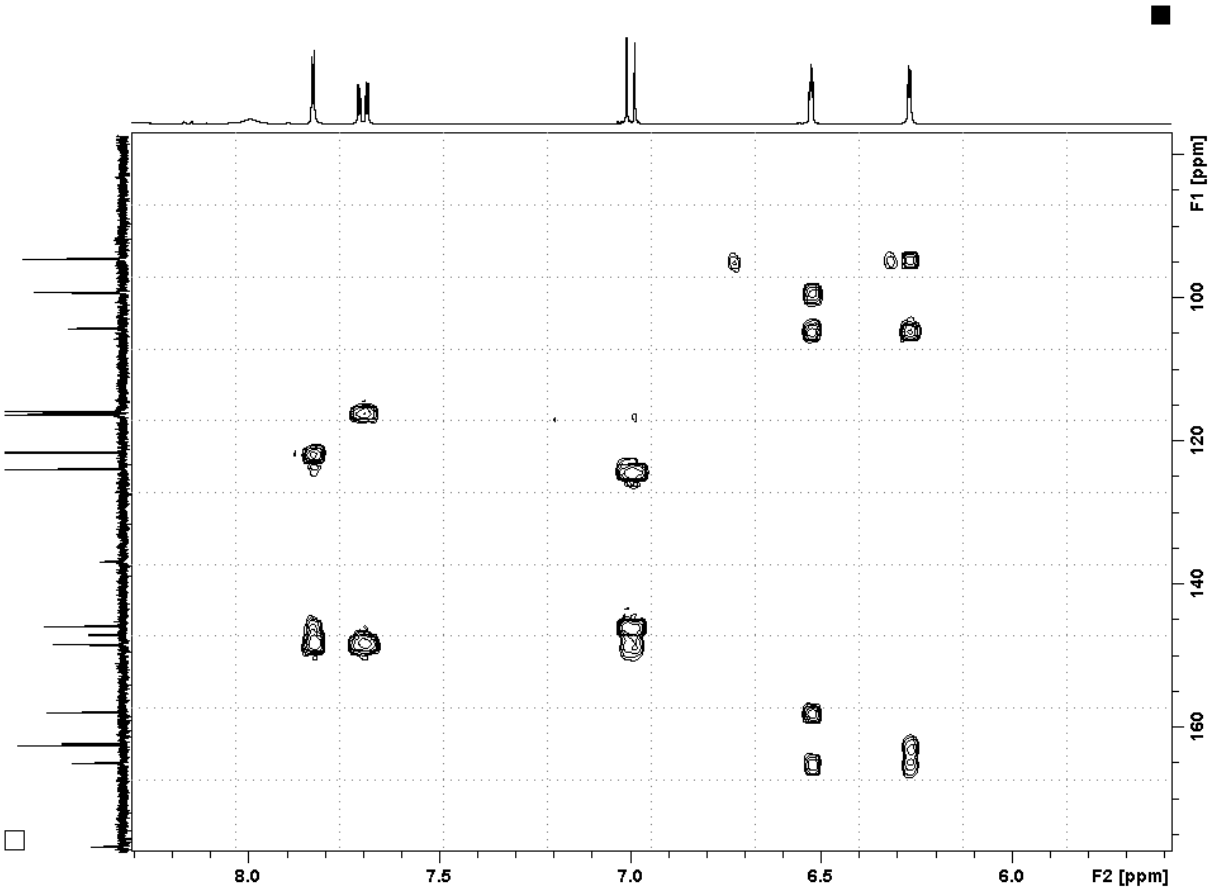


Figure 37: HMBC correlation of compound N-2.

The comparison of these data with those reported in the literature suggested the identification of compound N-2 as 2-3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one or Quercetin.

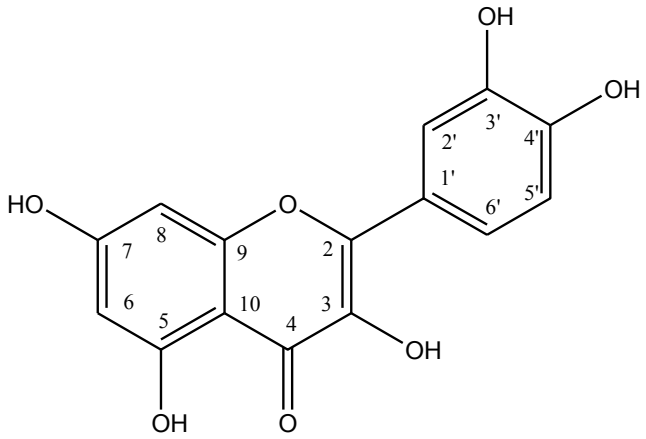


Figure 38: Structure of N-2, Quercetin

Table 14: ^{13}C NMR and ^{1}H NMR (600 MHz, acetone-d_6) spectra of compound N-2.

Position	**^{1}H ppm**	**^{13}C ppm**
2	-	158.3
3	-	137.3
4	-	177.0
5	-	162.9
6	6.30 (d, *J* = 2.1Hz, 1H)	99.6
7		165.3
8	6.52 (d, *J* = 2.1 Hz, 1H)	94.9
9	-	148.8
10	-	104.6
1’	-	124.3
2’	7.83 (d, *J* = 8.4Hz, 1H)	116.2
3’	-	147.4
4’	-	146.2
5’	7.00 (d, *J* = 8.4Hz, 1H)	116.6
6’	7.70 (dd, *J* = 2.2, 8.4Hz, 1H)	121.9

It is worth to notify that all NMR spectra were compared with those of original standard quercetin. In the figures 41, 42, 43 we show ^{1}H, ^{13}C and HMBC spectra.

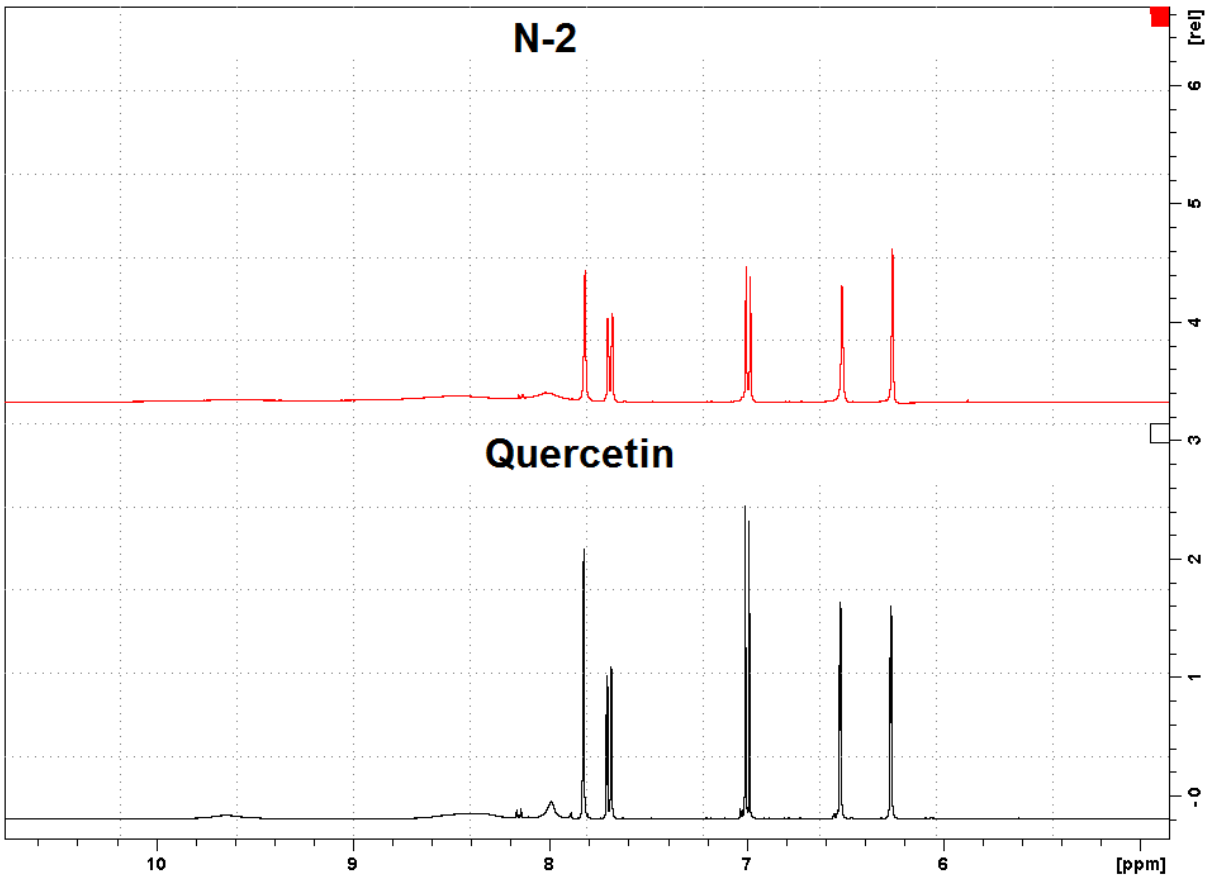


Figure 41: Comparison between ^{1}H spectrum N-2 and ^{1}H spectrum of original standard quercetin. Red = N-2, black = quercetin.

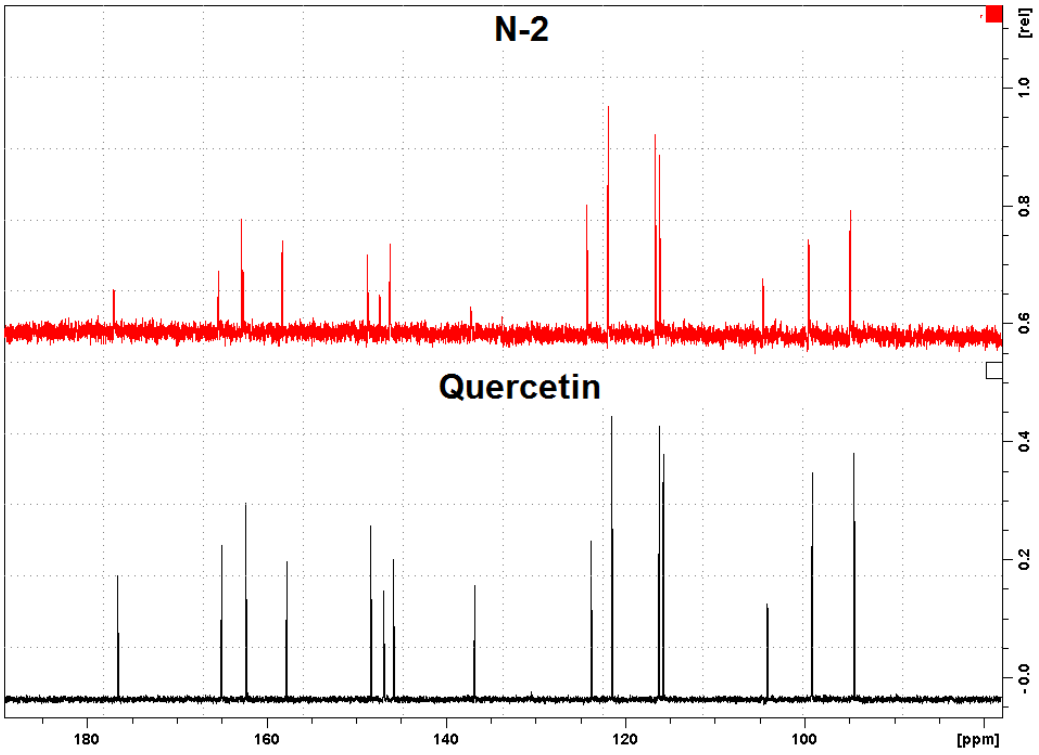


Figure 42: Comparison between ^{13}C spectrum N-2 and ^{13}C spectrum of original standard quercetin. Red = N-2, black = quercetin.

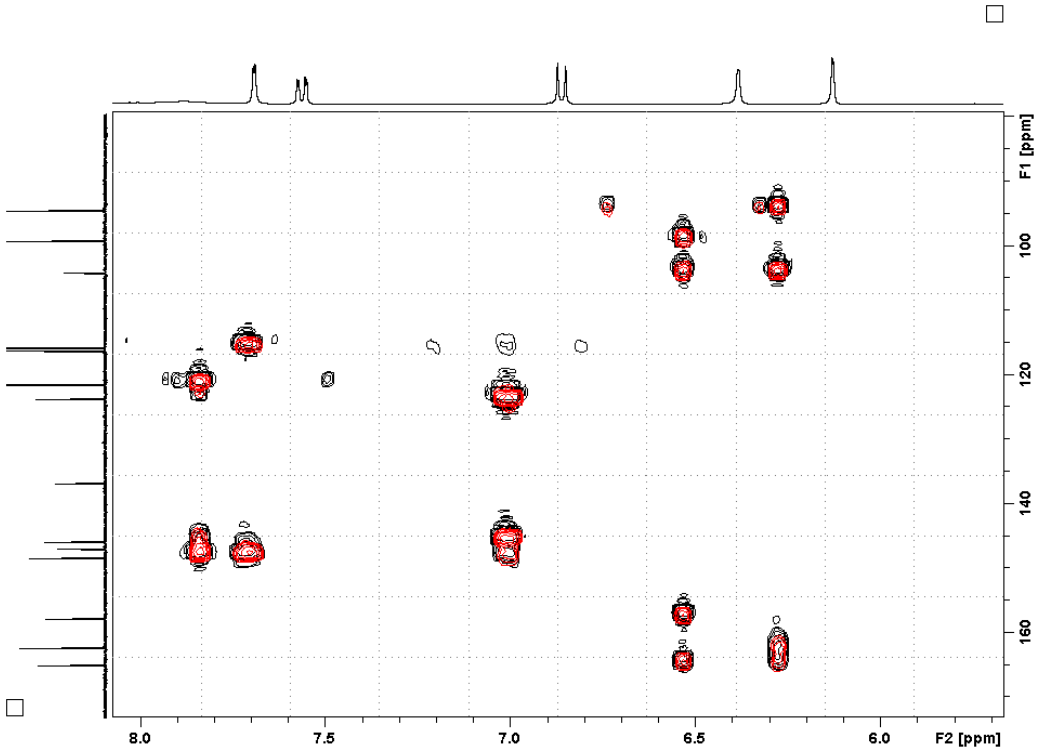


Figure 43: Comparison between HMBC spectrum N-2 and HMBC spectrum of original standard quercetin. Red = N-2, black = quercetin.

Characterization of compound N-3

^{1}H NMR and GC/MS were performed (Fig.44-48). A mixture of sterol compounds was observed. In particular, we can distinguish signals corresponding to olefinic proton of β-sitosterol (Fig. 47).

GC/MS analysis of N-3 showed that the spectrum exhibited M+ at m/z 414 corresponds to molecular formula $C_{29}H_{50}O$. The compound has a base peak at M/Z 43 due to the cleavage of a propyl group (C3H7) (Igwe and Okwu 2013) and fragment ions at m/z 381, 329, 303, 255, 213, 119, 107, 81, 57, 43, 41 similar to that for β-sitosterol obtained by Tripathi *et al.* (2013).

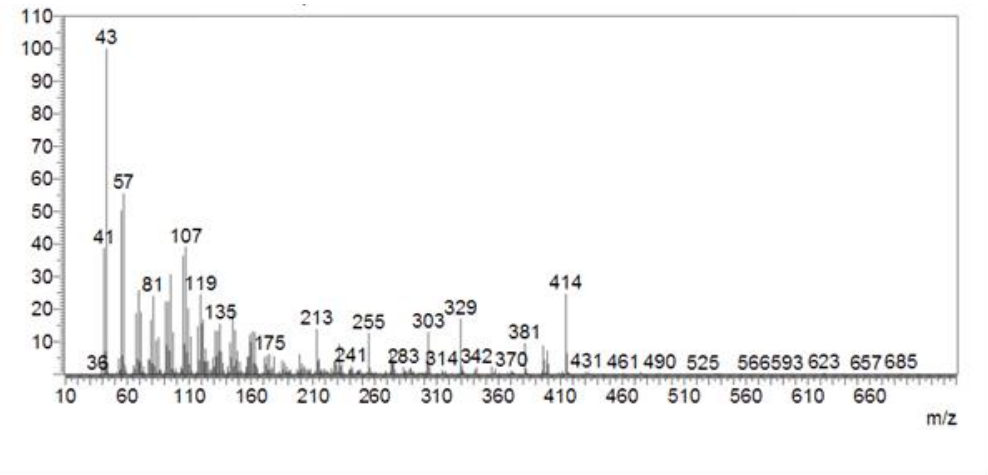


Figure 44: GC/MS spectrum of N-3.

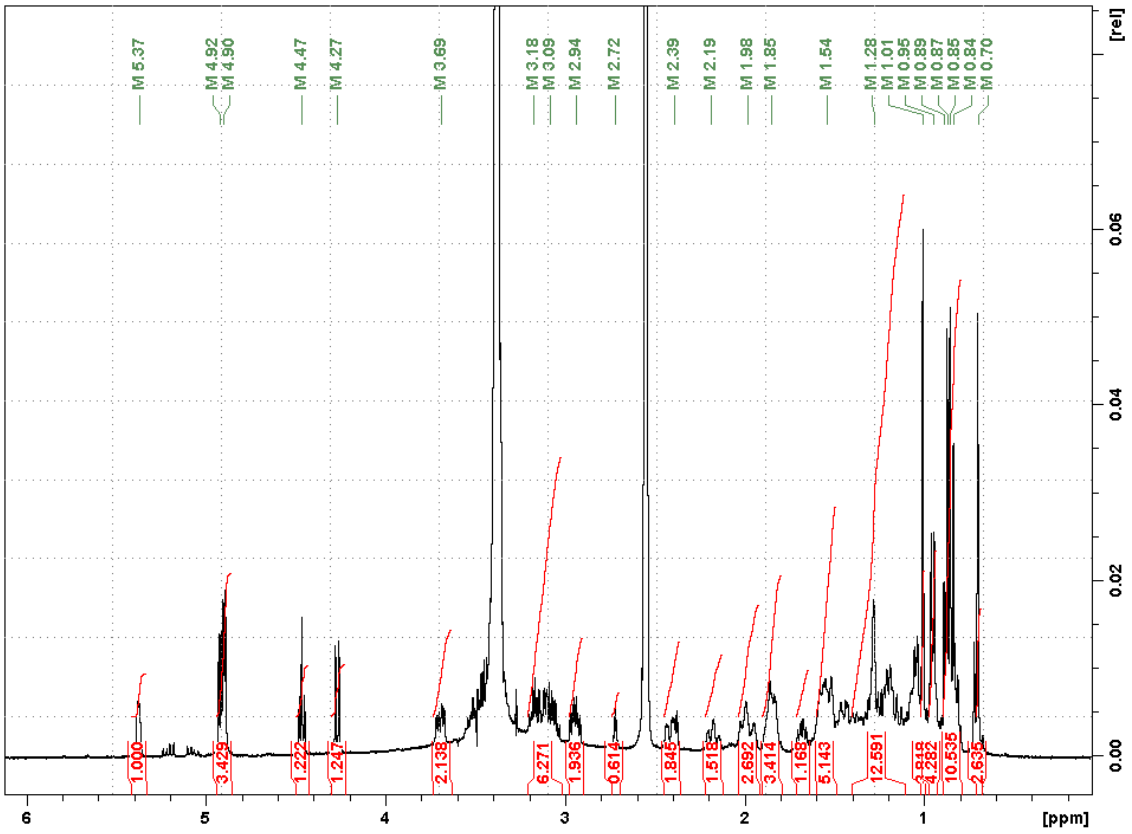


Figure 45: ^{1}H spectrum of compound N-3 in DMSO at 400 MHz

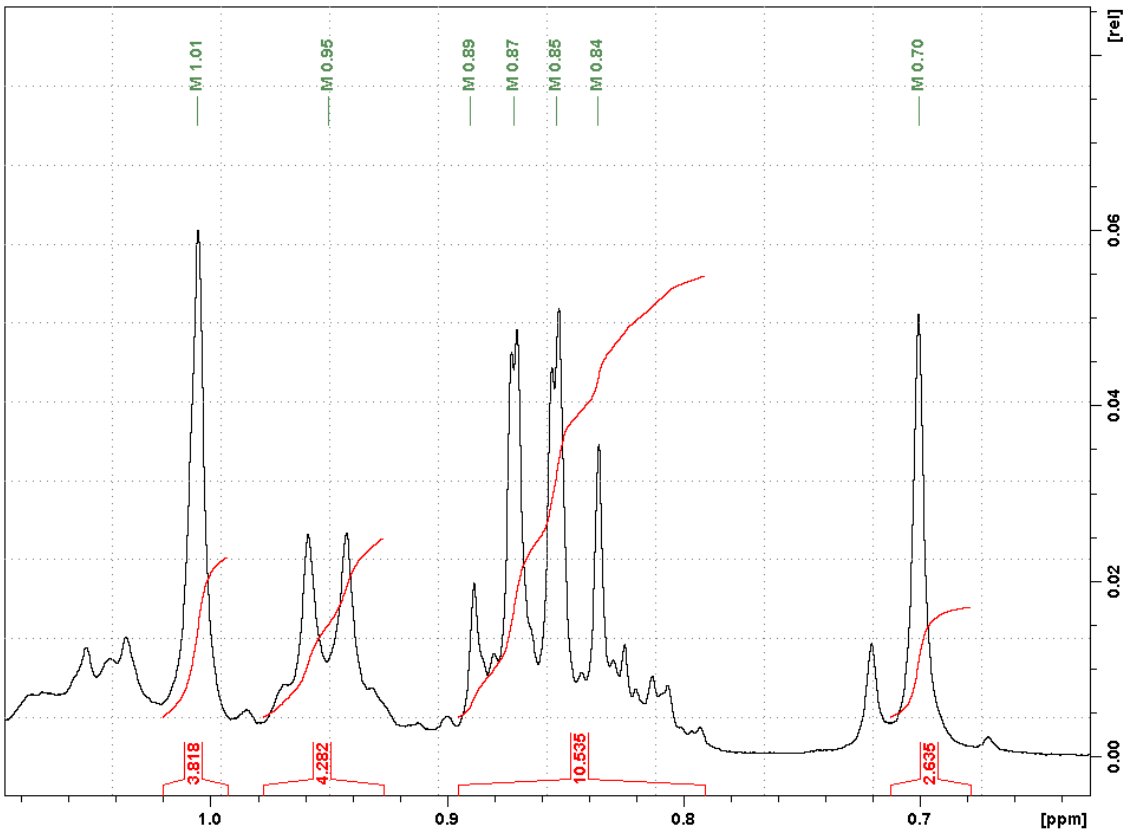


Figure 46: Zoom ^{1}H spectrum of compound N-3 in DMSO at 400 MHz.

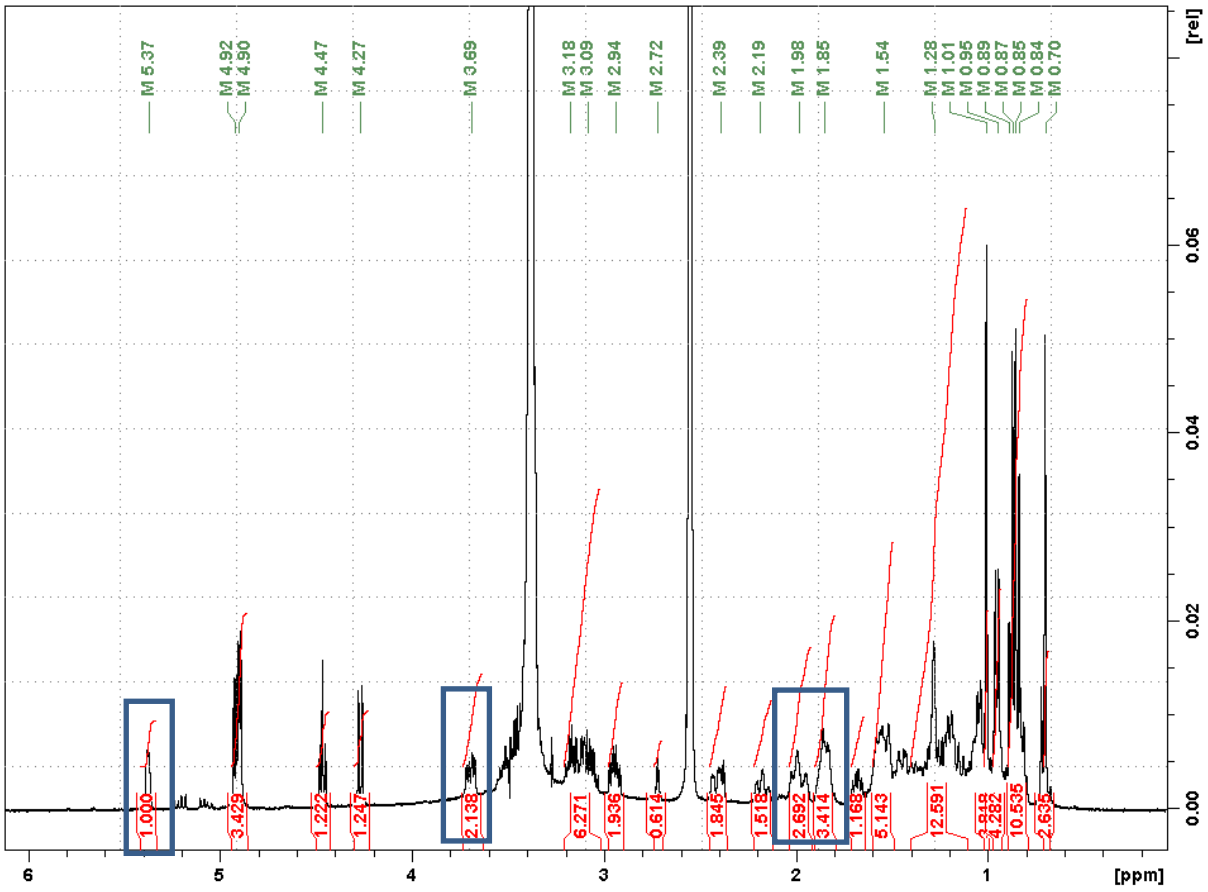


Figure 47: Selected signals of ^{1}H spectrum of compound N-3 in DMSO at 400 MHz.

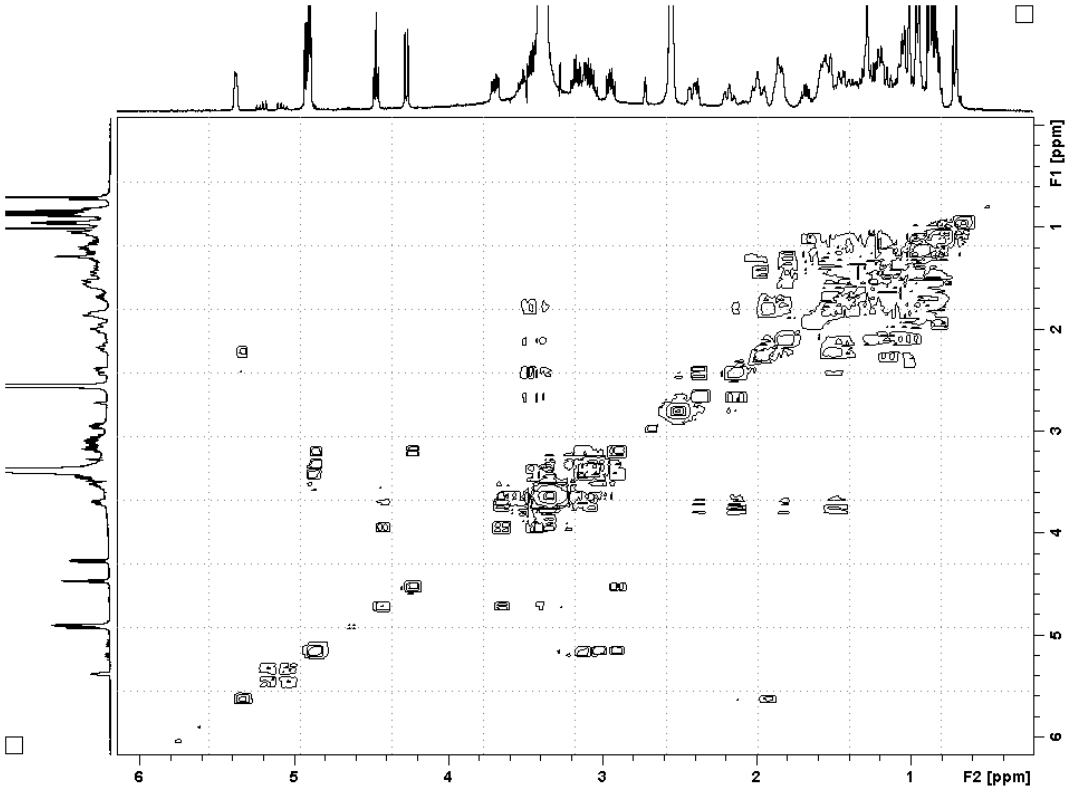


Figure 48: COSY spectrum of compound N-3 in DMSO at 400 MHz.

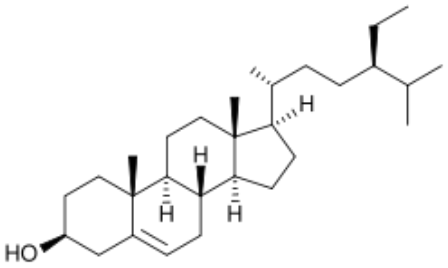


Figure 49: Structure of compound N-3, β-sitosterol

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3.3.2 Biological activity of the isolated compounds

3.3.2.1 Antioxidant activity of isolated compounds (N 1-3)

The three isolated compounds were evaluated for their antioxidant activity using the DPPH and ATBS assays and results are presented in Table 16. Only N-2 displayed remarkable antioxidant activity.

Table 15: Antioxidant activity of isolated compounds (N 1-3).

Code	Compound name	IC_{50} (mM)	
		DPPH	ABTS
N1	Astragalin	15.19± 0.7	10.19 ± 0.9
N2	Quercetin	3.95 ± 0.1	4.01±0.4
N3	β-sitosterol	197.122 ± 0.3	1000 ± 2.3

This result was in agreement with that obtained by Zhang *et al.* (2014) who found that quercetin displayed higher DPPH, ABTS radical scavenging abilities and better reducing power than quercetin glycosides. This could be attributed to the fact that larger polyphenols diffuse less readily in aqueous media reducing the overall free radical scavenging ability (Dubeau *et al.,* 2010). De Freitas et al proved that the binding affinity of polyphenols increases with the molecular weight and the number of hydrophilic hydroxyl groups. This binding can affect the electron donation capacity of the flavonoids by reducing the number of hydroxyl groups available for oxidation in the media (De Freitas *et al*., 2001).

3.3.2.2 Antityrosinase activity of isolated compounds (N 1-3)

Moreover, the potential of the isolated compounds for skin-whitening cosmetics with anti-tyrosinase activity was also carried out. Kaempferol glycoside showed moderate antityrosinase inhibitory effect while N-2 and N3 showed weak effect (Table 17). In contrast to this finding, Arung *et al*. (2011) evaluated the tyrosinase inhibitory activity of quercetin 4'-O-β-D-glucopyranoside which was isolated from the dried skin of *Allium cepa*. They found that quercetin 4'-O-β-D-glucopyranoside showed interesting activity with IC_{50} values of 4.3 and 52.7 µM, respectively.

Table 16: Antityrosinase activity of isolated compounds (N 1-3).

Code	Compound name	Antityrosinase activity (%)
N1	Astragalin	50.50
N2	Quercetin	14.75
N3	β-sitosterol	18.25

3.3.3 *In silico* anticancer validation of the isolated compounds

The current docking study was conducted to study the effect of the isolated compounds on human pregnane X receptor (PXR) in attempt to investigate their *in silico* potencies against cancer. The function of PXR, also known as steroid X receptor (SXR), is to respond to presence of xenobiotics (foreign toxic substances) and up regulate the expression of proteins involved in the detoxification and clearance of these substances from the body (Kliewer *et al*., 2002). It has been reported that PXR activates the overexpression of the multidrug resistance 1 (MDR1) gene that cause tumor cells to acquire drug resistance (Li *et al.,* 2016). So targeting PXR may provide a solution to enhance the efficacy of anticancer drugs.

In the current docking study, the three isolated phytochemicals were docked against PXR (PDB ID:) Table 18 shows the binding energies of the compounds (kcal/mole). β-Sitosterol was found to be the most selective compound and gave the highest binding energy (11.2 kcal/mol).

The docking visualization (Fig.50) presented that β-Sitosterol relays basically on the hydrophobic interactions with the greasy residues forming the enzyme binding site, namely; Leu206, Leu209, Phe281, Leu411, Ile414, and Phe420. Besides, a single hydrogen bond between the hydroxyl groups attached to ring A of the compound and Leu206 at a distance of 3.3 Å.

Table 17: Docking Binding free energies of the top ranked compounds with PXR.

	Compounds	Docking Energy (Kcal/mol)
1.	β-sitosterol	-11.2
2.	Quercetin	-6.1
3.	Astragalin	-5.2

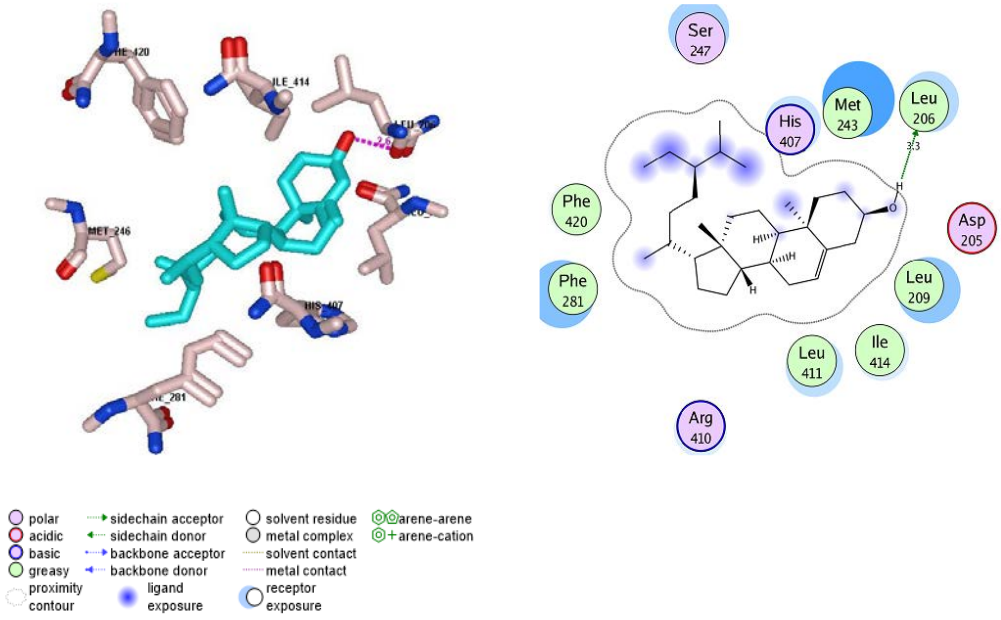

Figure 50: Interaction of β-Sitosterol and PXR.

(A) A picture generated by LigX module of MOE software; protein residues are coloured according to their nature. The map shows the 2D visualization of the interactions, represented by the hydrogen bonding (green dots) between the hydroxyl group of the compound and the amino acid Leu206, and van der waal contacts with the hydrophobic residues (coloured green). (B) 3D representation of compound β-Sitosterol within the active site of PXR (carbons are in pink, nitrogens in blue and oxygens in red), H-bonds are shown as dark pink dotted line.

Conclusion et Perspectives

Les plantes appartenant à la famille des Solanaceae sont généralement utilisées dans la médecine traditionnelle soudanaise pour le traitement de différentes affections. Cette étude a porté sur l'étude phytochimique et sur l'évaluation d'activités antibactériennes, antiprolifératives et antioxydantes in vitro d'extraits de feuilles méthanoliques et de fractions de glycoalcaloïdes stéroïdiens (SGAF) de *Solanum incanum* L., *S. schimperianum* Hochst, *S. nigrum* L., *Physalis lagascae* Roem. & Schult. et *Withania somnifera* (L) Dunal. Un screening phytochimique préliminaire des extraits de feuilles a également été réalisé en utilisant une technique de chromatographie gazeuse associée à la spectrométrie de masse. Une interprétation chimiotaxonomique des cinq espèces basée sur le screening par GC / MS a été proposée.

Une analyse phytochimique détaillée utilisant différentes techniques chromatographiques a été réalisée pour la feuille de *S. schimperianum*. De plus, les composés purs, isolés et identifiés, ont été testés pour leurs activités *in vitro* antioxydantes et antityrosinase. Ils ont également été soumis à une étude *in silico*. L'activité antibactérienne des extraits méthanoliques et des SGAF a été déterminée avec deux souches bactériennes à Gram-positifs et deux souches bactériennes à Gram-négatifs par la méthode des microdilutions. L'activité anti-proliférative a été déterminée sur des lignées cellulaires humaines cancéreuses (MCF7 et MDA-MB-231, HT29 et HCT116) en utilisant le procédé au bromure de thiazolyl-bleu tétrazolium (MTT). L'activité antioxydante a été évaluée en utilisant les méthodes à base du radical diphényle 2 pycril hydrazil (DPPH) et 2,2'-azino-bis 3-éthylbenzthiazoline-6-sulfonique (ABTS). L'extrait méthanolique de S. schimperianum a été analysé en utilisant la chromatographie liquide couplée à un spectromètre de masse Orbitrap avec une source d'ionisation par électropulvérisation (LC-MS).

Le screening phytochimique qualitatif a montré la présence de métabolites secondaires comme les alcaloïdes, les flavonoïdes, les tanins, les stérols / triterpénoïdes et les saponines. Nous n'avons pas identifié d'anthraquinones ni de d'hétérosides stéroïdiques cardiotoniques.

L'analyse par GC-MS, des extraits méthanoliques et des fractions SGAF des espèces étudiées, a révélé la présence d'alcaloïdes stéroïdiens, de saponines stéroïdiennes, de stéroïdes et d'autres composés comme les terpènes, les phénols et les alcanes. Leur répartition variait selon les espèces et, par conséquent, elles pouvaient servir de preuves pour établir des

relations chimiotaxonomiques préliminaires. Selon les indices de similitude de Jaccard, les cinq plantes sont regroupées en deux groupes principaux. Le groupe I contient *P. lagascae* et *W. somnifera* tandis que, les trois espèces de *Solanum*, *S. incanum*, *S. nigrum* et *S. schimperianum*, sont regroupées ensemble dans le groupe II. L'analyse du composé principal (PCA) a indiqué que le premier composé (Groupe 1) et le deuxième composé (Groupe 2) représentent 44,75 et 31,30% de variation entre les espèces végétales. Par ailleurs, le groupe 2 montre une similitude étroite de *S. incanum* avec *S. schimperianum* comparativement à sa relation avec *S. nigrum*. D'autre part, *P. lagascae* et *W. somnifera* se montrent très proches l'un de l'autre dans le groupe 1 et, par conséquent, ce résultat est en accord avec la classification morphologique des plantes de Solanaceae du Soudan proposées par Andrews (1956).

La sensibilité des bactéries à Gram-positif et à Gram-négatif en contact avec les différents extraits était variable (les valeurs d'IC_{50} variaient de 15-> 1000 µg / mL). Seul l'extrait méthanolique de la feuille de *S. schimperianum* a montré une activité antiproliférative intéressante contre les lignées cellulaires humaines testées avec des valeurs d' CI_{50} allant de 2,69 à 19,83 µg / mL tandis que l'activité la plus élevée de la fraction SGAF a été obtenue avec la feuille de *W. somnifera* (valeurs de l' IC_{50} comprises entre 1,29 et 5,00 µg / mL). Les fractions SGAF de toutes les espèces ont montré une activité antioxydante plus élevée que leurs extraits méthanoliques respectifs, quel que soit le test utilisé. La fraction SGAF de *S. schimperianum* a montré l'activité antioxydante la plus forte selon les deux tests avec une valeur IC_{50} $3,5 \pm 0,2_{DPPH}$ et $3,5 \pm 0,3_{ABTS}$ µg / mL. Le coefficient de corrélation (R^2), entre l'activité antioxydante et le contenu en polyphénols totaux des extraits méthanoliques des cinq plantes, suggère que les composés phénoliques ne seraient pas les principaux facteurs responsables de l'activité antioxydante des feuilles de ces plantes.

Douze amides de l'acide hydroxycinnamique (HCAA) ont été identifiés à partir de l'extrait méthanolique de feuilles de *S. schimperianum* et l'acétate de N-caffeoyl était le composé présentant la plus forte intensité. En outre, la présence d'alcaloïdes stéroïdiques a également été détectée, les présences de solanopubamine et de solanocapsine ainsi que des dérivés 3-amino des alcaloïdes stéroïdiques ont été détectées.

Le fractionnement par bio-guidage de l'extrait méthanolique de la feuille de *S. schimperianum* a révélé que trois fractions des neuf obtenues, à partir de la chromatographie sur colonne, ont une activité antioxydante. La purification sur colonne chromatographique de ces trois

fractions a conduit à l'isolement puis à l'identification de trois composés connus, à savoir: la quercétine, le kaempferol glycosylé et le β-Sitostérol. Par ailleurs, la quercétine a montré une activité antioxydante élevée, quelle que soit la méthode d'analyse utilisée, avec une valeur de IC_{50} de $3,95 \pm 0,2_{DPPH}$ et de $4,01 \pm 0,4_{ABTS}$ µg/ mL. Le glucosyl de kaempferol a montré un effet inhibiteur de la tyrosinase modéré (50,50%) tandis que son aglycone (14,75%) et le β-sitosterol (18,25%) ont eu un effet limité.

Les trois composés isolés ont été associés au récepteur humain X prégnane (PXR) dans le but d'étudier leur potentiel in silico contre le cancer. Le β-sitosterol s'est révélé être le composé le plus sélectif et a donné l'énergie de liaison la plus élevée (-11,2 kcal / mol). La visualisation de la fixation a montré que le β-sitosterol présente essentiellement des interactions hydrophobes avec les résidus formant le site de liaison enzymatique, à savoir : Leu206, Leu209, Phe281, Leu411, Ile414 et Phe420. En outre, une seule liaison hydrogène existe entre le groupe hydroxyle attaché au cycle A du composé et Leu206 à une distance de 3.3 Å.

En résumé, il est clair que les phénols ne sont pas les principaux éléments responsables de l'activité antioxydante des feuilles de ces plantes. *S. schimperianum* a montré l'activité antioxydante la plus importante dans les deux méthodes de dosage et, à notre connaissance, c'est la première fois que l'activité antioxydante de cette plante est rapportée. Les résultats obtenus par GC-MS et LC-MS ont montré la présence de composés connus pour leur activité biologique significative. Les résultats obtenus par GC-MS nous ont permis de suggérer que les alcaloïdes stéroïdiens, les saponines stéroïdiennes et les stéroïdes peuvent être considérés comme des marqueurs chimiotaxonomiques pour le genre *Solanum*. Les résultats de l'étude *in silico* ont révélé que *S. schimperianum* pourrait être une source potentielle de molécules d'intérêt.

Perspectives

Suite aux résultats obtenus dans cette étude, les recommandations suivantes peuvent être suggérées :

- Poursuivre l'isolement et l'identification de nouveaux composés bioactifs à partir des extraits méthanoliques et des fractions SGAF des espèces citées ci-dessus et réaliser des tests d'activité biologique.

- Effectuer des études de chimie théorique sur les composés actifs pour étudier leur reconnaissance moléculaire avec l'enzyme PXR et pour étudier la possibilité d'optimisation la structure des inhibiteurs de cette enzyme.

- Étudier l'activité inhibitrice potentielle *in vitro* du β-sitostérol et d'autres composés isolés futurs contre l'enzyme PXR.

- D'autres plantes de la famille des Solanaceae devraient être étudiées ce qui permettrait d'obtenir des informations taxonomiques infragénériques détaillées qui, associées à des données chimiques et moléculaires, permettraient de faire évoluer les connaissances de cette famille.

Conclusion and Perspectives

Plants belonging to the Solanaceae family are generally used in Sudanese traditional medicine for the treatment of different ailments. This study aimed at phytochemical study and evaluation of *in vitro* antibacterial, antiproliferative and antioxidant activities of methanolic leaf extracts and steroidal glycoalkaloids fractions (SGAFs) of *Solanum incanum* L., *S. schimperianum* Hochst, *S. nigrum* L., *Physalis lagascae* Roem. & Schult. and *Withania somnifera* (L) Dunal. Preliminary phytochemical screening of leaf extracts was also carried out using standard qualitative technique and Gas column/mass spectroscopy technique. Chemotaxonomical interpretation of the five species based on GC/MS screening was proposed.

A detailed phytochemical analysis using different chromatographic technique was carried out for the leaf of *S. schimperianum.* Moreover, the pure isolated and identified compounds were tested for their *in vitro* antioxidant and antityrosinase activities. They were also subjected to *in silico* study. Antibacterial activity of the methanolic extracts and SGAFs was determined against two Gram-positive and two Gram-negative bacteria by microdilution method. Anti-proliferative activity was determined against human cell lines (MCF7 and MDA-MB-231, HT29 and HCT116) by the thiazolyl blue tetrazolium bromide (MTT) procedure. Antioxidant activity was evaluated by diphenyl 2 pycril hydrazil (DPPH) and 2,2'-azino-bis 3-ethylbenzthiazoline- 6-sulphonic acid (ABTS) scavenging radical methods. Methanolic extract of *S. schimperianum* was analyzed using liquid chromatography coupled to an Orbitrap mass spectrometer with an electrospray ionization source (LC-MS).

Qualitative phytochemical screening showed the presence of secondary metabolites like alkaloids, flavonoids, tannins, sterols/triterpenoids, and saponins and they all devoid of anthraquinones and cardiac glycosides.

GC-MS analysis of methanolic and SGAFs extracts of the studied species revealed the presence of steroidal alkaloids, steroidal saponins, steroids and other compounds like terpenes, phenols and alkanes. Their distribution varied among the species and thus they could provide evidence to assess preliminary chemotaxonomic relationships. Based on Jaccard similarity indices, the five plants are grouped into two main clusters. Cluster I contains *P. lagascae* and *W. somnifera* whereas, the three *Solanum* species, *S. incanum*, *S. nigrum* and *S. schimperianum*, were grouped together in Cluster II. Principle component

analysis (PCA) indicated that the first (Group 1) and second components (Group 2) accounts for 44.75 and 31.30% of the variation between the plant species. Moreover, Group 1 indicates the close similarity of *S. incanum* to *S. schimperianum* compared to its relation to *S. nigrum*. On the other hand, *P. lagascae* and *W. somnifera* are shown to be very close to each other in group 2 and hence supporting the morphological classification of Solanaceae plants from Sudan proposed by Andrews (1956).

The sensitivity of Gram-positive and Gram-negative bacteria to different extracts was variable (IC_{50} values in the range of 15->1000 µg/mL). Only the methanolic extract of *S. schimperianum* leaf demonstrated interesting anti-proliferative activity against the human cell lines tested with IC_{50} values in the range of 2.69 to 19.83 µg/mL while the highest activity from the SGAFs was obtained from *W. somnifera* leaf with IC_{50} values in the range of 1.29 to 5.00 µg/mL. In both assays the SGAFs of all species demonstrated higher scavenging activity than their respective methanolic extracts. The SGAF of *S. schimperianum* displayed the strongest antioxidant activity in both assays with IC_{50} value $3.5 \pm 0.2_{DPPH}$ and $3.5 \pm 0.3_{ABTS}$ µg/mL. The correlation coefficient (R^2) between the antioxidant capacities and the total phenolic contents of the methanol extracts suggested that the phenolic compounds could not be the main contributor to the antioxidant capacities of leaves of these plants.

Twelve known hydroxycinnamic acid amides (HCAAs) were tentatively identified from the methanolic extract of *S. schimperianum* leaf and N-caffeoyl agmatine appeared with the highest intensity. Moreover, the presence of steroid alkaloids was also detected and the presence of solanopubamine and solanocapsine as well as dehydro derivatives of the 3-amino steroid alkaloids was suggested.

Bioassay guided fractionation of the methanolic extract of *S. schimperianum* leaf revealed that three fractions from the nine ones obtained from the column chromatography demonstrated antioxidant activity. Repeated column chromatography of these three fractions resulted in the isolation and then identification of three known compounds namely: Quercetin, Astragalin and β-Sitosterol. Furthermore, Quercetin displayed high antioxidant activity in both assays with IC_{50} value $3.95 \pm 0.2_{DPPH}$ and $4.01 \pm 0.4_{ABTS}$ µg/mL. Kaempferol glycoside (50.50%) showed moderate antityrosinase inhibitory effect while its aglycon (14.75%) and β-Sitosterol (18.25%) showed weak effect.

The three isolated compounds were docked against human pregnane X receptor (PXR) in attempt to investigate their *in silico* potency against cancer. β-Sitosterol was found to be the most selective compound and gave the highest binding energy (-11.2 kcal/mol). The docking visualization presented that β-Sitosterol relays basically on the hydrophobic interactions with the greasy residues forming the enzyme binding site, namely; Leu206, Leu209, Phe281, Leu411, Ile414, and Phe420. Besides, a single hydrogen bond between the hydroxyl group attached to ring A of the compound and Leu206 at a distance of 3.3 Å.

Thus, in summary it was clear that phenols were not the main contributors to the antioxidant capacity of leaves of these plants. *S. schimperianum* displayed the strongest antioxidant activity in both assays and, to the best of our knowledge, this is the first report on the antioxidant activity of this plant. GC/MS and LC-MS results identified the presence of compounds that were known for their significant biological activity. Moreover, the obtained results of GC/MS suggested that steroidal alkaloids, steroidal saponins and steroids could be considered as chemotaxonomic markers for the genus *Solanum*. Results of *in silico* study suggested that *S. schimperianum* could be a potential source of lead molecules.

Future perspectives

According to the results obtained in the current study the following recommendations were suggested.

- To isolate and identify new potent bioactive compounds from both the methanolic and SGAFs extracts of the studied species and tested their biological activity.

- To carry out a structural computational-chemistry based studies on the active compounds to investigate the PXR enzyme-inhibitor molecular recognition and to investigate the possibility of structural optimization of inhibitors.

- To study the *in vitro* inhibitor potentiality of β-Sitosterol and other future isolated compounds against PXR enzyme.

- More Solanaceae plants should be investigated and consequently, detailed infrageneric taxonomic information joined to chemical and molecular data would be needed for the classification and evolution of this family.

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Scientific Production

Poster

1. Phytochemical and Biological Evaluation of Solanum schimperianum Hochst. Leaves. The 15th Symposium of the Natural Products Research Network for Eastern and Central Africa (NAPRECA), Khartoum, Sudan 7 – 10th December 2013.

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Phytochemical and Biological Evaluation of *Solanum schimperianum* Hochst. Leaves

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INTRODUCTION

Genus *Solanum* (to which is belong *S. schemperianum* Hochst, Fig 1) is the largest group of the family Solanaceae which distributed all over the world. *Solanum* specices are very rich in specific constituents that may have important role in the biological activity, these constituents are known as steroidal alkaloids(SGAs), they are a class of plant natural products and have a fairly complex nitrogen containing nucleus. Most recently plants belonging to Solanaceae family have been the topic of active research investigation in many phytochemical and pharmacological laboratories for the presence of these biologically steroidal alkaloids El-Sayed and Hassan,(2006).

Figure 1 : *S. schimerianum*

AIMS

The study aimed to evaluate the chemical constituents of *S. schiperianum* leaves and their potentiality as Anticancer ,antioxidant and antibacterial agents

MATERIALS & METHODS

Plant materials

S.schimperianum leaves were collected from Erkowit, eastern Sudan in April 2012.

Methanol Extract

The defatted powdered leaves were macerated in methanol (95%) for 72h.

Butanol Extract

Glyco-alkaloids were extracted by the method described by Moh –Ud-Din (2010).

Phytochemistry

- Phytochemical study was carried out using Shimadzu GC-MS QP 20-10A system chromatography.

Biological assays

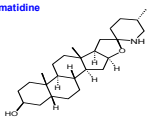
- Cytotoxicity assay was performed against two selected cell lines, Breast cancer MGF7 and Hepatocellular HepG2 (Maul,1992).
- DPPH and ABTS radicals scavenging assays were used for evaluation of antioxidant property.
- Antibacterial test was carried out using disc-diffusion method against four bacterial strains.

RESULTS & DISCUSSION

1. *Butanolic Extract*

Five compounds were isolated from *S.schimperianm* butanolic extract ,two of them were identified as glyco-alkaloids ,they were Soanocapsine and Tomatidine (Fig 2).

- 1-Tomatidine



2-Solanocapsine

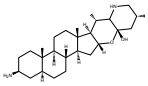
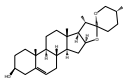


Fig. 2 : Isolated alkaloids from butanolic extract.

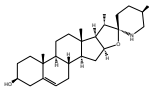
2.Methanolic Extract

Seven compounds were isolated from the methanolic extract, three of them were identified as glyc-alkaloids ,they were: Diosgenin,sarsapognin and Solasodie (Fig. 3).

- 1-Diosgenin



2-Solasodine



3-Sarsapognin

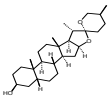


Fig. 3 Isolated alkaloids from methanolic extract.

3. Antioxidant activity

- Butanolic extract showed high antioxidant activity with DPPH and ABTS assays ,while the methanolic extract showed a moderate activity (Fig 4).

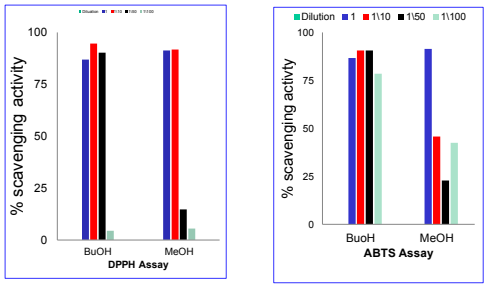


Fig.4 Antioxidant activity of S. schimperianum leaves extracts.

Table 1: Major compounds in butanol and methanol extract using GC-MS.

Butanolic Extract	Area %	Methanolic Extract	Area %
Solancapsine	92	Beta-Sitosterol	60.5
Tomatodine	5.4	Alph-tocopherol	58.1
2-Methyl-6-tridecyl-6	1.5	Vitamin E	43
2-(4Hydroxy 3-methoxphenyl	0.6	Gamma-Sitosterol	38.7
4H-1- Benzoxpyran 4-01	0.5	Diosgenin	27.4
		Sarsapognin	6.3
		Solasodine	4.1

2.Cytotoxicity activity

S. schimperianum butanolic extract has a significant cytotoxicity effect on the both cell lines, MGF7 and HepG2, in concentrations range between 0.1 to 0.36 mg/ml and 0.08 to 0.25, respectivly.These results are very interesting- because such a small alkaloids concentration can kill the resistant invasive breast carcinoma cell.

Extract	MGF7 Cell Line		HepG2 Cell Line	
	IC 20	IC50	LC20	LC50
Butanol Extract	0.02	0.05	0.01	0.04
Methanol Extract	2.13	2.49	4.3	4.3
Standard	0.11	0.26	0.08	0.25

4.Antibacterial activity

Methanolic extract exhibited no activity against certain types of tested bacteria ,whereas the butanolic extract showed weak activity towards *S.aureus, E.faecalis* and *P. aeruginosa.*

Table 3:Atibacterial activities of butanolic and methanolic extracts.

Bacteria	L (me) 5mg\ml	L (me) 20mg\ml	L (bu) mg\ml	L(bu) 20mg\ml
S. aureus (+)	o	0	9 mm	14 mm
E. faecalis (+)	o	0	7 mm	10 mm
P. aeruginosa (-)	0	0	7 mm	10 mm
E. coli (-)	0	0	0	0

CONCLUSION

- In conclusion, the leaves of the plant contains potent steroidal glyco-alkaloids which could have beneficial biological effects as anticancer and antioxidant agents.

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Antibacterial, antiproliferative and antioxidant activity of leaf extracts of selected Solanaceae species

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abstract

Plants belonging to the Solanaceae family are generally used in Sudanese traditional medicine for the treatment of different ailments. This study aimed at the evaluation of in vitro antibacterial, antiproliferative and antioxidant activities of methanolic leaf extracts and steroidal glycoalkaloids fractions (SGAFs) of Solanum incanum L., S. schimperianum Hochst, S. nigrum L., Physalis lagascae Roem. & Schult. and Withania somnifera (L) Dunal. Methods: Antibacterial activity of the methanolic extracts and SGAFs was determined against two Grampositive and two Gram-negative bacteria by microdilution method. Anti-proliferative activity was determined against human cell lines (MCF7 and MDA-MB-231, HT29 and HCT116) by the thiazolyl blue tetrazolium bromide (MTT) procedure. Antioxidant activity was evaluated by diphenyl 2 pycril hydrazil (DPPH) and 2,2'azino-bis 3-ethylbenzthiazoline-6-sulphonic acid (ABTS) scavenging radical methods. Methanolic extract of S. schimperianum was analyzed using liquid chromatography coupled to an Orbitrap mass spectrometer with an electrospray ionization source (LC–MS).

Results: The sensitivity of Gram-positive and Gram-negative bacteria to each extract was variable (IC_{50} values in the range of 15–N1000 μg/mL). Only the methanolic extract of S. schimperianum leaf demonstrated interesting anti-proliferative activity against the human cell lines tested with IC_{50} values in the range of 2.69 to 19.83 μg/mL while the highest activity from the SGAFs was obtained from W. somnifera leaf with IC_{50} values in the range of 1.29 to 5.00 μg/mL. In both assays the SGAFs of all species demonstrated higher scavenging activity than their respective methanolic extracts. The SGAF of S. schimperianum displayed the strongest antioxidant activity in both assays with IC_{50} value $3.5 \pm 0.2_{DPPH}$ and $3.5 \pm 0.3_{ABTS}$ μg/mL. The correlation coefficient (R^2) between the antioxidant capacities and the total phenolic contents of the methanol extracts suggested that the phenolic compounds could not be the main contributor to the antioxidant capacities of leaves of these plants. Twelve known hydroxycinnamic acid amides (HCAAs) were tentatively identified from the methanolic extract of S. schimperianum leaf and N-caffeoyl agmatine appeared with the highest intensity. Moreover, the presence of steroid alkaloids was also detected and the presence of solanopubamine and solanocapsine as well as dehydro derivatives of the 3-amino steroid alkaloids was suggested.

1. Introduction

The Solanaceae family is one of the largest and most complex families of the Angiosperms. It includes 2500 species in 100 genera (Olmstead et al., 2008). Plants of this family exhibit a wide variety of secondary metabolites with different biological activities, which render them very important from economic, agricultural, and pharmaceutical point of view (Zadra et al., 2012; Oliveira et al., 2006). In Sudan, the family is represented in nature by 9 genera and 30 species (Andrews, 1956).

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Most of these species are known to possess diverse medicinal uses in the Sudanese traditional medicine. Roots of Solanum incanum L. are used as antiasthmatic and for the treatment of dysentery and snake bite (Elghazali, 1986). Fruits and leaves of S. nigrum L. are used to treat fever, diarrhoea, and eye diseases (Anonym, 1982). Leaves of S. schimperianum Hochst are used to treat wounds. Roots and leaves of Withania somnifera and Physalis lagascae Roem. & Schult. are used as tonic, diuretic and as poultice for swellings (Elghazali et al., 1987).

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Plants of Solanaceae family are known to biosynthesize secondary metabolites with interesting biological activity such as hydroxycinnamic acid amides (HCAAs), steroid alkaloids, polyphenols and glycoalkaloids, presumably to protect themselves from damage by phytopathogens Rahman and Choudhary, 1998; Macoy et al., 2015). At higher concentrations glycoalkaloids are toxic in living organism (Lenka and Pavel, 2016). In contrast, some glycoalkaloids can also have beneficial effects from both ecological and human aspects having multidiscipline pharmacological applications as antibacterial, antioxidant, anti-inflammatory and anticancer (Taveira et al., 2014). Investigations of native Sudanese Solanaceae species are mainly in botanical aspect and there were scanty data about their phytoconstituents and biological activity. In this sense, the objective of this study was to evaluate the possible antibacterial, antiproliferative and antioxidant activities of leaf extracts of S. incanum, S. schimperianum, S. nigrum, P. lagascae and W. somnifera as well as LC-ESI/MS profile of S. schimperianum.

2. Materials and methods

2.1. Plant materials

Leaves of the studied plant were collected from eastern Sudan, Erkowit region (longitude: 36° 59′ 57.6″ E; latitude: 18° 42′ 4.8″ N), in April 2012. Botanical identification and authentication were performed and voucher specimens (No. 2012/4SN for S. incanum, No. 2012/4SI for S. nigrum; No. 2012/4SS for S. schimperianum; No. 2012/4PL for P. lagascae and No. 2012/4WS for W. somnifera) have been deposited in Botany Department Herbarium, Faculty of Science, University of Khartoum, Sudan.

2.2. Preparation of plant extracts

2.2.1. Methanolic extract

Twenty grams of the dry powder from each plant material were macerated in methanol (200 mL), at room temperature for 72 h. The extracts were evaporated under vacuum to dryness to obtain 1.9 g (S. incanum), 4.3 g (S. schimperianum), 4.8 g (S. nigrum), 4.4 g (P. lagascae) and 4.1 g (W. somnifera).

2.2.2. Steroidal glycoalkaloids fractions (SGAFs)

Extraction of SGA was carried out according to the method described by Mohy-Ud-Din (2010). Twenty grams of each powdered plant material were extracted with 5% aqueous acetic acid at room temperature for 30 min. Samples were then vacuum filtered through a Whatman no 4 filter paper. The polar fraction was then basified with NH_4OH (pH = 11.0) and extracted with water saturated n-butanol. Finally the solvent was evaporated with a rotavapor to dryness to obtain 2.1 g (S. incanum), 2.5 g (S. schimperianum), 2.9 g (S. nigrum), 2.4 g (P. lagascae) and 2.0 g (W. somnifera).

2.3. Antibacterial activity assay

2.3.1. Microorganisms

Well-established methods were used. The microorganisms panel selected for study corresponds to some of the etnopharmacological uses. Standard strains of microorganism, obtained from Medicinal and Aromatic Institute of Research, National Research Center, Khartoum, were used in this study. The bacterial species used were the Gramnegative Escherichia coli (ATCC 25922) and Pseudomonas aeruginosa (ATCC27853), and Gram positive Bacillus subtilis (ATCC 6633) and Staphylococcus aureus (ATCC 25923).

2.3.2. Minimum inhibitory concentration (MIC) assay

The two-fold serial microdilution method described by Eloff (1998) was used to determine the MIC values for each extract against bacteria growth. All dilutions were prepared under aseptic conditions. A volume of 100 µL of the extracts (1 mg/mL) dissolved in DMSO (5%, v/v) in duplicate was serially diluted two-fold with sterile distilled water and 100 µL of bacterial culture in MH Broth, corresponding to 10^6 CFU/mL, was added to each well. Gentamicin and amoxicillin were used as positive controls and DMSO as negative control. Plates were incubated overnight at 37 °C. Afterwards, 40 µL of 0.2 mg/mL of p-iodonitrotetrazolium violet (INT) was added to each well to indicate microbial growth. The colorless salt of tetrazolium acts as an electron acceptor and is reduced to a red colored formazan product by biologically active organisms. The solution in wells remains clear or shows a marked decrease in intensity of color after incubation with INT at the concentration where bacterial growth is inhibited. Plates were further incubated at 37 °C for 2 h and the MIC was determined as the lowest concentration inhibiting microbial growth, indicated by a decrease in the intensity of the red color of the formazan. The experiment was performed in triplicate.

2.4. Cell viability assay

2.4.1. Cell culture

Anti-proliferative activities of each extract were evaluated with four cell lines established from human breast carcinoma samples (MCF7 and MDA-MB-231) and from human colon adenocarcinoma samples (HT29 and HCT116). HCT116 and HT29 cells were cultivated in Dulbecco's minimum essential medium (DMEM, Eurobio, Courtaboeuf, France) supplemented with 10% (v/v) fetal calf serum (Eurobio), 1% Penicillin/ streptomycin (Eurobio) and 2 mM L-glutamine (Eurobio). MCF7 and MDA-MB-231 cells were grown in RPMI medium with the same additives. Cells were routinely seeded at 100,000 cells/mL and maintained weekly in a humidified atmosphere of 5% CO_2 at 37 °C.

2.4.2. MTT procedure

Cell viability assay was performed using the thiazolyl blue tetrazolium bromide (MTT) procedure as described by Mosman (1983). In brief, cancer cells were seeded in 96-well plate at 10,000 cells/well for HT29, MCF-7 and MDA-MB231 cells, at 5000 cells/well for HCT116 cells (Greiner-Bio-One GmbH, Friekenhanusen, Germany). Twenty four hours after seeding, 100 µL of medium containing increasing concentrations of each extract (final concentration range from 0.5 to 400.0 µg/mL) were added to each well for 72 h at 37 °C. Dried extracts were firstly diluted with DMSO to a final concentration at 50

mg (w/v)/mL or 200 mg (w/v)/mL. After incubation, the medium was discarded and 100 µL/well of MTT solution (0.5 mg/mL diluted in DMEM or RPMI medium) were added and incubated for 2 h. Water-insoluble formazan blue crystals were finally dissolved in DMSO. Each plate was read at 570 nm. IC_{50} was calculated using GraphPad Prism (GraphPad Software, La Jolla, CA, USA). Data are expressed as IC_{50} ± SD obtained from quadruplicate determinations of two independent experiments (n = 8). As a control, we tested usually fenofibrate, a member of fibrate family, on HT29 and HCT116 cells, in order to confirm a moderate effect on HT29 cell viability (IC_{50} = 28.3 ± 0.9 µM) whereas IC_{50} is N50 µM when HCT116 cells are used.

2.5. Antioxidant activity studies

2.5.1. DPPH radical-scavenging test

Antioxidant activity of extracts was estimated using in vitro 2,2diphenyl-1-picrylhydrazyl (DPPH) scavenging radical method (Yagi et al., 2013). Test samples were dissolved separately in methanol to get test solution of 1 mg/mL. Series of extract solutions of different concentrations (1, 5, 10, 20, 40, 60, 80 and 100 µg/mL) were prepared by diluting with methanol. Assays were performed in 96-well, microtiter plates. 140 µL of $0.6.10^{-6}$ mol/L DPPH were added to each well containing 70 µL of sample. The mixture was shaken gently and left to stand for 30 min in dark at room temperature. The absorbance was measured spectrophotometrically at 517 nm using a microtiter plate reader (Synergy HT Biotek®, logiciel GEN5). Blank was done in the same way using methanol and sample without DPPH and control was done in the same way but using DPPH and methanol without sample. Ascorbic acid was used as reference antioxidant compound. Every analysis was done in triplicate.

The ability to scavenge DPPH radical was calculated by the following equation:

DPPH radical scavenging activityð%Þ ¼

1-Abs_{sample}–Abs_{blank}=ð$Abs_{control}$Þ 100:

Where;

Abs_{sample} is the absorbance of DPPH radical þ sample;

Abs_{blank} is the absorbance of sample þ methanol;

$Abs_{control}$ is the absorbance of DPPH radical þ methanol:

The IC_{50} value was calculated from the linear regression of plots of concentration of the test sample against the mean percentage of the antioxidant activity. Results were expressed as mean ± SEM and the IC_{50} values obtained from the regression plots (Sigma PlotsR 2001, SPSS Science) had a good coefficient of correlation, (R^2 = 0.998).

2.5.2. ABTS radical-scavenging test

A second in vitro method was performed to estimate antioxidant potential of the extracts: 2,2′-azino-bis 3-ethylbenzthiazoline-6-sulphonic acid (ABTS) scavenging radical assay, based on the method of Re et al. (1999). Test samples were dissolved separately in methanol to get test solution of 1 mg/mL. Series of extract solutions of different concentrations (1, 5, 10, 20, 40, 60, 80 and 100 µg/mL) were prepared by diluting with methanol. The ABTS radical cation ($ABTS^{*+}$) was produced by reacting 7 mM stock solution of ABTS with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12 h before use. The obtained ABTS radical solution was diluted with methanol to an absorbance of 0.700 ± 0.02 at 734 nm. 190 µL of ABTS radical solution were added to each well containing 10 µL of sample. The mixture was shaken gently and left to stand for 15 min in dark at room temperature. The absorbance was measured spectrophotometrically at 734 nm using a microtiter plate reader (Synergy HT Biotek®, logiciel GEN5). The $ABTS^{*+}$ scavenging capacity of the extract was compared with that of ascorbic acid and the percentage inhibition calculated as:

ABTS radical scavenging activity ð%Þ

¼ $Abs_{control}$–Abs_{sample}=ð$Abs_{control}$Þ 100:

Where;

$Abs_{control}$ is the absorbance of ABTS radical ð¼ 0:700

0:02Þ; Abs_{sample} is the absorbance of sample þ ABTS

radical:

The IC_{50} value was calculated from the linear regression of plots of concentration of the test sample against the mean percentage of the antioxidant activity obtained from triplicate assays. Results were expressed as mean ± SEM and the IC_{50} values obtained from the regression plots (Sigma PlotsR 2001, SPSS Science) had a good coefficient of correlation, (R^2 = 0.9926).

2.6. Determination of total phenol

Total phenols contents in the methanol extract were recorded using modified Folin Ciocalteu method (Wolfe et al., 2003). An aliquot of the extract was mixed with 5 mL Folin Ciocalteu reagent (previously diluted with water at 1:10 v/v) and 4 mL (75 g/L) of sodium carbonate. The tubes were vortexes for 15 s and allowed to stand for 30 min at 40 °C for color development. Absorbance was then measured at 765 nm (Shimadzu UV-2550 UV–VS spectrophotometer). Sample extracts were evaluated at a final concentration of 0.1 mg/mL. Total phenolic contents were expressed as gallic acid equivalents in milligram per gram sample (mg/g).

2.7. LC–MS analyses

Mass analyses of the extract were carried out on a Q Exactive Plus mass spectrometer (ThermoFisher Scientific) equipped with a heated electrospray ionization (HESI-II) probe (ThermoScientific). The instrument parameters were as follows: spray voltage 3.5 kV, sheath gas flow rate 36, auxiliary gas flow rate 11, spare gas flow rate 1, capillary temperature 320 °C, probe heater temperature 320 °C, and S-lens RF level 50. All mass spectrometry parameters were optimized for sensitivity to the target analytes using the instrument control software program. Acquisition was acquired at Full-scan with DD-MS^2 and PRM modes. Full-scan mass measurements were carried in positive mode with resolution of 70,000, AGC target at 1e6, maximum ion time (IT) at 50 ms and a scan range of 100–1000 m/z. For the DD-MS^2 analysis, the following parameters were used: microscans 1, resolution 17,500, ACG target at 1e5, maximum IT of 30 ms, loop count 5, MSX count 1, isolation window 2 m/z, utilizing a stepped NCE at 10, 30 and 60.

The targeted acquisition of the 12 HCAAs were carried out on parallel reaction monitoring (PRM) mode, with the following instrument settings: microscans at 1, resolution at 35,000, AGC target at 5e5, maximum ion time at 100 ms, MSX count at 1, isolation window at 1.0 m/z, normalized collision energy (NCE) at 20. Data acquisition and processing were carried out with Xcalibur 3.0 software (ThermoScientific).

3. Results and discussion

3.1. Antibacterial activity

Methanolic extracts and SGAFs of S. incanum, S. schimperianum, S. nigrum, P. lagascae and W. somnifera leaves were evaluated for their in vitro antibacterial activity against Gram positive bacteria; S. aureus and B. subtilis, and Gram negative bacteria; E. coli, and P. aeruginosa and results are presented in Table 1.

The sensitivity of tested Gram-positive and Gram-negative bacteria to different extracts was variable. The antibacterial activity of the

Table 1
Antibacterial activity of leaf extracts of five Solanaceae plants.

Plant species	B. subtilis	S. aureus	E. coli	P. aeruginosa
	Minimum inhibitory concentration (μg/mL) Methanol extract			
S. incanum	250	15	15	N1000
S. schimperianum	126	N1000	N1000	N1000
S. nigrum	N1000	500	N1000	63
P. lagascae	N1000	N1000	32	N1000

W. somnifera	N1000	N1000	N1000	15
Steroidal glycoalkaloids fraction (SGAF)				
S. incanum	32	126	32	250
S. schimperianum	32	N1000	N1000	N1000
S. nigrum	N1000	15	15	63
P. lagascae	500	15	63	31
W. somnifera	N1000	15	15	15
Amoxicillin	4	4	–	–
Gentamicin	–	–	4	4

SGAFs of S. incanum, S. schimperianum and P. lagascae against B. subtilis exceeded the effect of their corresponding methanolic extract by 7.8fold, 3.9-fold and N2-fold respectively while that of S. nigrum, P. lagascae and W. somnifera SGAFs against S. aureus was higher by 33.3-fold for the first and N66.7-fold for the two last than their respective methanolic extracts. SGAFs of S. nigrum and W. somnifera against E. coli increased by N66.7-fold and S. incanum and P. lagascae against P. aeruginosa increased by N4-fold and N32.3-fold than their respective methanolic extracts. In contrary, the antibacterial activity of SGAF of S. incanum against S. aureus and E. coli reduced by 8.4-fold and 2.1-fold respectively compared to their respective methanolic extracts. The same observation was obtained for P. lagascae (2-fold) against E. coli. However, the antibacterial activity of S. schimperianum against S. aureus, E. coli and P. aeruginosa was comparable in the two types of extracts, the same was true for the antibacterial activity of S. nigrum and W. somnifera against P. aeruginosa. These results supported the previously reported antibacterial activity for crude extracts of S. nigrum (Jimoh et al., 2010), S. incanum (Britto and Senthinkumar, 2001; Taye et al., 2011), S. schimperianum (Al-Oqaila et al., 2012) and W. somnifera (Alam et al., 2012).

3.2. Anti-proliferative activity

Methanolic extracts and SGAFs of S. incanum, S. schimperianum, S. nigrum, P. lagascae and W. somnifera leaf were tested, in vitro, for their potential anti-proliferative activity against HT29, HCT116, MCF7 and MDA-MB231 cell lines (Table 2). Results of the methanolic extracts showed that only S. schimperianum leaf demonstrated interesting antiproliferative activity against the four cell lines with IC_{50} values in the range of 2.69 to 19.83 μg/mL. The highest anti-proliferative activity was obtained against HT29 (2.69 ± 0.56 μg/mL) followed by HCT116 (5.70 ± 0.51 μg/mL), MDA-MB231 (7.01 ± 0.25 μg/mL) and MCF7 (19.83 ± 3.83 μg/mL) respectively. However, all other methanolic extracts showed anti-proliferative activity against the four cell lines with IC_{50} values N 50 μg/mL.

The anti-proliferative activity of SGAFs was variable. Potent antiproliferative activity was observed for the SGAF of W. somnifera leaf where the highest activity was obtained against HCT116 (1.29 ± 0.06 μg/mL) followed by MCF7 (2.34 ± 0.68 μg/mL), HT29 (4.23 ± 0.25 μg/mL) and MDA-MB231 (5.00 ± 0.75 μg/mL) respectively. Ichikawa et al. (2006) reported that, withanolides (bioactive compounds of W. somnifera) inhibit cyclooxygenase enzymes, lipid peroxidation, and proliferation of tumor cells through the suppression of nuclear factor-κB (NF-κB) and NF-κB-regulated gene products. P. lagascae leaf SGAF displayed the second highest anti-proliferative activity where the highest anti-proliferative activity was obtained against HCT116 (4.79 ± 0.77 μg/mL) followed by MCF7 (5.09 ± 0.24 μg/mL), MDA-MB231 (6.90 ± 0.30 μg/mL) and HT29 (7.45 ± 0.59 μg/mL)

Table 2
Anti-proliferative activity of leaf extracts of five Solanaceae plants.

Plant species	HT29	HCT116	MCF7	MDA-MB231
	Methanol extract			
S. incanum	N50	N50	N50	N50
S. schimperianum	2.69 ± 0.56	5.70 ± 0.51	19.83 ± 3.83	7.01 ± 0.25
S. nigrum	N50	N50	N50	N50
P. lagascae	N50	N50	N50	N50
W. somnifera	N50	N50	N50	N50
Steroidal glycoalkaloids fraction (SGAF)				
S. incanum	N50	N50	N50	N50
S. schimperianum	9.13 ± 0.67	13.39 ± 0.61	23.21 ± 3.08	17.70 ± 2.00
S. nigrum	N50	N50	N50	N50
P. lagascae	7.45 ± 0.59	4.79 ± 0.77	5.09 ± 0.24	6.90 ± 0.30
W. somnifera	4.23 ± 0.25	1.29 ± 0.06	2.34 ± 0.68	5.00 ± 0.75

Table 3
IC50 values of DPPH and ABTS free radical scavenging activity of leaf extracts of five Solanaceae species.

Plant species	DPPH(IC50 μg/mL)		ABTS (IC50 μg/mL)	
	Methanol extract	SGAF	Methanol extract	SGAF

S. incanum	177.9 ± 5	160.4 ± 7	616.4 ± 4	45.7 ± 2
S. schimperianum	156.1 ± 3	3.5 ± 0.2	521.3 ± 3	3.5 ± 0.3
S. nigrum	179.1 ± 1	111.9 ± 3	250 ± 3	144.9 ± 2
P. lagascae	199.0 ± 1	81.9 ± 3	782.3 ± 5	95 ± 3
W. somnifera	168.9 ± 7	87.1 ± 0.5	1674.1 ± 8	49.3 ± 1
Ascorbic acid	1.5 ± 0.1		1.2 ± 0.2	

respectively. This is the first time to illustrate the antiproliferative activity of P. lagascae, previous study on P. crassifolia demonstrated its potent and selective cytotoxicity against prostate cancer cells (Ya-ming et al., 2016). In contrast, to the methanolic extract, the SGAF of S. schimperianum leaf exhibited anti-proliferative activity with IC_{50} values (9.13–23.21 μg/mL) higher than those obtained for the methanolic extract (2.69–19.83 μg/mL). S. incanum and S. incanum leaves SGAFs were less active showing anti-proliferative activity against the four cell lines with IC_{50} values N 50 μg/mL. Ding et al. (2013) found that SGAs exhibited antitumor activity and induced apoptosis on human gastric cancer MGC-803 cells. They further stated that the number and type of sugar and the substitution of a hydroxyl on steroidal alkaloid backbone play an important role in the anti-proliferative activity.

Table 5
Detected HCAAs in leaf extract of Solanum schimperianum.

No	Name	tR (min)	Elemental composition	[M + H]	MS^2
1.	N-caffeoyl putrescine	2.13, 3.39	$C_{13}H_{18}N_2O_3$	251.13901	251.14, 234.11, 163.04, 115.09, 89.11, 72.08
2.	N-feruloyl putrescine	4.33, 5.74	$C_{14}H_{20}N_2O_3$	265.15466	260.14, 177.05, 145.03, 115.09, 89.11, 72.08
3.	N-coumaroyl agmatine	4.99, 6.72	$C_{14}H_{20}N_4O_2$	277.1659	260.14, 147.04, 131.13, 114.10, 89.11, 72.08
4.	N-coumaroyl tyramine	10.13, 10.53	$C_{17}H_{17}NO_3$	284.12811	164.07, 147.04, 121.065
5.	N-caffeoyl agmatine	3.57, 5.38	$C_{14}H_{20}N_4O_3$	293.16081	276.13, 163.04, 131.13, 114.10, 89.11, 72.08
6.	N-sinapoyl putrescine	5.09	$C_{15}H_{22}N_2O_4$	295.16523	278.14, 207.065, 175.04, 115.09, 89.11, 72.08
7.	N-caffeoyl tyramine	9.77	$C_{17}H_{17}NO_4$	300.12303	163.04, 147.04, 138.09, 121.065
8.	N-feruloyl agmatine	5.88, 7.76	$C_{15}H_{22}N_4O_3$	307.17647	290.15, 177.05, 157.11, 131.13, 114.10
9.	N-feruloyl tyramine	10.40, 10.82	$C_{18}H_{19}NO_4$	314.13868	194.08, 177.05, 164.07, 145.03, 121.065
10.	N^{ε}-feruloyl-lysine	5.04, 6.46	$C_{16}H_{22}N_2O_5$	323.16015	177.05, 147.11, 145.03, 84.08
11.	N-sinapoyl agmatine	6.18, 8.56	$C_{16}H_{24}N_4O_4$	337.18703	320.16, 207.065, 131.13, 114.10
12.	N-feruloyl-3-methoxytyramine	10.63, 11.09	$C_{19}H_{21}NO_5$	344.14924	194.08, 177.05, 151.07, 145.03

3.3. Antioxidant activity

The methanolic extracts and SGAFs of S. incanum, S. schimperianum, S. nigrum, P. lagascae and W. somnifera leaves were evaluated for their in vitro antioxidant activity using DPPH and ATBS assays and results are presented in Table 3. Generally, it was clear that the SGAFs of all species demonstrated higher scavenging activities than their corresponding methanolic extracts and the scavenging of the ABTS radical was found to be generally different than that of DPPH radical. The SGAFs increased the DPPH and ABTS scavenging capacity respectively by 1.1and 13.5-fold for S. incanum, 1.6- and 1.7-fold for S. nigrum, 2.4- and 8.2-fold for P. lagascae and 1.9- and 34-fold for W. somnifera comparatively with the methanolic extracts. Furthermore, S. schimperianum displayed the strongest antioxidant activity in both assays where a sharp increase by 45- and 140-fold was observed for SGAFs. Several studies have reported variations in the biological activities of extracts prepared using different extraction techniques (Dhanani et al., 2017). Zheng and Wang (2001) and Jimoh et al. (2010) reported that factors like stereoselectivity of the radicals or the solubility of the extracts in different testing systems affect the capacity of extracts to react and quench different radicals. Wang et al. (1998) found that some compounds which have ABTS scavenging activity did not show DPPH scavenging activity.

3.4. Total phenolic content of leaf methanolic extracts.

The total polyphenolic contents were expressed as mg gallic acid equivalent (GAE)/g of dry material and results are listed in Table 4.

Table 4
Total polyphenolic content of leaf methanolic extracts of five Solanaceae species.

Plant species	Concentration (mg GAE/100 g)
S. incanum	0.20 ± 0.04
S. schimperianum	0.40 ± 0.04
S. nigrum	0.26 ± 0.02
P. lagascae	0.48 ± 0.04
W. somnifera	0.40 ± 0.03

The amount of total phenolics, varied in the studied species and ranged from 0.20 to 0.48 mg GAE/g. P. lagascae had the highest polyphenolic content followed by S. schimperianum and W. somnifera (0.40 mg GAE/g) while S. nigrum (0.26 mg GAE/g) and S. incanum (0.20 mg GAE/g) had the lowest content.

Previous studies demonstrated that the antioxidant activity of S. incanum leaf (Konaté et al., 2011), S. nigrum leaf (Jimoh et al., 2010; Loganayaki et al., 2010) and W. somnifera leaf (Fernando et al., 2013) was mainly attributed to the phenolic content of extracts. In this study, the correlation coefficient (R^2) between the antioxidant capacities and the total phenols of the methanol extracts was also determined using Pearson's correlation coefficients. The R^2 between the antioxidant capacities obtained from DPPH and ABTS assays were 0.3727 and 0.1757 respectively. Thus, it was clear that the antioxidant capacity of these plants did not correlate with their phenolic content, which suggested that the phenolic compounds could not be the main contributors to the antioxidant capacities of leaves of these plants.

3.5. LC–MS analyses

Based on the results obtained from antiproliferative assay, leaf methanolic extract of S. schimperianum was subjected to LC–MS analyses. Twelve known hydroxycinnamic acid amides (HCAAs) were detected and N-caffeoyl agmatine appeared with the highest intensity (Table 5, Fig. 1). Furthermore, the positive HR-ESI-MS Full-scan mass spectrum revealed the presence of several isobaric, high intensity quasi-

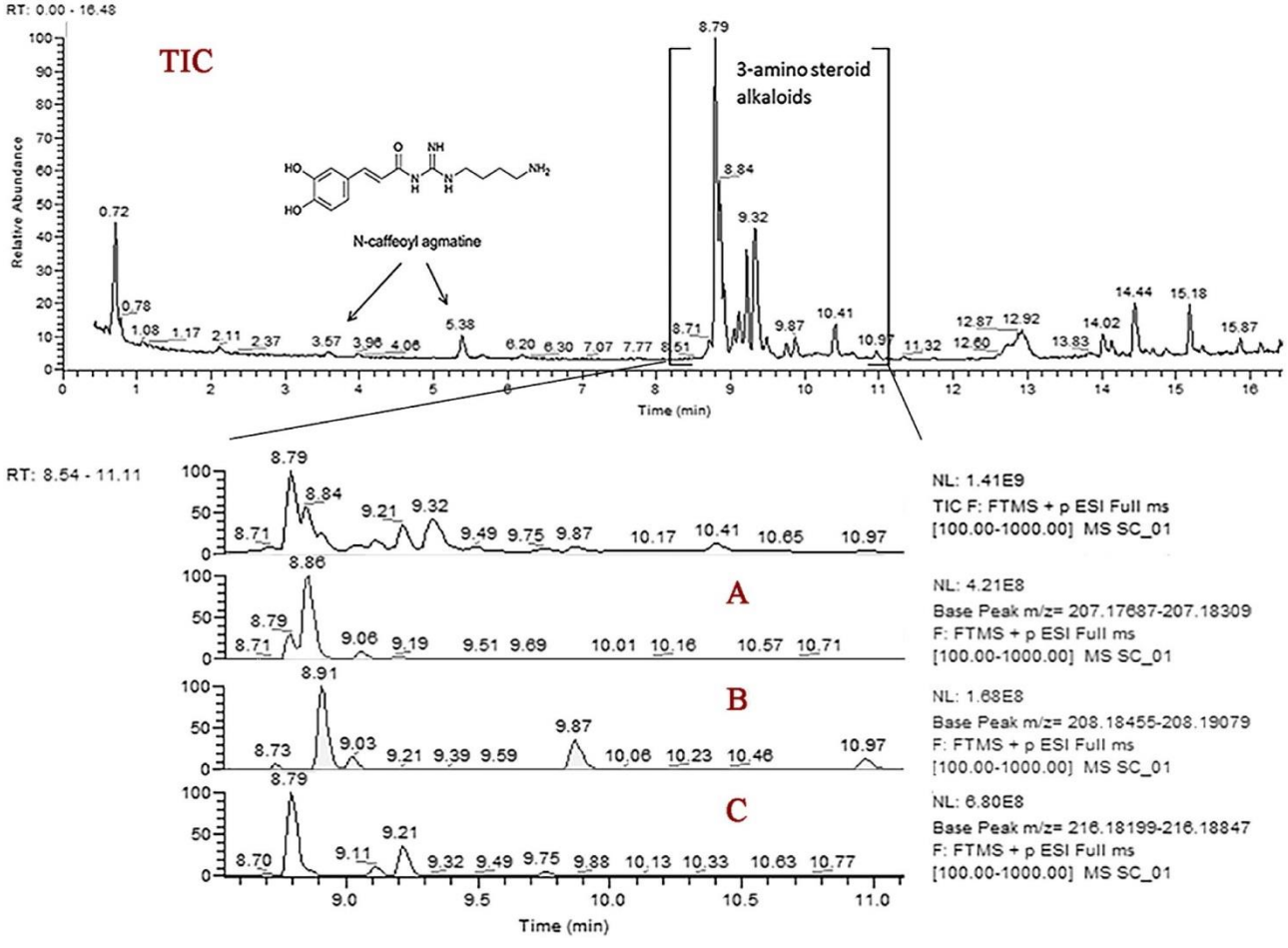
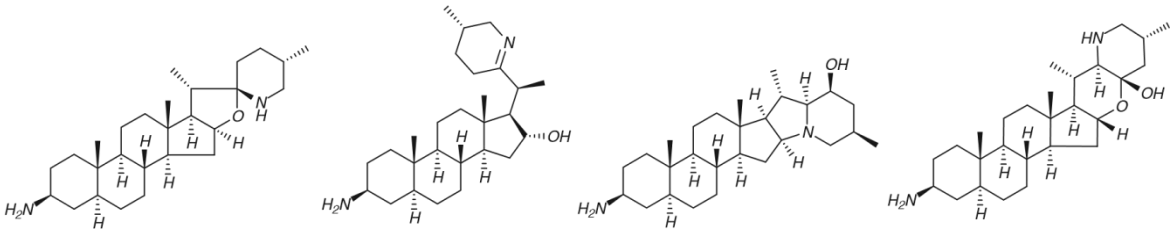


Fig. 1. Total ion chromatogram (TIC) and extracted ion chromatograms for steroid alkaloids. (A) Extracted ion chromatogram for base peak at m/z 207. (B) Extracted ion chromatogram for base peak at m/z 208. (C) Extracted ion chromatogram for base peak with m/z 216.



Soladunalinidine Solacallinidine Solanopubamine Solanocapsine

Fig. 2. Some 3-amino steroid alkaloids found in Solanaceae.

molecular ions at m/z 207.17998, 208.18777, and 216.18523, appearing predominantly as doubly charged $[M + 2H]^{2+}$ ions (Fig. 1). The mass errors between the theoretically calculated and measured masses ranged from 0.50 to 5.00 ppm. Based on high-resolution accurate mass measurements and analysis of their isotopic patterns, the elemental composition of the three abovementioned ions were determined as $C_{27}H_{44}ON_2$, $C_{27}H_{46}ON_2$ and $C_{27}H_{46}O_2N_2$, respectively. In the MS^2 of all three $[M + H]^+$ parent ions at m/z 413.35229, 415.36771 and 431.36389, a subsequent loss of ammonia ($[M + H\text{-}NH_3]^+$) and water ($[M + H\text{-}(H_2O + NH_3)]^+$) was observed. The elemental composition, together with the MS^2 data permitted us to assign the abovementioned chemical compositions to 3-amino steroid alkaloids (Fig. 2).

There have been several reports in the literature describing isolated 3-amino steroid alkaloids from the genus Solanum, having an elemental composition of $C_{27}H_{46}ON_2$, as Solacallinidine (Bird et al., 1979a) and Soladunalinidine (Bird et al., 1979b) (Fig. 2). Moreover, another isobaric compound, the 3β-amino steroid alkaloid Solanopubamine, was recently isolated by Al-Rehaily et al. (2013) in high quantity from the aerial parts of S. schimperianum. This data allowed us to tentatively assign one of the discovered ions having an elemental composition $C_{27}H_{46}ON_2$ as Solanopubamine (Fig. 2). The ions with elemental composition $C_{27}H_{44}ON_2$ can be putatively ascribed as dehydro derivatives of the 3-amino steroid alkaloids mentioned above. The ions having elemental composition $C_{27}H_{46}O_2N_2$ can be assigned tentatively as Solanocapsine, found previously in S. pseudocapsicum (Aliero et al., 2006).

A variety of HCAAs has been found throughout the genus Solanum and was found to represent the main phenylpropanoid constituents in 12 Solanum species (Sun et al., 2015a, b). However, the HCAAs reported in this study were also detected previously from the roots where, N^{ε}-feruloyllysine, as well as HCAAs of agmatine, cadaverine and sinapoyl putrescine were reported in genus Solanum for the first time (Voynikov et al., 2016). HCAAs have been reported to possess good activity against wide range of microbial pathogens (Guzman, 2014). Feruloyl dopamine, feruloyl tyramine and feruloyl tryptamine were found effective against S. aureus 209 and S. pyogenes with MIC values between 190 and 372 µM (Georgiev et al., 2012). N^{ε}-feruloyl lysine exhibited MIC of 349 µM against S. aureus 3359 and ATCC 6538 P (Voynikov et al., 2016). Solanopubamine was found to exhibit good antifungal activity against Candida albicans and C. tenuis with MIC of 12.5 µg/mL (AlRehaily et al., 2013).

However, the steroidal alkaloid detected in this study might not be the main contributor to the antiproliferative activity of this species. Previous study showed that solanopubamine was found inactive against several cancer (SK-MEL, KB, BT-549, SK-OV-3, HL-60) cell lines (Al-Rehaily et al., 2013). A derivative of solanocapsine, Omethylsolanocapsine, isolated from S. pseudocapsicum leaf was found to possess cytotoxic properties against HeLa cell lines, with IC_{50} values of 39.90 ± 0.03 and 34.65 ± 0.06 by MTT and SRB assays, respectively (Dongre et al., 2007). On the other hand, several data in the literature are reporting the antiproliferative activity and mechanism of action of hydroxycinnamic acids and derivatives against several cancer cell lines (Rocha et al., 2012; Eun-Ok et al., 2014; Tavares-da-Silva et al., 2016).

4. Conclusion

Generally, the antibacterial and free radical scavenging activities of SGAFs of leaf of investigated Solanaceae species was higher than those obtained in their corresponding methanolic extracts. SGAFs of leaf of S. schimperianum displayed the strongest antioxidant activity in both assays. Based on the antiproliferative activity of the fractions, further investigation as for S. schimperianum was warranted. HCAAs and steroid alkaloids were identified by LC–MS in methanolic extract of S. schimperianum. Thus, the results of this study suggested that Solanaceae plants from Sudan could be a good natural source of chemopreventive and/or chemotherapeutic agents.

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