بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Basic and Applied Studies on the Chemical Conistituents and Bioactivity of Argel (Solenostemma argel Del Hayne).

دراسات أساسية وتطبيقية على المكونات الكيميائية والفعالية الحيوية للحرجل

A Thesis Submitted in Fulfillment of the Requirements

for the Degree of Ph.D. in Chemistry.

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استهـــــلال

قال تعالى:

﴿ سَنُرِيهِمْ آيَاتِنَا فِي الآفَاقِ وَفِي أَنفُسِهِمْ حَتَّى يَتَبَيَّنَ لَهُمْ أَنَّهُ الْحَقُّ

أَوَلَمْ يَكْفِ بِرَبِّكَ أَنَّهُ عَلَى كُلِّ شَيْءٍ شَهِيدٌ ﴾

(سورة: فصلت - الآية: ٥٣)

Dedication

To my

Parents,

Husband, Son and Daughters,

Brothers and Sisters.

Acknowledgement

I would like to express my deepest appreciation and thanks to my supervisor Professor Mohammed El.mukhtar, for encouraging and guiding me to accomplish my research. Special thanks to my co-supervisors Professor Ahmed Alsadiq and Professor Taj-Alsir Ibraheem for their comments and suggestions.

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Abstract

At the present work extraction, fractionation, phytochemical screening and biological activity of the extract for the stems of *Solenostemma argel* (*S. argel*) were studied. The results showed that *S. argel* contains medicinal species such as phytosterols, triterpenoids, alkaloids, flavonoids, and saponins. Chemical research of the stems showed the presence of some minerals, micro element: Fe (1.055%), Zn (0.052%), Mn (0.059%), Ca (21.10%), Mg (29.95%), and Cu (0.014%), and macro elements: N (1.96%), P(0.48%), S (0.050%) and K (0.816%), by using atomic absorption spectrophotometer (AAS) to determine heavy metal concentrations.

The aqueous extract of the stem of *S. argel* and its other organic fractions were tested against the activity of third larvae of *Tribolium castaneum*. Among these, the chloroform extract ($CHCl_3$) showed high activity. Its activity was investigated further, using the diffusion method, against the growth of four types of bacteria (two Gram-positive and two Gram-negative), and two types of fungi. These experiments proved that the $CHCl_3$ extract of *S. argel* had a high antimicrobial activity.

Fractionation of the $CHCl_3$ extract was done by column chromatography (CC), thin layer chromatography (TLC), and further purification of semi-pure compounds was achieved using preparative thin-layer chromatography (PTLC) to obtain pure compounds. Isolated compounds were characterized using nuclear magnetic resonance (^{1}H- NMR, ^{13}C-NMR, HMBC combined with HSQC NMR), mass spectroscopy (MS) and infrared (IR) for structure elucidation. From the structure elucidation, it was obvious that the two compounds were phytosterols, which were identified as novel compounds, named stigma sterol glucoside and beta sitosterol glucoside, and another compound was triterpenoids that was identified as novel compound, named beta amyrin.

المستخلص

في هذا العمل تمت دراسة الاستخلاص والتجزئة والمسح الفايتوكيميائي واختبار الفعالية الحيوية لمستخلص سيقان نبات الحرجل. أوضحت النتائج ان نبات الحرجل يحتوي على مختلف المركبات الطبية مثل الفيتوستيرولات، الترايتربنويدات، الالكلويدات، الفلافونويدات والصابونينات. أظهر البحث الكيميائي للسيقان وجود بعض المعادن، عناصر كبرى مثل الحديد (1,055%)، الزنك (0,052%)، المنجنيز (0,059%)، الكالسيوم (21,10%)، الماغنيزيوم (29,95%) والنحاس (0,014%)، وعناصر صغرى مثل النيتروجين (1,96%)، الفسفور (0,48%)، الكبريت (0,050%) والبوتاسيوم (0,816%). ولقد تم تعيين تراكيز الفلزات الثقيلة بالمضوائية الطيفية الامتصاصية للذرات.

أجريت تجارب الفعالية لجزء المستخلص المائي وبقية الاجزاء العضوية المستخلصة من سيقان الحرجل ضد نشاط اليرقة الثالثة لحشرة التريبوليوم كاستانيوم ومن بين هذه المستخلصات اظهر الكلوروفورم فعالية حيوية عالية. فحصت فعاليته اكتر باستخدام طريقة الانتشار ضد أربعة أنواع من البكتريا (نوعين موجبة الجرام ونوعين سالبة الجرام) ونوعين من الفطريات. ولقد اثبتت هذه التجارب أيضا ان مستخلص الكلوروفورم احتوى على فعالية عالية ضد نشاط الميكروبات.

تم تجزئة مستخلص الكلوروفورم بواسطة كروماتوجرافيا العمود، كروماتوغرافيا الطبقة الرقيقة، وتم تحقيق مزيد من التنقية للمركبات شبه النقية باستخدام كروماتوجرافيا الطبقة الرقيقة التحضيرية للحصول على مركبات نقية. وقد تم تشخيص المركبات المعزولة باستخدام الرنين المغناطيسي النووي احادي وثنائي الابعاد، مطيافية الكتلة والاشعة تحت الحمراء للتعرف على التركيب البنائي. من التركيب البنائي تم التوصل الى مركبين من الفيتوستيرول، والتي تم تحديدها كمركبات جديدة، هما ستيقما ستيرول جلوكوزيد وبيتا سيتوستيرول جلوكوزيد، ومركب آخر من الترايتيربينويدات الذي تم تحديده كمركب جديد هو بيتا أميرين.

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List of Abbreviations

B. s.	*Bacillus subtilis.*
E. c.	*Escherichia coli*.
P. s.	*Pseudomonas aeruginosa*.
S. a.	*Staphylococcus aureus.*
A. n.	*Aspergillus niger*
C.a	*Candida albicans*
^{1}HNMR	Proton Nuclear Magnetic Resonance
^{13}CNMR	C-13- Nuclear Magnetic Resonance
COSY	Correlated Spectroscopy
DMSO-d_6	Deuterated Dimethyl Sulfoxide
DEPT	Distortion less Enhancement by Polarization Transfer
DMSO	Dimethyl Sulfoxide
HSQC	Hetero nuclear Single Quantum Correlation
HMBC	Hetero nuclear Multiple Bond Correlation
LC-MS	Liquid Chromatography- Mass Spectrometry
λ_{max}	Maximum Fluorescence emission wavelength
$[M]^+$	Molecular ion
Rf	Mobility Relative to Front
PTLC	Preparative Thin Layer Chromatography
TLC	Thin Layer Chromatography
S. argel	*Solenostemma argel*

Chapter One

Introduction and Literature Review

Chapter One

Introduction and Literature Review

1.1 Introduction

Man's dependence on plants for the basics of his existence has been of paramount importance in his life since the beginning of the human race. Although different natural products contribute to his well-being, the plant kingdom is most important to human well-being, the increasing use of plant materials as a way to improve health and to heal specific elements is known as herbs. In general, plants used as medicinal plants or herbs. Recently, researchers in many countries and numerous publications have a growing interest in developing the cultivation of medicinal plants.

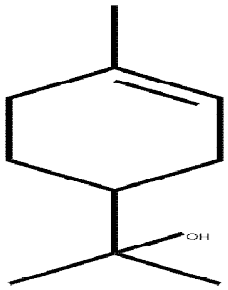
In green medicines, in all the available literature, medicinal plants are defined as plants that act as sources of various types of chemical compounds with complex structures that have the potential to treat many diseases, while the botanical definition of a herb is a plant whose stem is not woody and tenacious. The meaning of the word herb is open to controversy by botanists and herbalists (AOAC, 1990).

1.1.1 Natural products classification

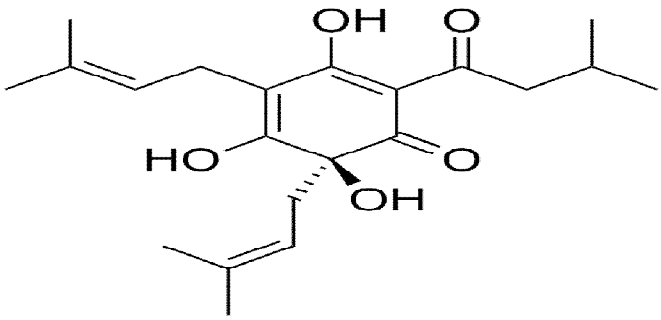
It is based on the type of chemical skeleton they are aliphatic or non-aliphatic fatty compounds of open chain as fatty acids, sugars and great amount of amino acid**.** Acyclic and cyclo aliphatic compound as terpenoids, steroids and some alkaloids**.** Aromatic or benzoic compounds as such phenols, quinines, *etc.* Heterocyclic compounds such as alkaloids, flavonoids and nucleic acid bases (Kamel *et al.*, 2000).

1.1.1.1 Terpenoids:

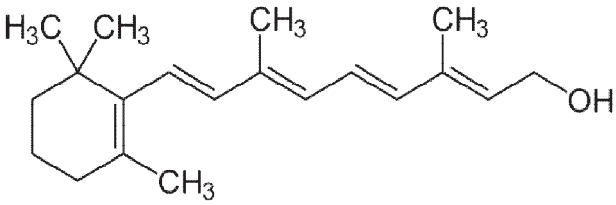
Practically all terpenoids are more or less based upon the specific 'Isoprene Molecule' (Hassan *et al., 2008*), were divided into several types:



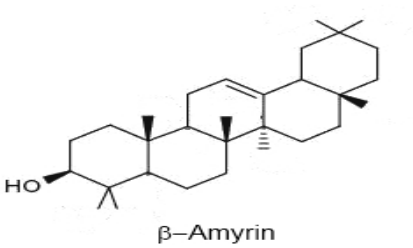
Mono terpenoids: two isoprene units [C10H16]



Sesqui terpenoids: three isoprene units [C15H24]

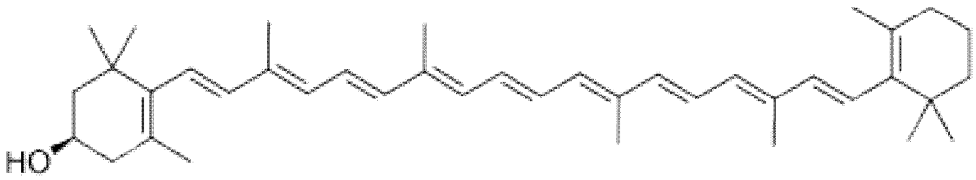


Di terpenoid: four isoprene units [C20H32]



β–Amyrin

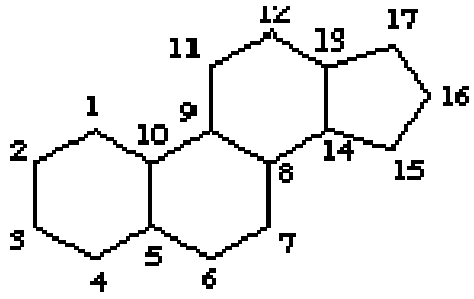
Triterpenes are a class of chemical compounds consisting of three terpene units with the molecular formula C30H48; it can also be ruminated as it consists of six isoprene units. Animals, plants, and fungi all produce triterpene, including squalene, which is a precursor to all stimulants**.** (Broun and Massey, 1929); (Macahig and Dayrit, 2012)



Tetra terpenoids: eight isoprene units [C40H64]

1.1.1.2 Steroids

Steroids are the most important natural products. They are well distributed in animals and plants and have been extensively studied since the isolation of cholesterol. The steroids found in two sources. (Khalid, 1974); animals (cholesterol) and Plants (stigma sterol)

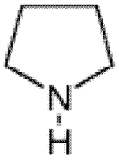


1.1.1.3 Alkaloids

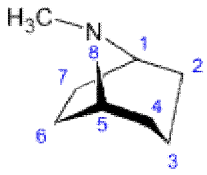
First, introduce common and well-known terms (Alkaloid) to critically assign all of these natural materials and specifically react almost like a base or alkali**.** An attempt was made to classify “alkalis” based on heterocyclic, classified as Pyrimidine alkaloids**,** Pyridine alkaloids (or piperdine alkaloids-) Pyridine_Pyrrolidine alkaloids, Tropane alkaloid**,**

Quinoline alkaloids**,** Iso quinolone alkaloid **,**Indole alkaloids and Imidazole alkaloids**.**

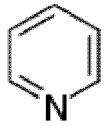
These previously mentioned eight typical N-heterocyclic basic ring categories of ‘alkaloids‘(Khalid, *et al* 1974).



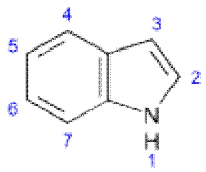
Pyrimidine



Tropane alkaloids



Pyridine

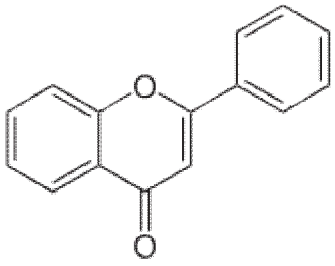


Indole alkaloids

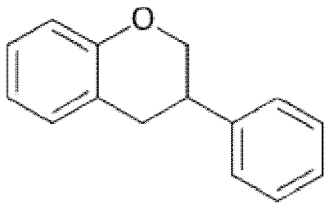
1.1.1.4 Flavonoids

The flavonoids from the Latin word "flavous" which means yellow, is generally used to describe a wide range of natural products that process 15 carbon atoms with a C6-C3-C6 carbon frame, comprising two benzene rings linked through a pyrene heterocycle.

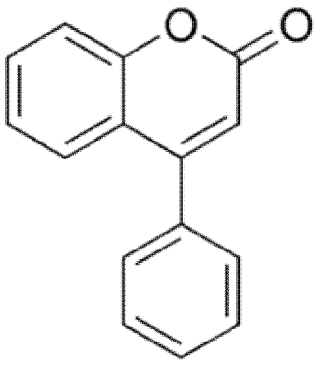
Classification of flavonoid aglycones includes flavones, flavonols, flavanones, Flavanonols are flavones, anthocyanidins, chalcones, catechins, and bioflavonoid**.**



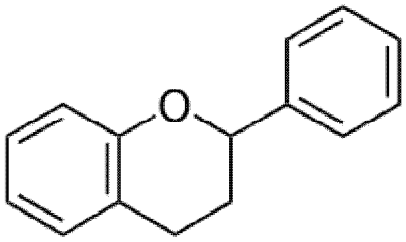
Flavone



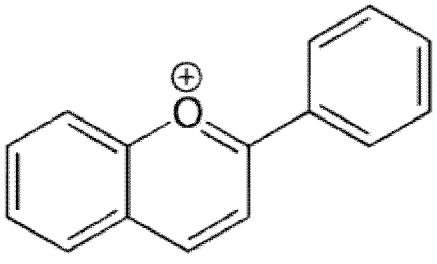
Isoflavan structure



Neoflavonoids structure



Flavan structure



anthocyanidins

The *Asclepiadaceae* family treated as a subfamily (*Asclepiadoideae)* in *Apocynaceae* (Endress and Bruyns, 2000) includes 348 genera, with about 2,900 species. It is mainly located in the tropics to the subtropics, especially in Africa and South America. This family is a rich source of indolin, alkaloids, steroidal alkaloids, pregnene, and glycosides that have been shown to possess anti-tumor and anti-cancer activities and are very similar to cardiac glycosides (Deepaks, 1989; Srivastava, *et al.*, 1993). Its other chemical components are cyanogen glycosides, saponins, tannins, coumarin, flavonoids, phenolic acids and triterpenoids.

Solenostemma argel (Del.) Hayne (*Apocynaceae)* is a desert plant widely distributed in Sudan and Egypt (Wadi Al-Laqi) with the common name "Hargel" (Al-Hadidi and Fayed, 1995) and in Sudan and is its richest source (Al-Ghazali, 1997). It is the most important of many Sudanese plants known for their potential medicinal benefits in herbal medicine.

Solenostemma argel is used to treat diabetes and jaundice. Its leaves have laxative properties, which may be due to the latex present in the stem parts (Al-Kamali and Khaled, 1996). In addition, an extract of the leaves of this plant showed a toxic activity on fungi (Abdel-Hadi and Aouf, 1993). It is used to treat some diseases of the liver, kidneys and allergies, as an incense in the treatment of measles, and sometimes it is crushed and used as a remedy for feeding wounds. It is an effective treatment for bronchitis and is used to treat nerve pain and sciatica. Its leaves are infused to treat stomach and intestinal cramps, stomach pain, colic, cold and urinary tract infections and is effective as an anti-syphilis if used for long periods of 40-80 days.

S. argel is used to suppress stomach pain, childbirth pains and loss of appetite. Its raw aqueous extracts have been shown to have insecticidal activity against mosquito larvae (Kamali, 2000). From previous phytochemical studies, it was found that its leaves are high in carbohydrates, low in crude fiber, protein, crude oil, and ash, high in potassium, calcium, magnesium, sodium, and low in copper, iron, manganese, and lead, and they contain phytic acid. (Marwan and Al-Khair, 2010). Kamel (2003) proved to have acetic phenolic glycosides.

Another study a chloroform extract showed that it had anti-inflammatory activity and contained a novel and another known pregnancy glycoside (solenoside A) and another known along with kaempferol 3-O-glucoside and 3-O-rutinoside (Innocenti et al., 2005). Its air fractions have been found to contain monoterpene glucose, pyrogen glucose, O-ap-apiofuranosyl alcohol (1 → 6) β-glucopyranoside, 2-phenyl-ethyl O-α-arabinopyranosyl (1 → 6) β-glucopyranoside, estragaline and kaferol. 3-O-neohesperidose (Kamil et al., 2000). In previous studies, (Threep et al., 1986; Abd al-Hadi *et al*., 1994) four compounds were isolated from their stalk but not identified and subjected to a primary screening for antimicrobial activity. A group of 14, 15-secopregnane glycosides, argelosides, were isolated from different parts of the plant.

From the fruits, Argylosides A-B were isolated, from the seeds of Argylosides C-G and from the leaves of Argylosides K-O. From different parts, a group of 15-ketognane glycosides were also isolated: A-B stemmosides from the leafy stem, C-D stemmosides from the cortex, and E-K stemmosides from the leaves. From the leaves, kaempferol-3-O-glucoside and kaempferol-3-O-rutinoside were isolated, as well as several

pregnene derivatives, and glycoside solenoside A. In addition, present in the leaves is β-sitosterol and triterpenoids α. Two princes and two beta princes. Moreover, from the aerial fractions polyhydroxy bergnan, Stimmen C, four acyl phenolic glycosides (Solar I to IV) as well as other flavonoid glycosides were isolated. It has been shown that many of these argylosides and stem stems to reduce cell proliferation in a dose-dependent manner in different human and mouse tumor models. The hot water extract of the aerial parts showed significant cytotoxicity in vitro and in vivo in a range of tumor models. The various extracts of the aerial parts showed hepatotoxic and nephrotoxic effects on albino rats, when administered orally within a week. In a chicken nutrition test, a diet containing 10% of *Solenostemma argel* leaves caused growth depression and hepatotoxicity.

The chloroform extract of the leaves showed a high local anti-inflammatory activity in the test of ears of croton oil in mice. The leaf chloroform extracts showed convulsive and uterotonic activity on the spontaneously contracted rabbit jejunum and rat uterus. The aqueous extract of the leaves produced a stimulating effect on the isolated aortic strip of the rabbit. When added to the isolated atrium of a guinea pig, the extract reduced the rate and force of shrinkage. Different parts of the methanol and water extracted aerial parts showed moderate to significant antibacterial and antifungal activity in the laboratory against a group of pathogenic organisms.

The aqueous extract or the dried aerial parts were found to be very effective in controlling the date scale (*Parlatoria blanchardi*) on the date palm in Sudan. Raw aqueous extracts of dried fruit peels, flower, root and

stem showed moderate insectcide activity against *Culex quinquefasciatus* mosquito third instar larvae. Peel fruit extract was more effective.

Methanol extract of aerial fractions was incorporated into *Culex pipiens* mosquito rearing medium, and showed significant and prolonged larval death and egg suppression. The extracts of aerial parts showed significant anti-trophic activity of the African cotton leaf worm (*Spodoptera littoralis*), in addition to a moderate larval mortality. Aerial fraction methanol and hexane extracts showed significant nematocidal activity against *Meloidogyne* incognita stage II juveniles. The merging of the leaves around the stems of the date palm (*Phoenix dactylifera L.*) showed a significant increase in flowering and yield.

1.2. Literature review

1.2.1 Scientific classification of *Solenostemma argel*

Kingdom: Plantae

Order: Gentianales

Family: *Apocynaceae*

Genus: *Solenostemma*

Species: *argel*

Binomial name: *Solenostemma argel*

1.2.2 Family *Apocynaceae*

Family *Apocynacea*, commonly known as the Dogbane family, consists of approximately 424 genera distributed among three sub-families known as *Rauvolfioideae, Apocynoideae* and *Asclepiadaceaes*. (AOAC. 1990). It includes many of the most well-known tropical Ornamental plants (Oleander, Frangipani, *Allamanda, Mandeville, and Vinca*)(Laurence *et al*., 1989) Many of these are large trees with buttress roots found in rainforests, while some are smaller, evergreen or deciduous trees, shrubs or climbers found in warm and temperate regions of the world (*Vinca*) (Miles and Misra., (1938). The sap of most of the plants is milky latex, which has economic importance for medicinal uses and for the production of rubber (Kavanagh, (1972).

Robert Brown (1810), because many of its members have milky latex, separated the *Asclepiadaceae*, also known as Milkweed family from the *Apocynaceae*. Miles (1938). By following recent work on DNA analysis and other morphological studies, both the families *Asclepiadaceae* and *Periplocaceae* have been merged into a sub-group of the family *Apocynaceae* (Kamel *et al.,* 2000 and Hassan *et al*., 2008). *Asclepiadaceae* is also classified into three tribes, *Asclepiadoideae*, the largest and cosmopolitan, *Periplocoideae* and *Secamonoideae* both are smaller sub-families and restricted to limited areas in Asia and Africa (Hassan *et al.*, 2001; Hamed, 2001).

1.2.3 Taxonomy of *Asclepiadaceae*

Asclepiadaceae plants are perennial herbs, shrubs, wood, climbers, twiners or trees, occasionally succulent, with a well-developed laticifer system (Plaza *et al.,* 2003). The leaves are usually opposite or whorled, rarely alternate, simple and generally entire. In some succulent taxa, they

are caduceus or vestigial. Minute stipules are normally present (Kamel, 2003).

The inflorescence is either a racial or cymose component more commonly than dichasia (usually an umbellate shape) or a monophasic branching (Plaza, 2003). The flowers are dioecious, radial, and are usually pentagonal (except for genosomes). The fruit is surreal (Mendel and Osborne, 1924). The cup consists of five cups partially compact and deeply divided with individual spouts in the back position. The corolla consists of five petals fused with twisted or valved lobes. Both the corolla and the stamens, or both, may bear secondary appendices of different species that form a double or single wreath. A highly morphologically altered annular ring, a term used for the coronal parts of Asclepiadacius flowers (Khalid *et al.*, 1974; Pearson, 1970), often terminates the corolla tube.

The filaments are short or may be completely absent. The anthers are usually fused to the gynoecium to form a gynostegium. The gynoecium consists typically of two distinct nearly superior or semi-inferior ovaries, two styles and a common single five-lobed, often much enlarged, stigma. Each ovary is unilocular, unicarpellate. The ovules are anatropous and pendulous, imbricated in several series on a ventral and parietal placenta. The enlarged stigma (discoid, conical or beaked) is non-receptive (Pearson, 1970). The fruit consists of a pair of follicles (often only one develops fully).

The seed is usually flat, elongated in shape and carries a microscopic coma with long silk bristles. The fetus is large and straight. The endosperm is thin and small (Pearson, 1970) (Plaza *et al.*, 2003). The most important anatomical features of the *Asclepiadaceae* family are the

presence of mammary tubes in most organs with the presence of phloem inside the relapse. The cork is superficially developed and the circumferential circulation in a broad ring containing isolated groups of fibers. Pots show simple holes. Narrow medulla rays have also been reported in xylem (Mcntyre, 2003; Price *et al*., 1987).

Figure (1): *Solenostemma argel*

Members of the *Asclepiadaceae* family are known to contain a number of bioactive compounds such as indoloquinoline alkaloids, flavonoids, triterpenes, cardenolides, gnane and C / D-cis-polyoxypregnane esters and glycosides (Khalid *et al*, 1974) (Ross *et al*., 1980). *Solenostemma argel* is a plant species in *Apocynaceae* that was

first described as a genus in 1825. It contains only one known species, *Solenostemma argel*, native to North Africa and the Arabian Peninsula. It is found in tropical Africa in the desert regions of Mali, Niger, Chad and Sudan. It is also widely distributed in Algeria, Libya, Egypt and Saudi Arabia.

1.2.4 Chemical Composition and Active ingredients

1.2.4.1 Chemical Composition

Solenostemma Argel contains flavonoids, triterpene, tannins, steroids, alkaloids, saponins, monoterpine, berinjan, fatty steroids, flavones, antocinan oxides, mucilage amino acids, poly-holosides, polyphenols, phytosterols and carotenoids. On the other hand, chemical research of the leaves showed the presence of carbohydrates, protein, and fiber and a lower proportion of minerals K, Ca, Mg, Na, Cu, Fe, Mn, and a non-nitrogenous protein. (WHO, 2002). Khaled and colleagues (1974) reported the presence of Kampferol and Steroidal glycosides in Hargill leaves. While Plaza *et al*. (2003) that protein, sugar, fiber, and vitamins are present with the minerals Na +, K +, Ca + 2, Mg + 2, Ni + 3, P + 3, and there are four new carrier glycosides from *Solenostemma Argel*.

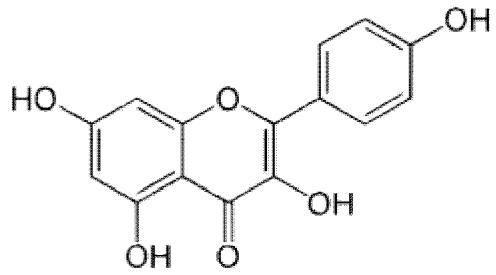
The presence of bioactive components such as phytates and phenolic compounds was found to have deleterious effects on the intrinsic properties of proteins (Jacob, 2003). Goldstein and Swain (1963) showed that tannins are phenolic polymers that deposit protein from an aqueous solution and they reduce or inhibit enzyme activity. Phytic acid represents a complex class of the naturally occurring organic form of phosphorous compounds that can significantly affect the functional and nutritional properties of foods (Fageer, 2003).

From previous phytochemical studies, it was found that its leaves are characterized by high potassium (0.54%), calcium (0.06%), magnesium (0.03%) and sodium (0.01%), but they are characterized by low ferrous (0.002%), lead (0.001%) and manganese (0.002 %).

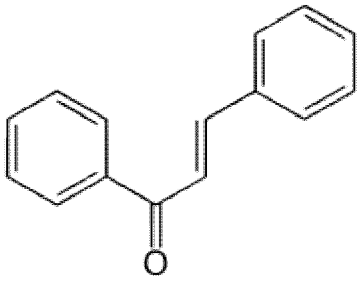
1.2.4.2 Active ingredients present in *Solenostemma argel*

Solenostemma argel belongs to the *Asclepiadaceae* family. This family includes many wild medicinal plants such as *Calotropisprocera, S.argel, Leptadineaspp* (El Tigani and Ahmed, 2009). Likewise, Ahmed (2003) claimed that *S.argel* is considered to be of medicinal importance in Sudan, Libya, and Chad. Additionally, *S.argel* is a plant or plant part valued for its medicinal, aromatic, or salty qualities. Plants produce herbs and contain a variety of chemicals that act on the body (Shayoub, 2003). In addition, it was found that tissue cultures produced compounds, not previously described, and higher plant cell cultures, which may provide an important source of economically important new compounds (Butcher, 1977; Constabel and Tyler, 1994).

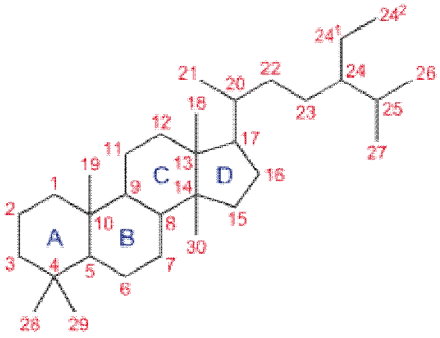
Moreover, chemical chromatographic examination and phytochemistry as well as tissue culture studies of the leaves, stems and flowers of *S.argel* revealed the presence of many biochemical constituents such as pyrogenic glycosides, flavonoids, kaempferol, quercetin, rutin, flavonols, flavonoids, and chalcon alkaloids. (Al-Tijani and Ahmed, 2009; Plaza *et al*., 2005; Shafiq and Michael, 2012). In their report on *S.argel,* (Khaled *et al*., 1974) demonstrated the presence of kaempferol, steroid glycosides and found that flavonoids in hargel leaves.



Kaempferol



Chalcones



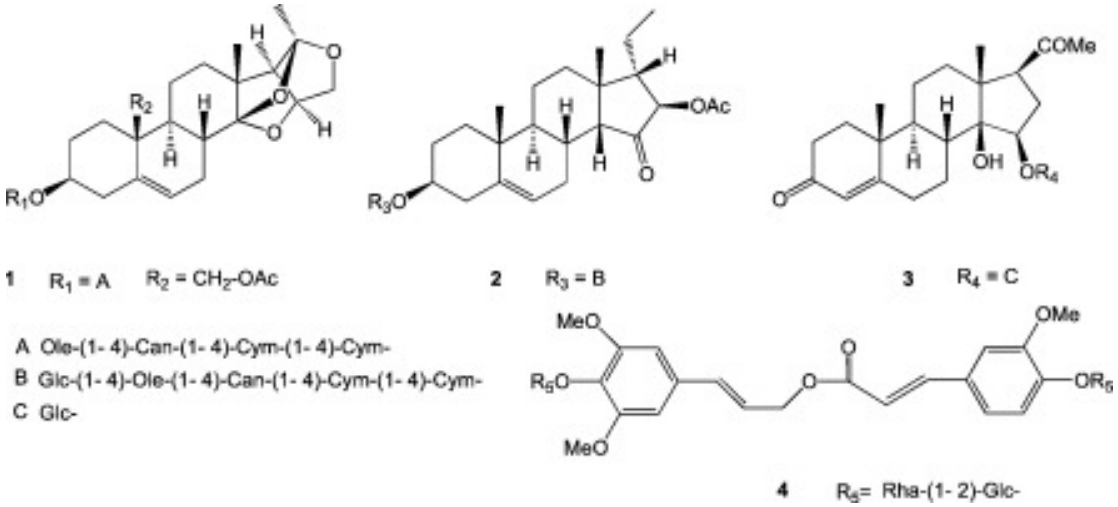
Steroid ring system

Mohamed *et al.*, (2012) reported that *Solenostemma argel* contains flavonoids, kaempferol, quercetin, rutin, flavonols, flavanones, chalcones and alkaloids. In addition, they contain pregnane ester glycosides.

S.argel was found to contain some flavonoids saponins alkaloids (Khalid *et al.*, 1974). Moreover, there are 2000 flavonoid found in *S.argel* in asmethoxil or hydroxyl group. Further studies are needed to investigate these flavonoids. Phytochemical studies of the leaves, stems and flowers revealed the presence of -amyrin and -sitosterol, 7-methoxy-3-22-dihydroxy-stigmastene, ethoxy derivative of vangurolic acid, an unidentified sterol.

Moreover, they detected the presence of flavonoids and saponins in the different organs and alkaloids and/or nitrogenous bases in the leaves, stems and flowers (Khalid *et al.,* 1974). Kamel (2003) proved that it contains acylated phenolic glycosides. Mahran *et al.* (1976) isolated -amyrin, -sitosterol-containin rutin and quercetin from *S.argel*. In addition, El-Tohami, MS (1996) claimed that *S.argel*.

Solenostemma argel contains an acidic resin, glycoside, choline, phytosterols and amyrins. Previous studies have reported the occurrence of monoterpene glycoside (Kamel *et al.,* 2000), pregnane derivatives (Hassan *et al.,* 2001*)* and pregnane glycosides including stemmosides A and B (Kamel *et al.,* 2000), (Hamed, 2001) and acylated phenolic glycosides in the leaves (Kamel, 2003) while 14, 15-secopregnane glycosides (Wollenweber and Dietz, (1981). and pregnane glycosides namely stemmosides C and D have been isolated from the pericarps (Kanadaswami *et al.,* 2005). Many previous studies have reported the presence of monoterpenes, pregnane glycosides and acylated phenolic glycosides in the leaves. In addition, there is an occurrence of four new pregnane glycosides from the pericarps of *S.argel* (Plaza *et al.*, 2003).

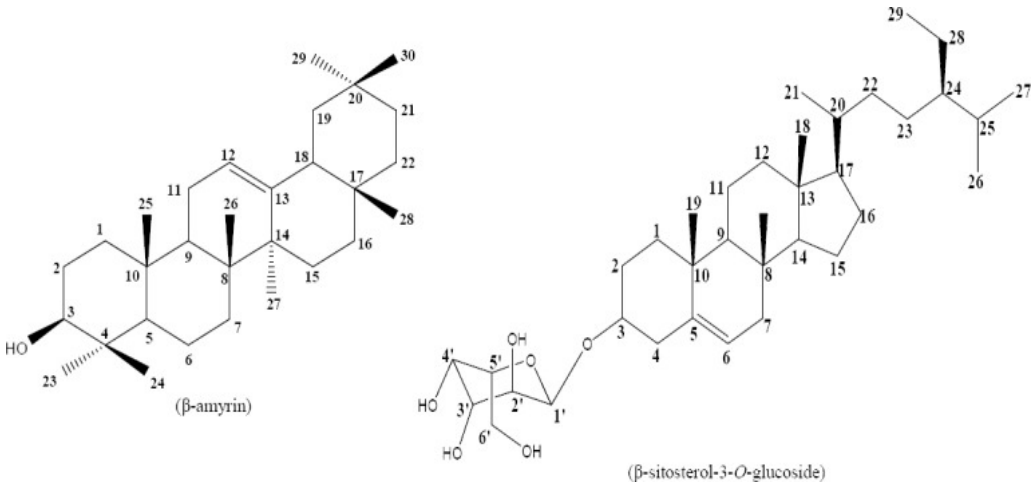


New pregnane and phenolic glycosides

from *Solenostemma argel*

Another study in its chloroform extract showed that it has an anti-inflammatory activity and it contains a new pregnene glycoside (solenoside A) and a known one besides kaempferol 3-O-glucoside and 3-O-rutinoside (Innocenti *et al*., 2005).

Mahran, *et al*., (1967), in their study on the leaves, stems, flowers and fruits of *Solenostemma argel*, reported the presence of a and b-amyrins, b-sitosterol,7-methoxy-3, 22-dihydrostigmastene, ethoxy-vangurolicacid in addition to an unknown steroid.

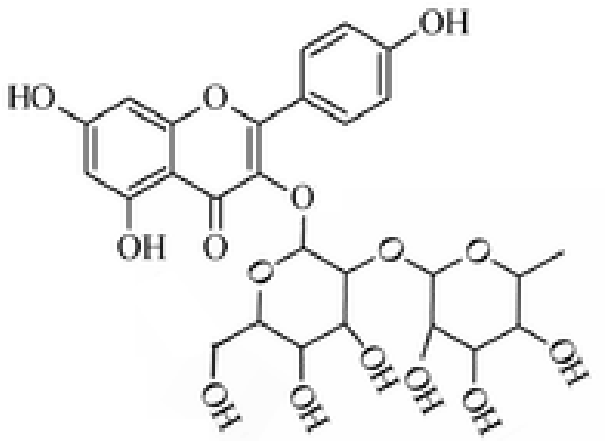


In another study (Mahran *et al*., 1969), using chromatographic examination of alcoholic extracts of *Solenostemma argel* leaves; they reported the presence of two crystalline substances called arginine and argiloside without detailed spectroscopy or structure determination. The study conducted in Hungary (Khaled *et al.*, 1974), on *Solenostemma argel*, showed that the light petroleum and chloroform extracts did not contain steroids or alkaloids and flavonoids, while the methanolic extract was found to be rich in flavonoids and steroids.

The only quaternary base that was found in significant quantities was choline. Chemical examination, which was identified in Romania (Yunus, 1988), on the leaves of hargel , showed the presence of lipids, flavones,

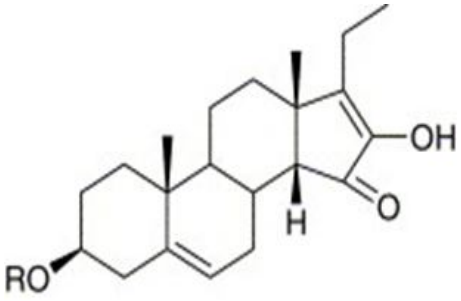
anthocyanin oxides, gum, tannins, amino acids, polyhlorosides, polyphenols, phytosterols, carotenoids. A biosynthesis study (Amariei *et al*. 1991), on *Solenostemma argel*, revealed the presence of rutoside, b-carotenean, and b-sitosterol.

Shafiq and Michael (2012) studied the chemical constituents of *Solenostemma argel* leaves, and identified a new natural flavonol glycoside: kaempferol 3, 40-di-Obd-glucoside with 3-glucuronide, 3-rutinoside, 7, 40-diglucoside, and glycon kaempferol.



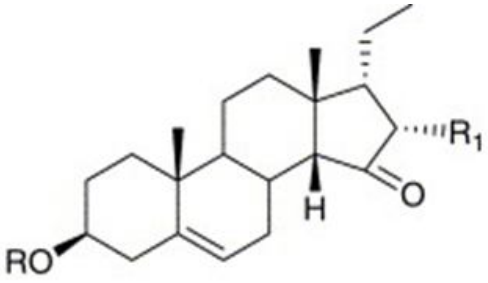
Kaempferol 3-O-rutinoside

Alberto Plaza*et al*., (2005) reported that seven new 15-keto pregnane glycosides, namely Stemmosides E–K, were isolated from *Solenostemma argel*. Stemmosides E–J were characterized by the occurrence of an uncommon 14-proton configuration while stemmosides E and F possess in addition a rare enolic function in C-16. On the other hand, stemmosides G–J display an unusual C-17 side chain. Electrospray ionization mass spectrometry (ESI-MS) and Nuclear magnetic resonance (NMR) established their structure experiments.



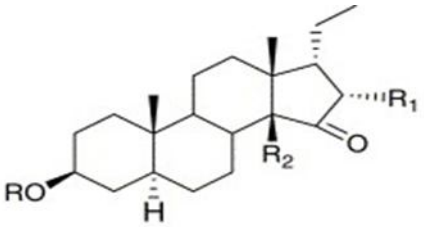
stemmoside E (**1**) R = A

stemmoside F (**2**) R = B



stemmoside G (**3**) R = B R_1= OH

stemmoside H (**4**) R = A R_1= OAc



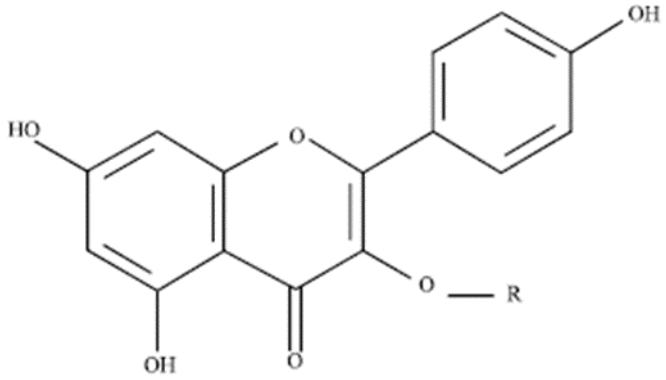
stemmoside I (**5**) R = A R_1 = OAc R_2 = H

stemmoside J (**6**) R = B R_1 = OAc R_2 = H

stemmoside K (**7**) R = B R_1 = H R_2 = OH

From the aerial fractions of *Solenostemma argel*, two monotrophic glucosides were isolated and identified as 6, 7-dehydroxy-dehydroenolol-3-or-beta-glucopranoside and 6, 7-dehydroxy-dehydroenolol-7-or-beta-glucopranoside. Bergen-glucoside was also isolated and designated as

pregnancy -5-en-3, 14-beta-dihydroxy-7, 20-dion 3-O-beta-glucopyranoside with compounds known as benzyl alcohol O-beta-apiofuranosyl- (1 -> 6).)-Beta-glucopyranoside, 2-phenyl ethyl O-alpha-arabinopyranosyl- (1 -> 6)-beta-glucopyranoside, astragaline and kaempferol-3-O-alpha-rhamnopyranosyl- (1 -> 2)-beta-glucopyranoside.



(S1) R =β-D-glucopyranosyl (1→2) β-D-xylopyranoside, (S3) R = α-L-arabinopyranosyl (1→2) β-D-galactopyranoside, (S9) R = H

Eight new 14, 15-secopregnane glycosides, namely argelosides C-J, possessing two ketal functions involved in three five-membered rings, have been isolated from the hairy seeds of *Solenostemma argel*. Their structures have been established by MS and NMR experiments, combined with quantum mechanical calculations of the C-13 chemical shifts for the interpretation of the experimental data.

Based on the results obtained, the structures of Argilocides A and B were revised to the effect that all compounds reduced cell proliferation in a dose-dependent manner.

From the aerial parts, pericarps and roots of *Solenostemma argel*, three new pregnane glycosides (1-3) with two known ones and a new phenolic glycoside (4) have been isolated. Extensive Nuclear magnetic resonance 1D - and 2D (NMR, 1D and 2D) and mass spectroscopic (MS) analysis established their structures. . Eight compounds were isolated and identified by NMR: kaempferol-3-O-glucopyranoside (1), kaempferol (2),

kaempferol-3-glucopyranosyl (1→6) rhamnopyranose (3) p-hydroxy benzoic acid (4), dehydrovomifoliol (5), 14,15-dihydroxypregn-4-ene-3,20-dion (6), 14, 15-dihydroxy-pregn-4-ene-3, 20-dione-15β-D-glucopyranoside (7) and solargin I (8). Two of them (compounds 2 and 3) could inhibit over 50 % of butyryl cholinesterase activity at 100 µM. Compound (2) displayed the highest inhibitory effect against acetyl cholinesterase (AChE) and butyryl cholinesterase (BChE) with a slight selectivity towards the latter.

Molecular cohesion studies supported the laboratory results and revealed that (2) had synthesized several hydrogen and-beta reactions that could explain the compound's effectiveness in inhibiting AChE and BChE. (Demak *et al* (2019)).

1.2.5 Traditional medicinal uses of *Solenostemma argel*

Solenostemma argel in folk medicine is used for the treatment of gastrointestinal diseases, high cholesterol disorders and diabetes, and is used externally in the form of compresses as anti-inflammatory and anti-rheumatic and by inhaling its smoke to treat measles and cold.

The trunk is generally used as an antispasmodic and for treating coughs. Moreover, "Hargal" infusion is used to treat jaundice, urinary tract infection and menstrual disorder (Kamali and Khalid, 1996). It is also used to treat stomach pain, as an anti-colic, as treatment for festering wounds, and as an anti-syphilis when used for a long period of 40 to 80 days (Paul, 1983; Hamishi and Meisa, 2006), and is an anti-inflammatory and anti-inflammatory. Rheumatology factor (Shayoub *et al.,* 2013). Again, the leaves are used as an antispasmodic, carminative, and anti-diabetic (Kamil *et al.,* 2000 and Hassan *et al.,* 2001).

In addition, it is used in indigenous medicine as an effective remedy for cough. The infusion of its leaves is used for gastro-intestinal cramps and infections of the urinary tract (El-Tohami, 1996). It is an effective remedy for bronchitis and is used to treat neuralgia and sciatica *(*Tharib *et al.*, 1986*)*.

Mudawi (2003) reported that the chloroform extract (600 – 800) mg/kg induced a delayed and gradual decrease in amplitude of the spontaneous contractions of pregnant or non-pregnant uterus. Similarly, El-Tahir *et al.* (2005) studied the pharmacological activities of *S.argel*, including spasmolytic and uterine relaxant activities. Plaza *et al*., (2005) found that pregnane glycosides isolated from this plant could reduce cell proliferation.

In addition, the plant has antimicrobial activity (Mohamed *et al*., 2012). Again, Ross and their co-workers (1980) illustrated the presence of antibiotic substances in the ethanol extracts of Hargel plant. Similarly, it was reported to have antimicrobial properties as well as antibacterial and antioxidant activity (Shafek and Michael, 2012).

Moreover, many studies confirmed that the *S.argel* have remedial effect against numerous diseases and health problems such as diabetes mellitus (Trojan *et al.,* 2012) and cancer (Amr *et al.,* 2009; Hanafi and Mansour, 2010).

1.2.6 Toxicity

Osman et al., (2014) argued that *S.argel* had incurred hepato renal toxicity in the experimental animals. Also in a feeding test with chicken, a diet containing 10 leaves of *Solemenstomma argel* caused a depression in growth and hepato toxicity (EL-sanusi and Adam, 2007). In addition Osman *et al.,* (2014) found that *S.argel*, could be proposed for seeking *S.argel* for treatment, used with dose much use them 600 mg/kg and the

levels of creatinine, urea, alkaline phosphatase (ALP) and aspartate amino transferase (AST) during the course of treatment.

On the other hand, Shayoub *et al.,* (2013) found that the different types of leaves, extracts or alkaloids of *Solenostemma argel* tablets showed a very good therapeutic effectiveness (71%-100%) and a great margin of safety (98%-100%). No side effects or adverse reactions were recorded and the patients did not complain of any undesirable or intolerable toxic or adverse effects of these preparations of *Solenostemma argel*.

1.2.7 Pharmacological activity

The pharmacological experiments to investigate the biological activity of *Solenostemma argel* leaves powder extracts and alkaloids on different experimental models, and confirmation of the effectiveness of *S. argel* preparations.

Solenostemma argel extracts showed inhibitory effects upon the spontaneous contractions as well as the induced contractions in contracting small intestine of rabbit and guinea pig small intestine model by sub-maximal doses of acetylcholine (1mg/ml) or barium chloride (0.002mg/ml).

The degrees of relaxation that occurred at these sub-maximal doses were estimated and compared with those produced by reference drugs such as oscine or palavering. Other experimental models, including isolated rabbit heart and intact African toad heart, showed depressant effects on the myocardium of different *S. argel* extracts, perfused continuously, leading to reduction of the heart rate and stroke.

1.2.8 Biological activity

The potency of *Solenostemma argel* (*asclepiadaceae*) as anti-inflammatory and antioxidant were investigated. Three solvent extracts were used for extracting polyphenol, flavonoids and tannins from the leaves of *Solenostemma argel*. Three complementary antioxidant systems: (DPPH· free radical scavenging, Fe^{2+}-chelating and iron reducing power) as well as the total phenolic, total flavonoids and total tannins contents of the extracts were also investigated.

In-vitro anti-inflammatory effects of *Solenostemma argel* were assessed by using albumin denaturation assay. *Solenostemma argel* acetone extract was found to exhibit greater scavenging activity than those extracted by other solvents. The same trend was achieved using Fe^{2+}-chelating and reducing power.

The results revealed that the aqueous, acetone and ethanol extracts of *Solenostemma argel* showed anti-inflammatory activity (74.19%, 69.44% and 66.58%, respectively) by inhibiting the heat induced albumin denaturation.

The outcomes from HPLC study showed that acetone extract from *Solenostemma argel* had the highest amounts of e-vanillic, benzoic, pyrogallol, chlorogenic and ellagic acids. Whereas, rosmarinic, quercetrin and narengin acids were the major flavonoids compounds in ***S. argel*** acetone extract.

The results obtained showed that the solvent plays a vital role in extracting plant components, and that *Solenostemma argel* is a very promising source of bioactive compounds. Therefore, it can be concluded that the relevant flavonoids and polyphenols contained in the

Solenostemma argel extract may be responsible for the anti-inflammatory and antioxidant activities that can be attributed to the root scavenging and the anti-inflammatory effects of some of its active components.

Several researchers in several countries have investigated the activity of insecticides for *Solenostemma argel*. Hajj Al-Tayeb and others, (2009) reported that aqueous argil extract was effective in controlling mosquito larvae *Culex spp.* In addition, *Anopheles spp.* Under laboratory conditions. In vitro, aqueous and organic extracts showed mortality, repellent and restriction effects against the macular pea beetle *Callosobruchus macules* (Mohammed *et al*., 2014). (2009) found that 10% concentrated Argel water filters gave 100% worker and soldier mortality from termites to cotton soil (Microtermes thoracalis Sjost) under laboratory conditions. Furthermore, spraying Argel shoot water filtrate at 1ounce/6liter of water/tree was recommended to control white scale insect (*Parlatoria Blanchardii Targ*.) and (*Asterolicanium phoenicis*) on date palm (Eldoush *et al*. 2011). Mardi and Suliman (2013) found that the aqueous extract of Argel shoots at 40g/L of water gave comparable performance to the synthetic insecticide Alpha-cypermethrin.

1.3 Research Objectives

- To phytochemically screen, the aqueous extract and solvent fractions of *S. argel.*
- To evaluate the biological activity of extract and solvent fractions.
- To isolate and characterize active ingredients using spectroscopic techniques.

Chapter Two

Materials and Methods

Chapter Two

Materials and Methods

2.1 Materials

2.1.1 Plant Material

Samples of Hargal (*Solenostemma argel*.) stems were obtained from Albakheet-Northern State of Sudan during 2017. The stems were freed from foreign materials, and then washed with water, dried and milled using laboratory mill into fine powder.

2.1.2 List of Chemicals:

From [LOBA Chemie, India] n-hexane, Chloroform, Ethyl acetate, Ethanol, p.anisaldehyde, glacial acetic acid, sulfuric acid, Carbone tetra chloride, Bismuth sub nitrate.

From [Sd fine-Chem limited, Mumbai] Methanol**,** Petroleum ether**,** n-butanol and Silica gel**.**

From [RIEDEL-DEHAEN AGSEELZE-HANNIVER, Germany] Acetic anhydride, Ammonium hydroxide, Mercuric chloride and Phenolphthalein indicator

From [Central Drug House (P) LTD (CDH)] Acetone, Hydrogen peroxide, Sodium hydroxide, Potassium Iodide, Sodium chloride, Starch, Sodium carbonate, Sodium thiosulphate, Potassium hydroxide and Ferric chloride.

2.1.3 Materials for chromatography

Thin-layer chromatography (TLC) analyses were carried out on pre-coated silica gel 60F254 glass plates with different sizes. 0.25 mm thick (E.Merch, Germany).

Different rates of solvent systems were used. Column chromatography was performed using silica gel F254 particle size 0.04 – 0.63 mm 230 – 400 mesh, (Fluka, Bushes, and Switzerland) and activated by heating at 105 ^{0}C for an hour prior to use.

Chemicals of Reagent for chromatography: p.anisaldehyde. Glacial acetic acid. Methanol. Concentrated sulfuric acid.

2.1.4List of Apparatus

2.1.4.1 Magnetic Stirrers Apparatus

Evaporation, Rotary Vacuum Evaporator, Bibby-sterilin ltd., Stone-Staffs ST15 OSA-UK solvents at 60 ^{0}C**.**

2.1.4.2 Ultra Violet Spectro photometric Analysis (UV)

Ultra Violet Visible Spectrometer using Spectrophotometer model 6595 Jenway Limited England UV- visible Spectrometer.

Ultra Violet Lamp**:** The ultra violet lamp used in visualizing TLC plates with a miner alight device, multiband UV – 254/366 nm obtained from UVP Inc. USA.

2.1.4.3 Infrared spectro photometric Analysis (IR)

Infrared (IR) spectra were generally obtained using potassium bromide pellets unless otherwise specified using Perkin – Elmer FTIR model 1600 spectrometer, USA spray probe.

2.1.4.4 Nuclear Magnetic Resonance (^{1}H NMR) and (^{13}C NMR) Spectrophotometer

NMR spectra were recorded in pyridine-d5 using a JEOL JNM A-400 spectrometer (400 MHz for 1HNMR and 100 MHz for 13C-NMR) with TMS as internal standard.

2.1.4.5 Mass Spectrometer (MS)

The mass spectra were recorded using a JEOL JMS-SX 102 spectrometer.

2.1.4.6 Atomic Absorption Spectrophotometer

Atomic absorption measurements were performed using Perkin-Elmer AAS, model A Analyst 100 spectrophotometer equipped with an iron hollow-cathode lamp, under the following conditions: wavelength, 302.1 nm, slit-width 0.2 nm, lamp current 30.0 mA, airflow rate and acetylene flow rate are adjusted at the standard conditions.

2.1.4.7 Freeze-dryer machine

Freeze-dryer machine manufactured by Hochvakuum Technik Dresden (Germany), Model TG16.10, Serial # 81405.0, blt 1989. Registration #036. Dryer 750mm wide x 1050mm high x 950mm deep.

2.1.5 Test microorganisms

2.1.5.1 Bacterial microorganisms

Bacillus subtilis.

NCTC 8236 (Gram + ve bacteria).

Staphylococcus aureus.

ATCC 25923(Gram +ve Bacteria).

Escherichia coli.

ATCC 25922(Gram -ve bacteria).

Pseudomonas aeruginosa.

ATCC 27853 (Gram -ve bacteria).

National Collection of Type Culture (NCTC), Colindale, England.

American Type Culture Collection (ATCC) Rockville, Maryland, USA.

2.1.5.2 Fungal microorganisms

Aspergillus niger ATCC9763

Candida albicans ATCC7596

2.1.5.3 Insects

Sorghum grain.

Adults of *Tribolium castaneum.*

2.2 Methods

2.2.1Extraction and Fractionation

Extraction and fractionation were performed according to the method described by Sukhdev *et al.*, (2008):

The dried powder was extracted from *Solenostemma argel* stems (2 kg) with hot water (3500 ml) for six hours using a magnetic stirrer apparatus (m s a). After filtration, the extract was shaken three times using 500 ml of n-hexane, each time using a separating funnel. The hexane layers were combined and evaporated under low pressure using a rotary evaporator. The aqueous layer was then shaken three times with 500 ml of chloroform each time with a separating funnel. The chloroform layers were collected and evaporated under low pressure using a rotary evaporator.

The aqueous layer was then shaken, three times with 500 mL ethyl acetate, each time using a separating funnel. The layers of ethyl acetate were combined and evaporated under low pressure using a rotary evaporator. Aqueous layer was finally shacked, three times with 500 ml of n-butanol, in each time using separatory funnel. n-butanol layers were combined together and evaporated under reduced pressure using rotary evaporator apparatus. Aqueous layer was lyophilized using freeze-drier machine to dryness and the yield percentage of each fraction was calculated.

2.2.2 Phytochemical screening

2.2.2.1 Test for carbohydrates

To 2 ml of crude and solvent extract fractions, 1 ml of Molisch's reagent and few drops of concentrated sulfuric acid were adde. The appearance of purple or reddish colour indicated the presence of carbohydrates (Sofowora, 1993)

2.2.2.2 Test for tannins

To 1 ml of crude and solvent extract fractions, 2 ml of 5% ferric chloride were added. Formation of dark blue or greenish black colour indicates the presence of tannins (Harborne, 1973).

2.2.2.3 Test for saponins

To 2 ml of crude and solvent extract fractions, 2 ml of distilled water were added and shaken lengthwise in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins (Smolenski, 1974).

2.2.2.4 Test for flavonoids

To 2 ml of each crude and solvent extract fractions, 1 ml of 2 N sodium hydroxide was added. The appearance of yellow colour indicated the presence of flavonoids (Kapoor, 1996).

2.2.2.5 Test for alkaloids

To 2 ml of crude and solvent extract fractions, 2 ml of concentrated hydrochloric acid were added. Then, few drops of Mayer's reagent were

added. The presence of green or white precipitate indicates the presence of alkaloids (Jana, 2010)

2.2.2.6 Test for glycosides

To 2 ml of crude and solvent extract fractions, 3 ml of chloroform and 10% ammonia solution were added. Appearance of a pink colour indicates the presence of glycosides (Sureshkumar *et al., 2009)*

2.2.2.7 Test for terpenoids

To 0.5 ml of crude and solvent extract fractions, 2 ml of chloroform were added and concentrated sulfuric acid was added carefully. Appearance of red-brown at the interface indicates the presence of terpenoids (Kapoor, 1996).

2.2.2.8 Test for coumarins

To 1 ml of crude and solvent extract fractions, 1 ml of 10% NaOH were added. Formation of yellow colour indicates the presence of coumarins. (Ayoola *et al.,* 2008)

2.2.2.9 Test for Steroids

To 1 ml of crude and solvent extract fractions, an equal volume of chloroform were added and followed by few drops of the concentrated sulfuric acid; appearance of brown ring indicates the presence of steroids (Boxi *et al.,* 2010).

2.2.2.10 Test for Anthraquinones

To 1 ml of crude and solvent extract fractions, few drops of 10% ammonia solution were added; appearance of pink precipitate indicates the presence of anthraquinones (Kapoor et al., 1996).

2.2.3 Minerals

1 g of dry plant was weighed and placed in a porcelain crucible and heated at 500 ° C overnight, cooled and dissolved in 5 mL, 20% HCL, the solution was heated if necessary, to dissolve the residue. The solution was filtered with acid washed filter paper in a 50 mL volumetric flask. The insoluble residue was warned, and the solution was well mixed and diluted to volume with deionized water.

2.2.4 Bioactivity studies

2.2.4.1 Anti-insectidal activity

Tribolium castaneum, were cultured in a 2`kgs of sorghum grain in glass containers covered with a muslin cloth and held, by the side, with an expansible rubber band to allow aeration, avoid suffocation of the insects and equally prevent insects escape. The culture was raised under ambient temperature and normal relative humidity.

2.2.4.1.2 Preparation of Stems extracts

Stems extract powder were carried out according to the method described by Sukhdev *et al.*, (2008).

2.2.4.1.3 Bioassay procedure

Six experiments were conducted to evaluate the insecticidal effect (mortality effects) of different parts of the plant against the (third instar) larvae of *Tribolium castaneum*, All the above experiments were performed separately in petri dishes according to the number of treatments. All six powders portions were applied separately at a concentration of 10: 100 grams (w / w) of powder: sorghum grain.

The petri-dishes were shaken manually to enable the powder to spread evenly over the grain. (Each one was replicated three times). Ten larvae were placed in each petri-dish; the experiment was subjected to the complete randomized design (C R D), and Mortality of larvae was recorded after 24 hours.

Larvae were taken, as dead if they did not move when touched or tiled. Untreated control treatment was considered grains and *Tribolium castaneum.*However, not botanical (experimental control). The mortality percentage of larvae was calculated by the method of Parugrug and Roxas (2008) using the following formula:

$$\text{Mortality\%} = \frac{\text{No.of dead insects}}{\text{Total No of insects}} \times 100$$

2.2.4.2 Anti-microbial activity

2.2.4.2.1 Preparation of bacterial suspensions

Aliquots of 1 ml of broth culture for 24 hours were distributed to test organisms aseptically on nutrient agar slopes and incubated at 37 ° C for 24 h. The bacterial growth was harvested and washed with sterile 100 ml normal saline, to produce a suspension containing approximately 108-109

° C per unit / ml. The suspension was stored in the refrigerator at 4 ° C until use.

The average number of organisms per ml of stock suspension was determined by a viable surface counting technique (Miles and Misra, 1938). Serial dilutions of the stock suspension were performed in sterile normal saline and a volume of 0.02 ml of the appropriate dilution was transferred by micropipette onto the surface of each dried nutrient agar plate.

The plates were allowed to stand for 2 hours at room temperature for the drops to dry and then incubated at 37 ° C for 24 hours. After incubation, the number of developing colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the applicable number of stock suspensions, expressed as the number of colonization units per suspended ml. Every time a new pending stock is prepared. All of the above experimental conditions were kept constant so that a suspension with very close viable numbers was obtained.

2.2.4.2.2 Preparation of fungal suspension

The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25 °C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally forming suspension in 100ml of sterile normal saline, and the suspension was stored in the refrigerator until used in vitro testing of extracts for antimicrobial activity.

2.2.4.2.3 Testing for antibacterial Activity

The cup-plate agar diffusion method (Kavanagh, 1972) was adopted with some minor modifications to assess the antibacterial efficacy of the prepared extracts. One mL of standard bacterial suspension (108-109 ° C / ml) was mixed well with 100 mL of molten sterile nutrient agar, which was maintained at 45 ° C. 20 mL of inoculated nutrient agar was distributed into sterile petri dishes.

The agar was left to install and in each of these dishes 4 cups (10 mm diameter) were cut using a sterile cork borer (No. 4) and the agar tablets were removed. The replacement cups were filled with 0.1 ml of each of the oil diluents in methanol using an automated microliter pipette, and left to circulate at room temperature for 2 hours. The plates were then incubated in an upright position at 37 °C for 18 hours. Two iterations were performed for each extract against each of the test organisms. After incubation, the average diameters of the resulting growth inhibition regions were measured and the mean values were recorded.

2.2.4.2.4 Testing for antifungal activity

The same method as for bacteria was adopted. Instead of nutrient agar, Sabouraud dextrose agar was used. The inoculated medium was incubated at 25 ºC for two days for the *Candida albicans* and three days for *Aspergillus niger*.

2.2.5 Chromatographic studies of chloroform extract

2.2.5.1 Column Chromatography (CC)

The chloroform extract was concentrated by distilling the solvent and evaporating it to dryness. The chloroform extract (8.9 g) was suspended in n-hexane and ethanol, then the resulting solutions were concentrated and the filter was filtered with an increased polarity of the solvent, i.e., non-polar - polar - high polarity.

2.2.5.1.1 Preparation of Column for isolation of active ingredients

Adsorbent silica gel (230-400 mesh)

Activation 105 ^{0}C for 1 hour

Length of column 100 cm

Diameter 3.5 cm

Length of adsorbent 75 cm

Rate of elution 5-10 drops/min

The glass column was filled with wet filler. The slurry of the absorbent (silica gel, 230-400 mesh) was prepared by mixing the absorbent in n-hexane and used as a stationary phase. It was then poured into a glass column (100 cm x 3.5 cm) (with a center glass disc bottom) and allowed to settle. The trapped air was removed by stirring with a glass rod. A small amount of sand was kept on top of the shaft to provide the latter with a flat base. Excess of the solvent was removed until the mobile phase level decreased to 1 cm above the top of the sand layer.

2.2.5.1.2 Preparation and loading of sample

The chloroform extract of *Solenostemma argel* was subjected to silica gel (230-400 mesh) column (100 cm x 3.5 cm) chromatography for the isolation of active ingredients, and elution was carried out from non-polar to polar solvents n-hexane (0:100), n-hexane: Chloroform (90:10), n-hexane: Chloroform (50:50), n-hexane: Chloroform (10:90), Chloroform (0:100), Chloroform: Ethyl acetate (90:10), Chloroform: Ethyl acetate (50:50), Chloroform: Ethyl acetate (10:90), Ethyl acetate (0:100), Ethyl acetate: MeOH (90:10), Ethyl acetate: MeOH (50:50), Ethyl acetate: MeOH (10:90), Ethyl acetate (0:100).The eluents (each of ten ml) were collected. Collection Fractions were evaporator using rotary evaporated to concentrate and the progress of separation was monitored by thin layer chromatography (TLC).

2.2.5.2 Thin Layer Chromatography (TLC)

Thin layer chromatography (TLC) was performed using a solvent system; n-hexane, chloroform, ethyl acetate and methanol as a mobile phase at different concentrations. No. spots were considered as criteria for fracture selection for pure compound isolation.

2.2.5.3 Preparative Thin Layer Chromatography (PTLC)

Full-size plates with a thick layer of silica were used for preparatory separation by thin layer chromatography. Up to 100 mg of compound was deposited in a thin horizontal line below the plate, and the plate was operated in an appropriate solvent system as normal. The location of the product was (ideally) determined by UV visualization imaging, and

marked with a pencil. A razor blade was used to scrape the product-containing silica from the plate.

The silica was placed in a frothy funnel and washed with a polar solvent (such as EtOAc). The pure product could be isolated from the filter. Model board dimensions: 20cm x 20cm, 2.5mm thickness SiO_2 was put into a TLC prep room. This was a large, heavy, and square glass room. It was helpful at this stage to narrow down the sample range by running a polar solvent (usually Et 2O or EtOAc) up the plate to the upper edge of the range, then evaporating the solvent (this should be repeated as needed, usually 3 times). Then in a dry room, approximately 100 ml of eluent was prepared. The ideal eluent for your compound should give an Rf of around 0.1 on a normal TLC.

This would allow multiple elutions if the first one did not separate well enough. The plate in the chamber was placed and the top was sealed with the lid or aluminum foil. A typical run took 40 mins to 1 hour. The plate was removed and visualized under UV. If your desired band(s) were not separated enough, run was repeated for another elution. If the plate needed to be stained see where the band(s) was, a thin line of the stain could be pipited down an edge of the plate, and heated.

This would reveal approximately, where the bands were located, across the entire width of the board. Another staining option was berberine (an alkaloid), which was prepared as a 0.1% solution in EtOH and should be sprayed onto the plate, and allowed to be visualized under UV light. Because it was a salt, berberine bound irreversibly to silica gel, allowing it to be removed easily. Every important strip was scraped off with a spoon or razor. Some found it easier to do this while the board was

still wet due to filtering (it had to work quickly), as dry silica, dust might tend to blow or move around more easily.

2.2.5.4 Reagent for chromatography

Anisaldehyde sulfuric acid spray was prepared by a p.anisaldehyde mixture (0.5 ml) with 10 ml of glacial acetic acid, followed by 85 ml of methanol and 5 ml of concentrated sulfuric acid in this arrangement. The reagent was sprayed onto TLC plates, which were then heated in an oven at 105 ° C until color development was complete.

2.2.6 Physical properties of the isolated compound

The appearance of the compound was observed based on visual observation and its melting point was determined on the GallenKamp instrument (Sanyo).

2.2.7 LC-MS Analysis

LC-MS preprocessing the preprocessing step in a LC-MS metabolomics platform is performed as mentioned above to optimize the resulting matrix of identified peaks and transform the data into a format that makes the subsequent statistical analysis easier and more robust. Since the preprocessing in LC-MS is more demanding comparing to NMR a series of software Introduction 171 packages have been developed for this purpose (Castillo *et al*., 2011), among them the most popular are XCMS (Smith *et al*., 2006), MZmine (Pluskal *et al*., 2010), MAVEN (Melamud *et al*., 2010), MetAlign (Lommen, 2009).

The preprocessing procedure can be separated into two main steps: the peak detection and the annotation of isotope and adduct peaks. The

peak detection procedure consists in the characterization of peaks in the three-dimensional space (time, mass, intensity) as defined by the LC-MS data and estimation of the peak intensity.

The major problem in peak detection procedure is the identification of the real peaks from the noise peaks and specifically for small abundant peaks close to noise level. According to the utilized software, different algorithms are applied for peak detection. For instance, XCMS software incorporates the matched filter algorithm (Danielsson *et al*., 2002) and cent Wave algorithm (Tautenhahn *et al*., 2008).

An important issue in the application of a given software is to understand the features and the underlying algorithms used in the software to allow optimal choice of parameters. After the peak detection step a number of features are generated including features corresponding to isotope and adduct ions that hamper the compound identification and subsequently the biological interpretation. In order to identify the isotope and adduct ions in a data matrix inheriting from the peak detection step, specific algorithms are incorporated according to the applied software. In the case of XCMS, CAMERA software is usually combined for this purpose, which has been proven efficient in isotope and adducts annotation even in high complex data (Kuhl *et al*., 2011)

2.2.8 Spectroscopic methods

2.2.8.1 Infrared spectroscopy (IR) Method

Liquid samples can be placed between two plates of potassium bromide salt. The plates are transparent to infrared light and no single drop of sample enters the KBr dish of potassium bromide and then is

compressed by another dish until it becomes a flat surface between these discs, the cell is placed in the device.

2.2.8.2 Ultraviolet visible spectroscopy (UV)

For determinations with a UV visible spectrophotometer, the sample was generally dissolved in a solvent. Unless otherwise directed in the study, the analyzers make decisions at room temperature using a 1 cm path length.

2.2.8.3 Nuclear Magnetic Resonance(NMR)

NMR spectra were recorded on a Bruker DRX-400 instrument (Rheinstetten, Germany), operating at 400 MHz for 1 hour and at 100 MHz for 13 ° C, using a 3 mm wide-band reverse probe (BBI) or 5 mm dual ^{1}H / ^{13}C Sensor using the standard Brooker pulse sequence. Spectra of the "undistorted enhancement by polarization transfer" (DEPT-135, DEPT-90) were also recorded. The chemical shifts are given in ppm and coupling constants (J) in Hz. The multiplicity was indicated as s for singlet, d for doublet, t for triplet, q for quartet 56 GENERAL EXPERIMENTAL METHODS and m for multiplet. Additionally two-dimensional NMR experiments (2D-NMR) were performed where necessary.

The 2D-NMR experiments included correlation spectroscopy (COSY) for 1H1H correlation, hetero nuclear single quantum coherence (HSQC) for direct $^{1}H^{13}C$ correlation and hetero nuclear multiple bond coherence (HMBC) for long-range $^{1}H^{13}C$ correlation. Structure elucidation was assisted by a ^{13}CNMR database (NMR Predict version 4.8.57, Modgraph®). Deuterated solvents including $CDCl_3$ (99.8% D),

DMSO-d6 (99.9 % D) and CD3OD (99.8% D) were purchased from Sigma-Aldrich.

Micro flows NMR were done at the Laboratory of Pharmacognosy and Phytochemistry, Deanship research at Prince Sattam bin Abdulaziz University,

2.2.8.4 Mass Spectrometer (MS)

Mass Spectrometry High resolution mass spectra was obtained with an Agilent™ 6530 quadruple-time-off light mass spectrometer (QTOF-MS) equipped with an Agilent™ Jetstream source that was used with the following parameters: gas temperature 325 °C, sheath gas flow 11L/min. Capillary, fragmentor and skimmer voltages were set to 3500 V, 150 V and 65 V, respectively, and OCT 1RF Vpp was set at 750 V.

The mass spectrometer was operated in positive and negative ions mode with an accuracy of 20000. The instrument was calibrated and tuned with a tune mix (G1969-85000) and during acquisition; Accuracy was monitored with Agilent ™ ES-TOF reference block solution kit (G1969-85001). Mass Hunter® (Agilent ™ Technologies) software was used for acquisition and processing. Spectrometer was operated in positive and negative ion mode at 20000 resolution. The instrument was calibrated and tuned with a tune mix (G1969-85000) and during acquisition; the accuracy was monitored by using ES-TOF reference mass solution kit (G1969-85001) from Agilent™. Mass Hunter® (Agilent™ Technologies) software was used for acquisition and processing.

Chapter Three

Results and Discussion

Chapter Three

Results and Discussion

3.1 Results

3.1.1 Yield of aqueous extract

The dried stems of *S argel (*2.5 Kg) aqueous extract yielde 400 g of extract (Table.1)

Table.1 Yield percentage of aqueous extract

Sample	Weight of sample	Weight of extract	Yield percentage
Stem of *Solenostemma argel*	2500 g	400 g	16%

3.1.2 Yield of solvent fractions

Aqueous extract with water yielding 400 g yield of aqueous extract were fractionated by organic solvents (Table. 2)

Table 2 Weights of solvent fractions of aqueous extract

Hexane fraction	chloroform fraction	Ethyl acetate fraction	n- butanol fraction	Aqueous fraction
102.6 g	8.9 g	17.5 g	98.7 g	177.9 g

3.1.3 Result of phytochemical screening

Tables 3.3 – 3.11 show phytochemical screening test of the aqueous extract.

Table 3. Test of alkaloids

Extract	Test and result	Deduction
crude extract	No precipitate with Mayer's and Dragendefts reagent	No alkaloid in crude extract
Hexane fraction	precipitate with Mayer,s and Dragendefts reagent	Presence of alkaloid in Hexane fraction
CHCl3 fraction	Heavy precipitate with Mayer,s and Dragendefts reagent	Presence of alkaloid in CHCl3 fraction
Ethyl acetate fraction	Heavy precipitate with Mayer,s and Dragendefts reagent	Presence of alkaloid in Ethyl acetate fraction
n-butanol fraction	precipitate with Mayer,s and Dragendefts reagent	Presence of alkaloid in n-butanol fraction
Aqueous extract	precipitate with Mayer,s and Dragendefts reagent	No alkaloid in Aqueous extract

Table. 4 Test of saponins

Extract	Test and result	Deduction
crude extract	The appearance of foam	Indicated the presence of saponins in crude extract and their fractions
Hexane fraction		
CHCl3 fraction		
Ethyl acetate fraction		
n-butanol fraction		
Aqueous extract		

Table. 5 Test of glycosides

Extract	Test and result	Deduction
crude extract	Pink color	Presence of glycoside
Hexane fraction	Pink color	Presence of glycoside
CHCl3 fraction	Pink color	Presence of glycoside
Ethyl acetate fraction	Pink color	Presence of glycoside
n-butanol fraction	White color	No glycoside
Aqueous extract	White color	No glycoside

Table. 6 Test of terpenoids

Extract	Test and result	Deduction
crude extract	Brown color	There's no terpenoids
Hexane fraction	Red color	Presence of terpenoid
CHCl3 fraction	Red to brown	Presence of terpenoid
Ethyl acetate fraction	Red to brown	Presence of terpenoid
n-butanol fraction	Red to brown	Presence of terpenoid
Aqueous extract	Pale white color	No terpenoids

Table. 7 Test of coumarins

Extract	Test and result	Deduction
crude extract	The appearance of red color	Indicated the presence of coumarins in crude extract and their fractions
Hexane fraction		
CHCl3 fraction		
Ethyl acetate fraction		
n-butanol fraction		
Aqueous extract		

Table. 8 Test of steroids

Extract	Test and result	Deduction
crude extract	The appearance of dark ring	Indicated the presence of steroids in crude extract
Hexane fraction	The appearance of brown ring	Indicated the presence of steroids in Hexane fraction
CHCl3 fraction	The appearance of brown ring	Indicated the presence of steroids in CHCl3 fraction
Ethyl acetate fraction	The appearance of brown ring	Indicated the presence of steroids in Ethyl acetate fraction
n-butanol fraction	The appearance of yellow ring	Indicated the presence of steroids in n-butanol fraction
Aqueous extract	Pale white color	No steroids Aqueous extract

Table. 9 Test of flavonoids

Extract	Test and result	Deduction
crude extract	Yellow color	Indicated the presence of flavonoids in crude extract and their fractions
Hexane fraction		
CHCl3 fraction		
Ethyl acetate fraction		
n-butanol fraction		
Aqueous extract		

Table. 10 Test of tannins

Extract	Test and result	Deduction
crude extract	No precipitate and no green or dark color	Indicated the absent of tannin
Hexane fraction	No color	Indicated the absent of tannin
CHCl3 fraction	Dark blue color	Indicated the presence of tannin
Ethyl acetate fraction	Greenish black	Indicated the presence of tannin
n-butanol fraction	No precipitate and no green or dark color	Indicated the absent of tannin
Aqueous extract	No precipitate and no green or dark color	Indicated the absent of tannin

Table. 11 Test of anthraquinones

Extract	Test and result	Deduction
crude extract	Gave negative test	Indicated the absent of anthraquinone in crude extract and their fractions
Hexane fraction		
CHCl3 fraction		
Ethyl acetate fraction		
n-butanol fraction		
Aqueous extract		

3.1.4 Assessment of Antimicrobial Activity of Extracts

3.1.4.1 Testing of Extracts for bacterial Activity

Four types of bacteria *Escherichia coli* (*E.c*), *Pseudomonas aeruginsa (p.s*), *Staphylococcus aureus (S.a), Bacillus subtilis (B.s),* were treated with the plant extract fractions. The inhibition zone are shown in (Table. 12). The results indicated low activity with n- hexane and water extract against the four bacteria. Chloroform extract and Ethyl acetate extract showed high activity with four bacteria.

Table. 12 Preliminary screening of antibacterial activity of *Solenostemma argel* stems against standard organisms (*E.c, P.s, S.a, and B.s*) and **Inhibition zone (in mm)**

Fractions	*E.c*	*p.s*	*S.a*	*B.s*
crude extract	14	13	13	11
Hexane fraction	12	14	10	14
$CHCl_3$ fraction	19	18	20	22
Ethyl acetate fraction	18	20	18	19
n-butanol fraction	14	15	11	12
Aqueous extract	10	11	09	11

(10-14) low activity, (14-18) medium activity and (over 18) high activity.

The Inhibition zone of *E.c, S.a, B.s* and *P.s* bacteria caused by $CHCl_3$ extract fraction are shown in figures 2, 3, 4, and 5, respectively.

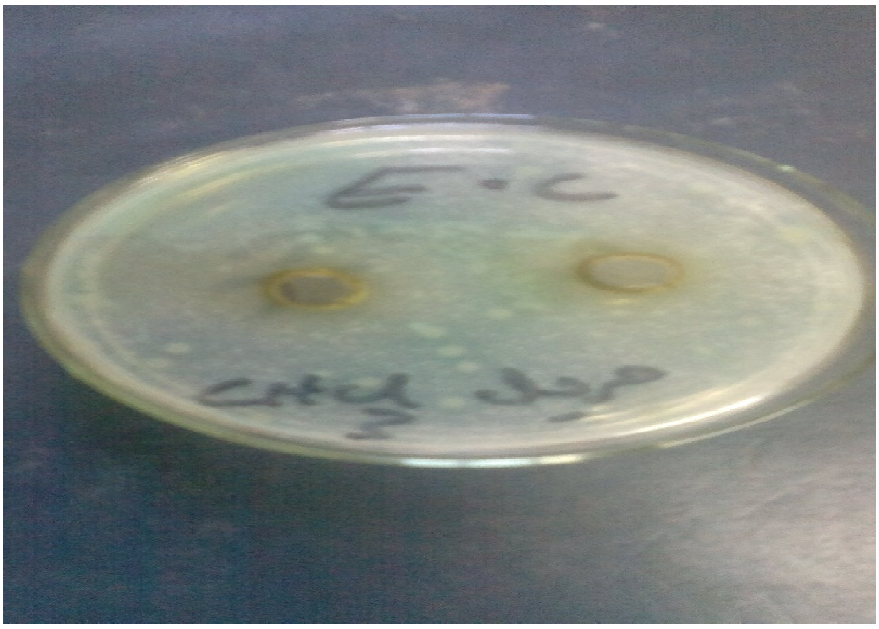


Figure (2): Inhibition zone of bacteria *E c* with CHCl3 extract

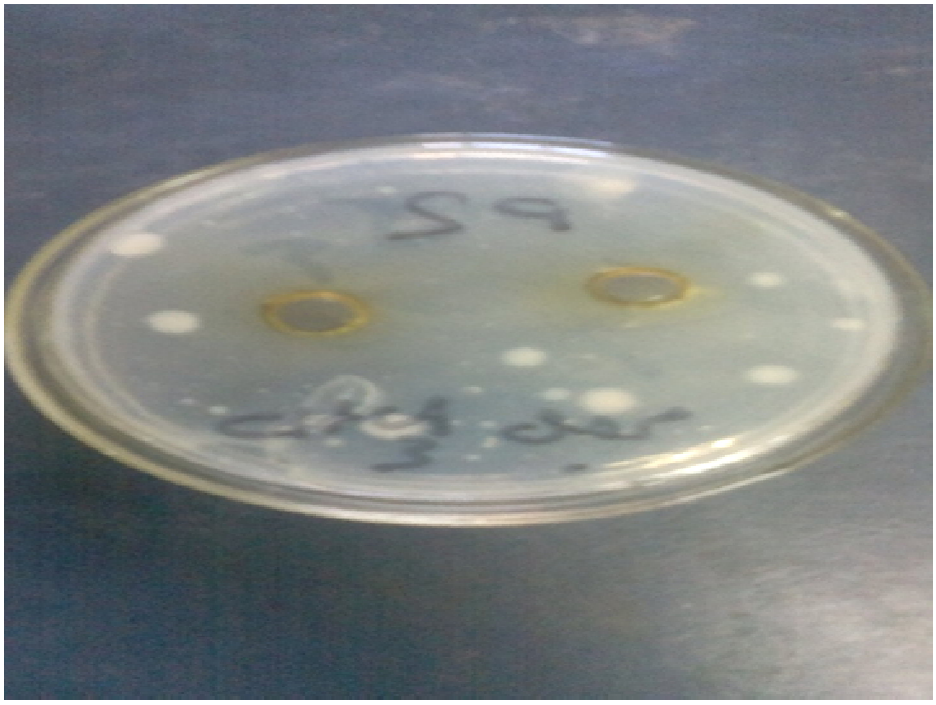


Figure (3): Inhibition zone of bacteria *S a* with $CHCl_3$ extract

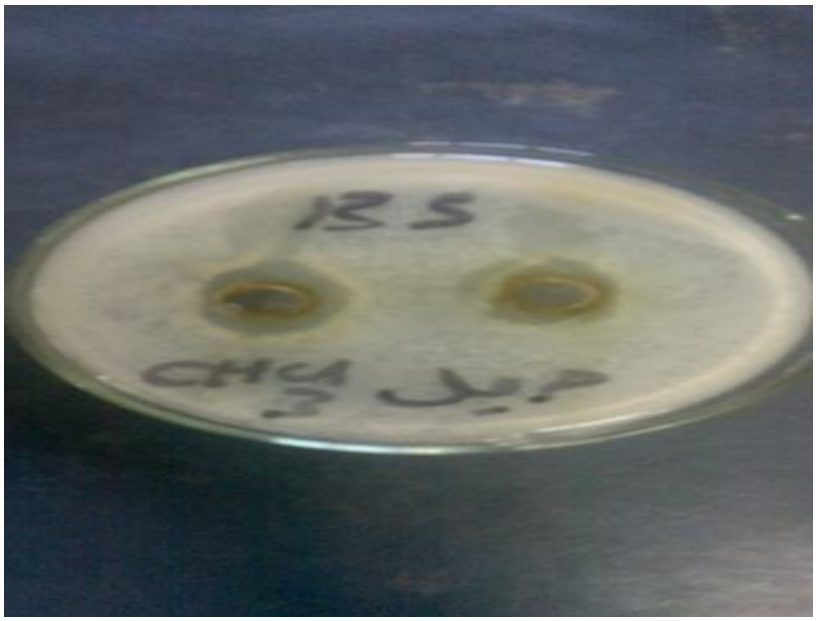


Figure (4): Inhibition zone of bacteria *B s* with CHCl3 extract

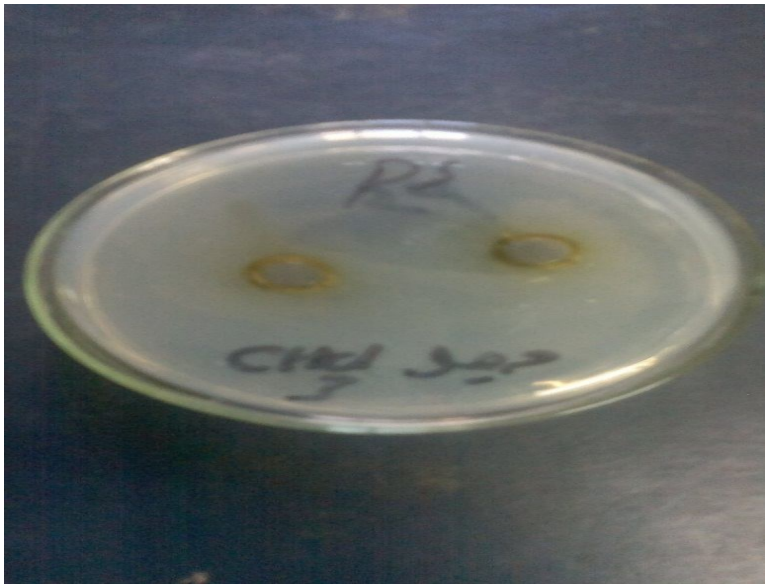


Figure (5): Inhibition zone of bacteria *P s* with CHCl3 extract

With ethyl acetate, the inhibition zones of these four bacteria are shown in figures 6, 7, 8 and 9 respectively.

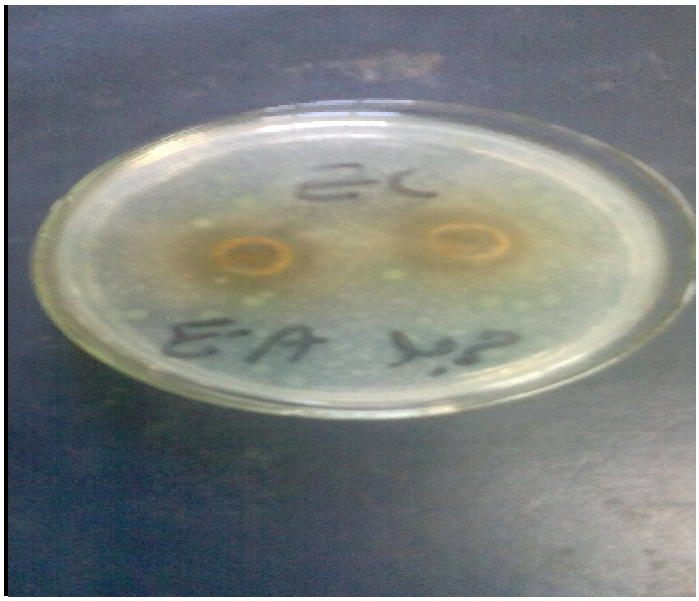


Figure (6): Inhibition zone of bacteria *E c* with ethyl acetate extrac

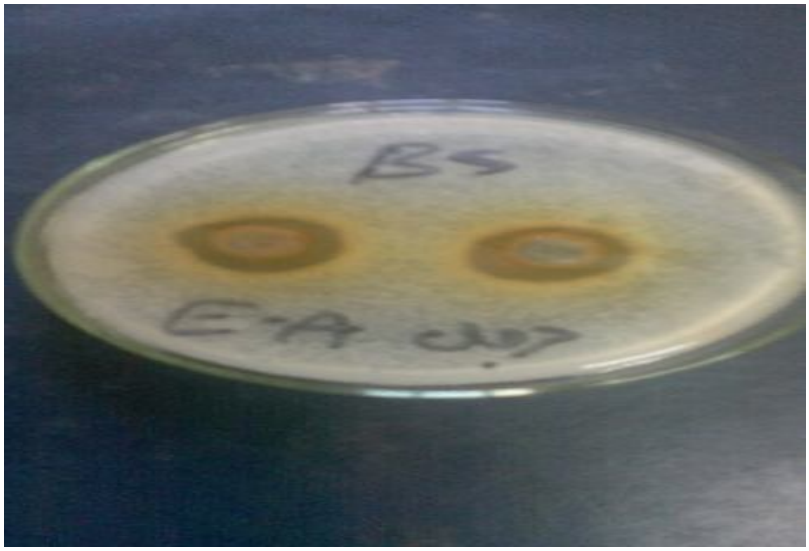


Figure (7): Inhibition zone of bacteria *B s* with ethyl acetate extract

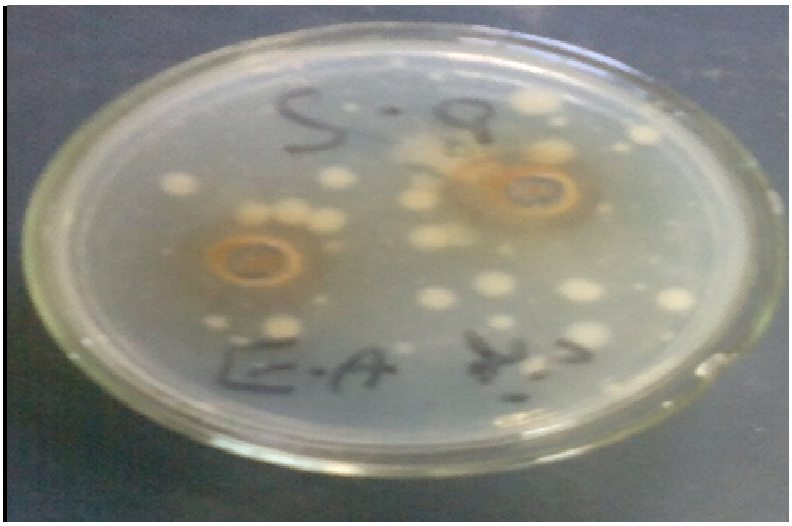


Figure (8): Inhibition zone of bacteria *S a* with ethyl acetate extract

Figure (9): Inhibition zone of bacteria *P s* with ethyl acetate extract

3.1.4.2 Testing of Extracts for Antifungal Activity

Here again, n- hexane and water extract of *S. argel* showed negative activity against the growth of standard fungi: *Candida albicans* (*C.a*) *and Aspergillus niger (A.n)* Table. 13

Table. 13 Preliminary screening for antifungal activity of *Solenostemma argel* (stems) extracts against standard organisms *Candida albicans* (*C.a*), *Aspergillus niger (A.n).*

Inhibition zone (in mm)

Fractions	*A. n*	*C. a*
crude extract	**17**	**16**
Hexane Fractions	**15**	**15**
$CHCl_3$ Fractions	**20**	**15**
Ethyl acetate Fractions	**18**	**16**
n-butanol Fractions	**14**	**13**
Aqueous extract	**12**	**10**

(10-14) low activity, (14-18) medium activity and (over 18) high activity.

The Inhibition zone of *A. n.* fungi caused by ethyl acetate extract and $CHCl_3$ fractions of *S. argel* stem are shown in figure. 10 and 11 respectively.

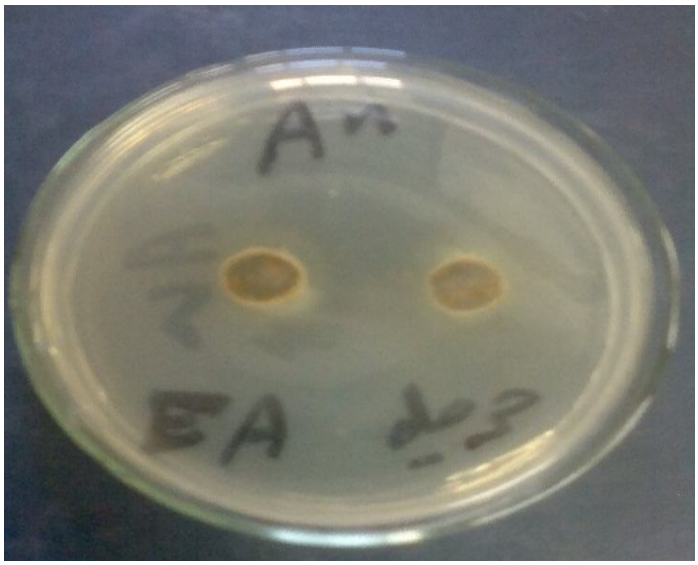


Figure (10): Inhibition zone of *A n* fungi with ethyl acetate extract

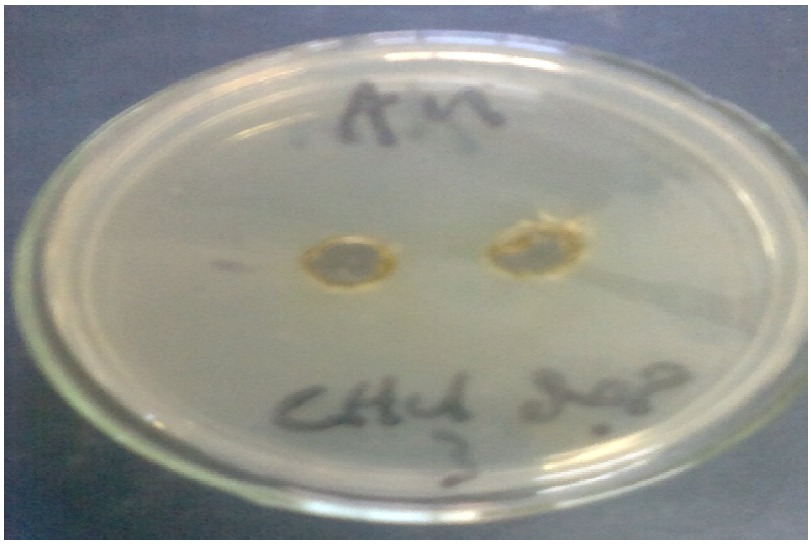


Figure (11): Inhibition zone of *A n* fungi with CHCl3 extract

3.1.5 Testing the Anti-insecticidal Activity

To assist the anti-insecticidal activity of the extracts, the mortality of the third larval instars of *Tribolium castaneum* was counted during 24hours as shown in Table. 14

Table. 14 Results of mortality of third larval instars of *Tribolium castaneum*, in 24 hrs.

Fractions	R_1	R_2	R_3	$\sum$	Mortality%
n- hexane	2	1	2	5	16.7%
$CHCl_3$	3	4	3	10	33.3%
Ethyl acetate	3	4	2	9	30%
n- butanol	1	0	0	1	3.3%
Aqueous extract	0	1	1	2	6.7%
Crude extreact	1	1	0	2	6.7%
Control	0	1	1	2	6.7%

3.1.6 Results of the samples analysis of minerals

Table 15 shows the results of the elemental content determined in stems of *S. argel*

Table. 15 Results of the minerals content of stems of *S. argel* plant

Micro elements (1mg) in 50 ml.						
Sample	Ca	Cu	Fe	Mg	Mn	Zn
Argel	21.10	0.014	1.055	29.95	0.059	0.052
Macro elements (1mg).						
Sample	N%	P%	S%	K%	----------	
Argel	1.96	0.48	0.050	0.81618	----------	

3.1.7 Result of Column chromatography

The Chloroform crude extract was further purified by column chromatography, and elution was carried out from non-polar to polar solvents by gradient elution method.

Isolation of active ingredient from chloroform extract of *Solenostemma argel.*

Different fractions namely F1, F2, F3…and F33 were eluted (Table. 16). Fraction F1, F2, F7, F14, F16, F20, F27, F29 and F32 did not yield residue after evaporation and residue of the fractions showed less quantity.

Fraction F9 eluted with chloroform and ethyl acetate (90:10) showed two spots on TLC, (1.24gm), which were subjected for preparative thin layer chromatography, PTLC, to obtain of two purified components, compound A (0.70) and compound B (0.49).

Fraction F21 –F24 eluted with chloroform and methanol (90:10) showed single spot on TLC and purified by PTLC to obtain compound C. in considerable quantity (0.83 g).

From compounds A, B and C the respective mobile phase were evaporated and were hence subjected for further investigation by spectral analyses, IR, UV, NMR and MS.

Table. 16: Different fractions of Column Chromatography and their Mobility Relative to Front (Rf) values:

S. No	Mobile phase	Ratio %	Total fractions obtained	No of spots	Rf value
1	n-hexane	100:0	-	-	-
	n-hexane:$CHCl_3$	90:10	-		
	n-hexane:$CHCl_3$	50:50	-		
	n-hexane: $CHCl_3$	10:90	Fra. 1 Fra. 2 Fra. 3	- - 1	- - 0.901
S. No	Mobile phase	Ratio %	Total fractions obtained	No of spots	Rf value
	$CHCl_3$	100: 00	Fra. 4 Fra. 5 Fra. 6 Fra. 7	1 2 1 -	0.975 0.894..0.865 0.865
	$CHCl_3$:ethyl acetate	90:10	Fra. 8 Fra. 9 Fra. 10 Fra. 11	1 1 1 1	0.876 0.888..0.853 0.843 0.821
	$CHCl_3$:ethyl acetate	50:50	Fra. 12 Fra. 13 Fra. 14	2 1 -	0.811..0.871 0.843 -
	CHCl3:ethyl acetate	10:90	Fra. 15 Fra. 16 Fra. 17 Fra. 18	1 - 2 3	0.888 - 0.777 0.862..0.874..0.833
	Ethyl acetate	100:00	Fra. 19 Fra. 20	1 -	0.854 -

S. No	Mobile phase	Ratio %	Total fractions obtained	No of spots	Rf value
	ethyl acetate: MeOH	90:10	Fra. 21 Fra. 22 Fra. 23 Fra. 24 Fra. 25	1 1 1 1 1	0.811 0.811 0.811 0.811 0.804
	ethyl acetate: MeOH	50:50	Fra. 26 Fra. 27 Fra. 28 Fra, 29	1 - 1 -	0.767 - 0.666 -
	ethyl acetate: MeOH	10:90	Fra. 30 Fra. 31 Fra. 32 Fra. 33	2 1 - 1	0.877..0.854 0.767 - 0.765

Figures 12 and 13 show the T L C profile of total plant extract and considerable fractions of the active isolated compounds, respectively. Figures 14 and 15 however show the PTLC profile of fractions 21 and 9.

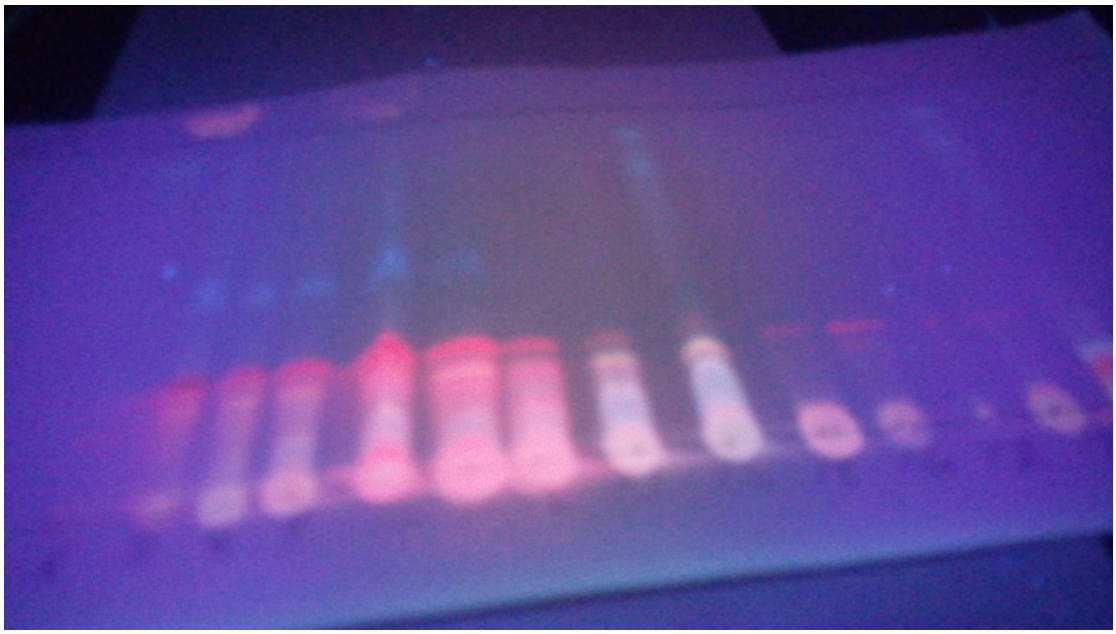


Figure (12): TLC profile of total plant extract

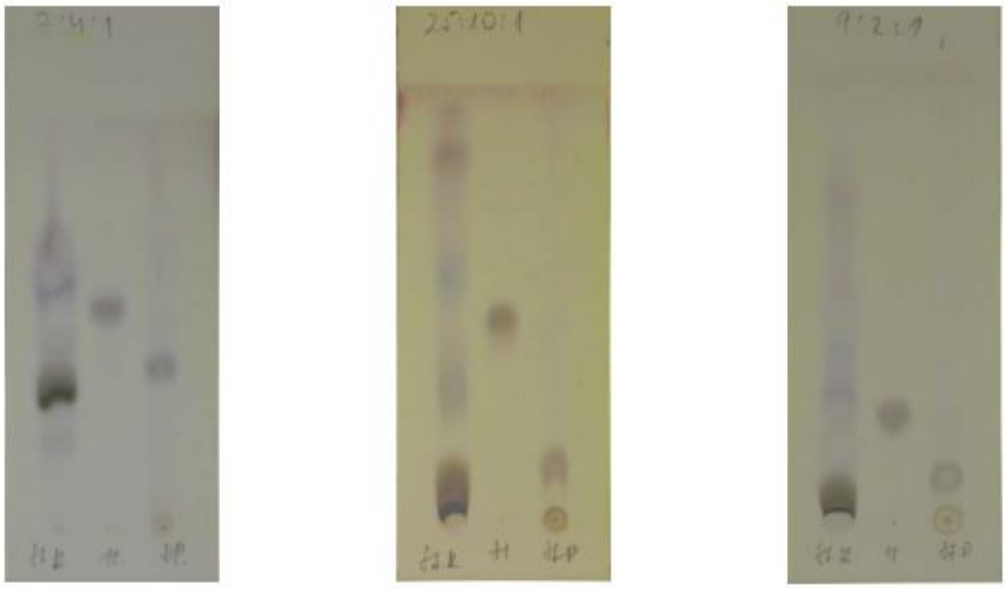


Figure (13): TLC profile of considerable fractions

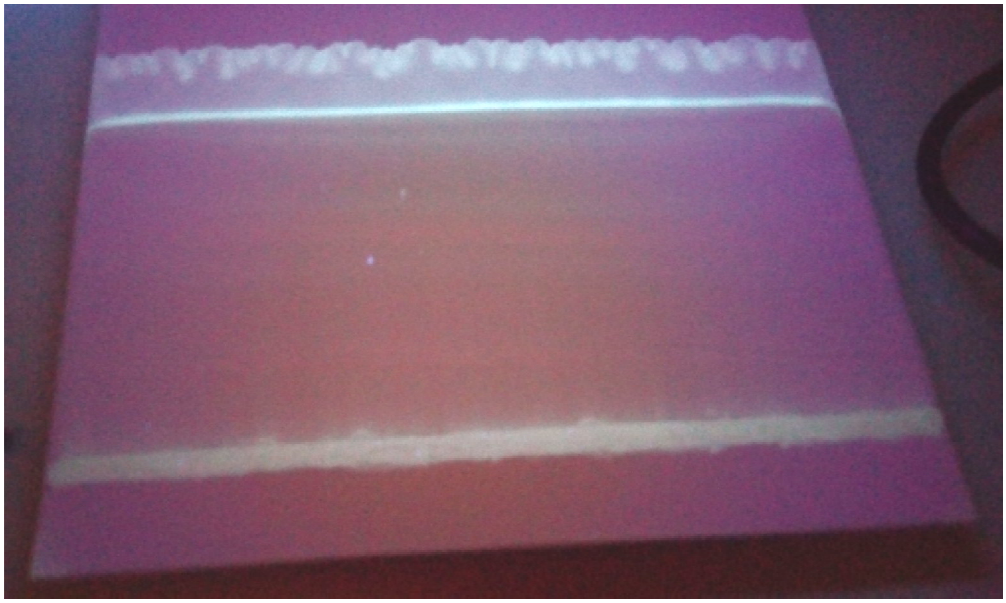


Figure (14): PTLC profile of fraction 21

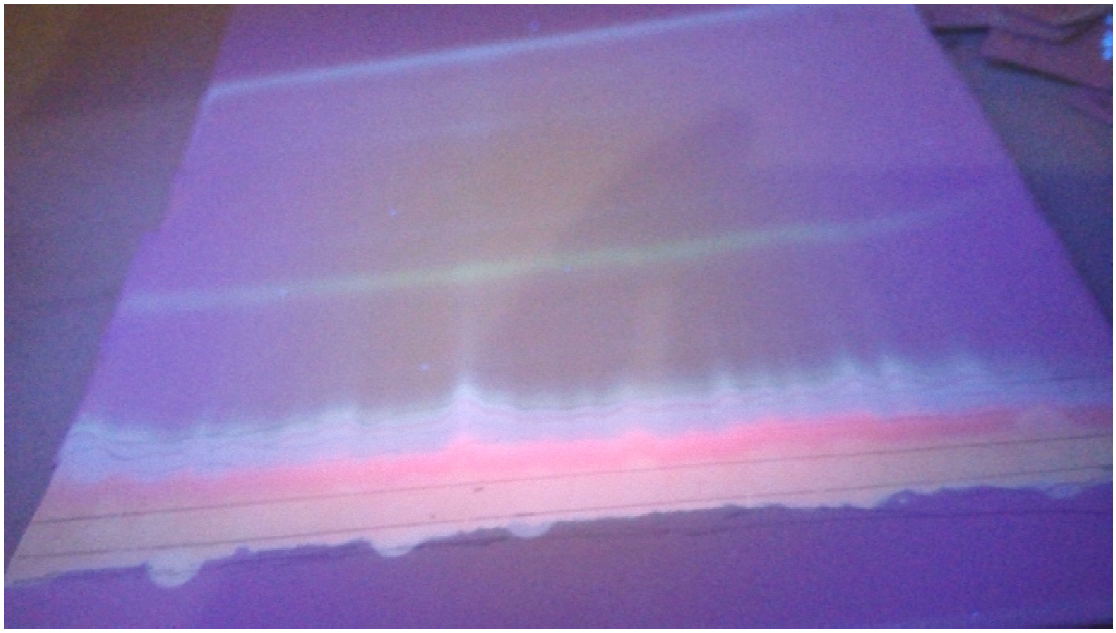


Figure (15): PTLC profile of fraction 9

3.1.8 Spectral data

3.1.8.1 Spectral data of compound A

Compound A is a pale white powder, melts at 278 ^{o}C -280 ^{o}C, separating at Rf value of 0.544, and giving a positive Liebermann- Burchard test proved to be of steroid base. Its structure was elucidated as ((E)-2-((17-(5-ethyl-6-methylhept-3-en-2-yl)-10,13-dimethyl 2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta [a] phenanthren-3-yl)oxy)-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol), to be stigma sterol glucoside

UV spectrum

The UV spectrum of compound (A) recorded in methanol shows an absorption peak at 210 nm.

IR spectrum

The IR spectrum of compound (A), shows broad band at 3437 cm^{-1} which clearly indicated the O-H str. Vib; the spectrum shows aromatic C=C str. vib. of carbon at 1564 cm^{-1} , bending band appearing at 2927 cm^{-1} indicating the presence of aliphatic C-H str.vib. and CO phenolic str. vib. appearing at 1063 cm^{-1}.

Mass spectrum: m/z 574.8 Molecular Formula: $C_{35}H_{58}O_6$

NMR spectrum

^{1}H NMR (500 MHz, DMSO-d6, δ, ppm)

1.76 & 0.96 (2H, m, H-1), 1.52 & 1.37 (2H, m, H-2), 3.46 (1H, m, H-3), 1.89 & 1.14 (1H, d, H-4), 5.32 (1H, d, H-6), 1.92 & 1.46 (2H, m, H-7), 1.51 (1H, m, H-8), 0.95 (1H, m, H-9), 1.22 & 1.16 (2H, m, H-11), 1.92 & 1.13(1H, m, H-12), 1.09 (1H, m, H-14), 1.48 & 0.97 (2H, m, H-15), 1.13& 1.63 (H, m, H-16), 1.04 (1H, m, H-17), 0.67 (3H, s, H-18), 0.98 (3H, s, H-19), 2.02 (1H, m, H-20), 0.99 (H, d, H-21), 5.17 (1H, d, H-22),

5.04 (1H, d , H-23), 1.50 (1H, m, H-24), 1.63 (1H, m, H-25), 0.82 (3H, d, H-26) 0.80 (3H, d, H-27), 1,01 &1,03 (2H, d, H-28), 0.81 (3H, d, H-29), 4.22 (1H, d, H-1'), 3,37 (1H, m, H-2'), 3.11 (1H, m, H-3'), 3.06 (1H, m, H-4'), 3.37 (1H, m, H-5'), 3.57 & 3.46(1H, m, H-6'), 4.89 (1H, s, 2'OH), 4.92 (1H, s,3,OH), 4.85 (1H, s, 4'OH), 4.43 (1H, s, 6'OH).

^{13}C NMR (500 MHz, DMSO-d6, δ, ppm)

37.3618 (C-1), 31.9440 (C-2), 77.4323 (C-3), 39.6987 (C-4), 140.9957 (C-5), 121.7283 (C-6), 29.7979 (C-7), 31.9940 (C-8), 50.1527 (C-9) 36.7704 (C-10), 21.6522 (C-11), 38.8403 (C-12), 42.2836 (C-13), 55.8662 (C-14), 24.4278 (C-15), 29.0539 (C-16), 56.7914 (C-17), 12.3905 (C-18), 19.3916 (C-19), 40.0325 (C-20), 21.4996 (C-21), 138.5921 (C-22), 129.3590 (C-23) 51.1256 (C-24), 31.8773 (C-25), 21.1180 (C-26), 19.6491 (C-27), 25.5871 (C-28), 12.6766 (C-29), 101.2972 (C-1'), 70.6220 (C-2'), 77.2892 (C-3'), 73.9985 (C-4'), 73.0828 (C-5'), 61.6178 (C-6').

The NMR data measured for compound A (Table. 17) were closely similar to those reported for stigmasterol glucoside **(**Feiha,*et al*., 2009**),** ((E)-2-((17-(5-ethyl-6-methylhept-3-en-2-yl)-10,13-dimethyl 2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl)oxy)-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol).

Figure. 16 shows the elucidated structure of compound A (stigmasterol glucoside).

3.1.8.2 Spectral data of compound B

Compound B obtained having a White crystalline solid, melting at 144 °C -146 °C, separating at Rf of 0.499, and giving a positive Liebermann-Burchard test, was elucidated by (2-{[14-(5-ethyl-6-methylheptan-2-yl)-

2,15-dimethyltetracyclo[8.7.0.0^{2},^{7}.0^{11},^{15}]heptadecan-5-yl]oxy}-6-(hydroxymethyl)oxane-3,4,5-triol (Beta-Sitosterol-3-O-Glucoside)) , to be beta Sitosterol Glucoside

UV spectrum

The UV spectrum of compound (B) recorded in methanol showed an absorption beak at 210 nm.

IR spectrum

The IR spectrum of compound (B) shows broadband at 3422 cm^{-1} that clearly indicated the O-H str. vib. . The spectrum shows aromatic C=C str. vib. of carbon at 1415 & 1568 cm^{-1}. Bending band appearing at 2926 cm^{-1} indicated the presence of aliphatic C-H str.vib. and CO phenolic str. vib. appearing at 1050 cm^{-1}.

Mass spectrum: m/z 578.8 Chemical Formula $C_{35}H_{62}O_6$

NMR spectrum

^{1}H NMR (500 MHz, DMSO-d6, δ, ppm)

^{1}H NMR (500 MHz, DMSO-d6, δ, ppm): 0.52(3H, s, H-18), 0.96(3H, s, H-19), 0.91(3H, m, H-21), 0.82(3H, m, H-26), 0.78(3H, m, H-27), 0.67(3H, m, H-29), 3.64(1H, m, H-3), 5.33(1H brs, H-6)

^{13}C NMR (500 MHz, DMSO-d6, δ, ppm)

37.30(C-1), 29.56(C-2), 77.42(C-3), 42.22 (C-4), 138.51(C-5), 129.46(C-6), 31.85(C-7), 31.80(C-8), 51.07 (C-9), 37.02 (C-10), 20.19(C-11), (C-12), 43.29(C-13), 55.68(C-14), 24.36(C- 15), 25.34(C-16), 54.95(C-17), 12.59(C-18), 19.32(C- 19), 36.70(C-20), 19.58(C-21), 34.38(C-22), 28.60(C-23), 49.15(C-24), 29.72(C-25), 21.41 (C-26), 21.72(C-27), 23.09(C-28), 13.26 (C-29), 101.32, 73.95, 77.22, 77.16, 70.57, and 61.58 (six carbons–glucose unit).

The NMR of compound B (Table 17) were closely similar to those reported for *β*-sitosterol glucoside **(**Feiha,*et al*., 2009**).**

Figure. 17 shows the elucidated structure of compound B (*β*-sitosterol glucoside).

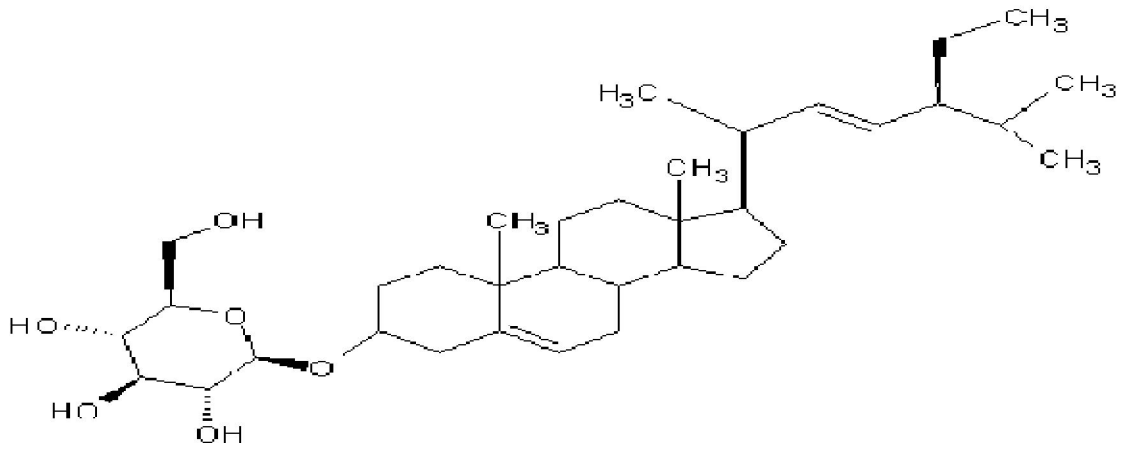


Figure (16): stigma sterol glucoside

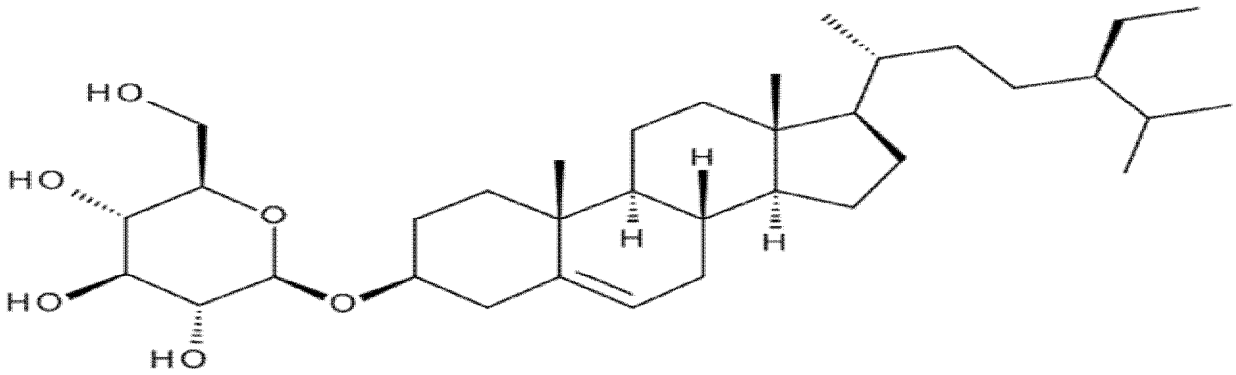


Figure. 17: β-Sitosterol -3-O- Glucoside

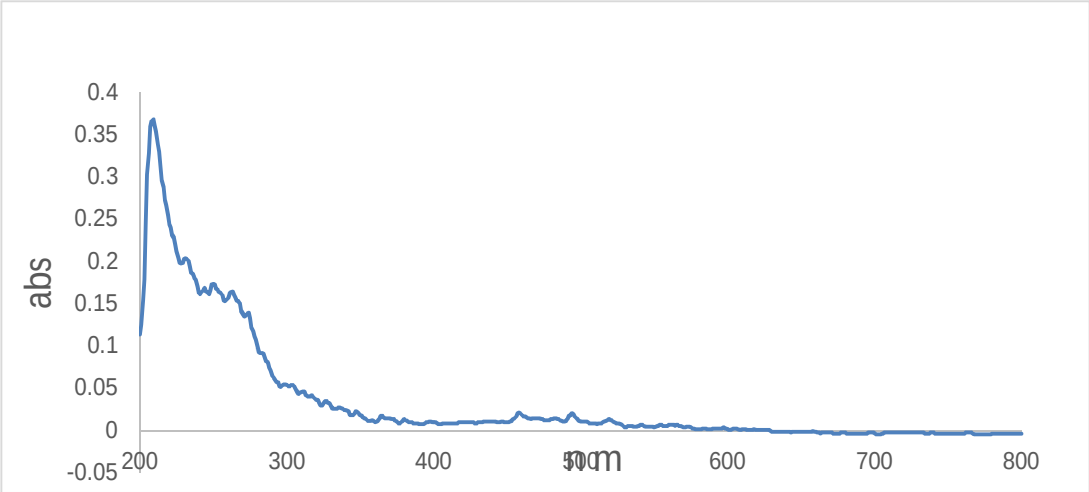
Table 17: NMR spectral data (δ ppm) of compounds 1 and 2 in DMSO.

Parameter				
	Compound 1.		Compound 2.	
Position	**δ H (ppm); multiplicity; J (Hz)**	**δ C (ppm)**	**δ H (ppm); multiplicity; J (Hz)**	**δ C (ppm)**
C-1	1.76 (m, 2H)	38.79	1.39 (m,2H)	37.28
C-2	1.50 (m, 2H)	33.82	1.39 (m, 2H)	29.44
C-3	3.42 (dd, j = 8.4)	70.53	3.64 (m, 1H)	77.42
C-4	1.80 (br d, j = 10.2)	36.76	1.39 (m, 2H)	42.23
C-5	-	140.92	-	137.99
C-6	5.32 (br d, j = 4.5)	121.65	5.33 (d, j = 7.8, 1H)	127.75
C-7	1.46 (m, 2H)	29.73	1.39 (m,2H)	31.85
C-8	1.55 (s, 1H)	31.88	1.39 (m,1H)	31.90
C-9	0.99 (s, 1H)	50.10	1.39 (m,1H)	51.07
C-10	-	36.94	-	37.06
C-11	1.39 (m,2H)	36.69	1.39-2.36(m,2H)	22.19
C-12	1.93 (m, 1H)	22.58	1.39-2.36(m,2H)	38.89
C-13	-	42.21	-	42.99
C-14	1.04 (m,1H)	40.47	1.39 (m,1H)	55.88
C-15	1.79 (m, 2H)	56.74	1.39 (m, 2H)	34.79
C-16	1.81 (m, 2H)	12.31	1.39 (m,2H)	44.29
C-17	1.14 (m, 1H)	55.81	1.39 (m, 1H)	55.68
C-18	0.96 (s, 3H)	12.06	0.52 (s, 3H)	12.59
C-19	0.91(s, 3H)	19.57	0.96 (s, 3H)	24.35

C-20	2.02 (m, 1H)	24.36	1.39 (m, 1H)	54.95
C-21	0.91 (d, j = 7.2, 3H)	31.20	0.91 (d, j = 5.6, 3H)	12.59
C-22	5.18 (dd, j = 8.4 & 15)	129.30	0.82 (m, 2H)	19.32
C-23	5.04 (dd, j = 8.4 & 8.1)	121.58	0.82 (m, 2H)	36.70
C-24	1.84 (s, 2H)	31.85	1.51 (m,1H)	49.15
C-25	1.63 (m, 1H)	37.31	1.94 (m,1H)	25.44
C-26	0.82 (d, j = 6.3)	21.42	0.82 (d, j = 7.2, 3H)	21.38
C-27	0.79 (d, j = 6.9)	28.99	0.78 (d, j = 8.0, 3H)	21.15
C-28	1.44 (s, 2H)	25.36	0.82 (m, 2H)	29.85
C-29	0.94 (d, j = 8.1)	20.18	0.67 (d, j = 8.0, 3H)	21.41
C-1,	4.23 (d, j = 7.8)	98.28	5.03 (m, 1H)	101.30
C-2.	3.37 (m, 1H)	70.53	3.06-3.64 (m,1H)	73.90
C-3,	3.14 (m, 1H)	77.23	3.06-3.64 (m,1H)	77.42
C-4,	3.06 (m, 1H)	73.99	3.06-3.64 (m,1H)	70.57
C-5,	3.04 (m, 1H)	73.08	3.06-3.64 (m,1H)	77.16
C-6'	3.66 (m, 1H)	61.53	3.06-3.64 (m,2H)	61.56
C-2'OH	4.77 (s, 1H)			
C-3'OH	4.75 (s, 1H)			
C-4'OH	4.83 (s, 1H)			
C-6'OH	3.89 (t, j = 6.0)			

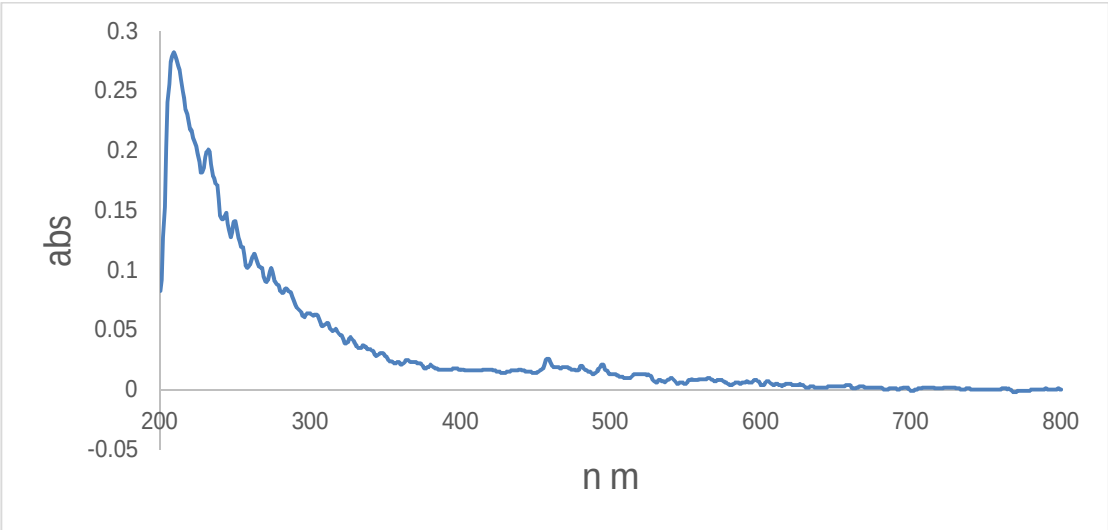
[a] ***J*** in parentheses in Hz

Figures. 18 and 19 show UV spectrum of stigma sterol glucoside and beta sitosterol glucoside respectively



Sa A	length	Abs
	210 nm	0.368

Figure. 18: UV spectrum of stigma sterol glucoside



Sa C	length	Abs
	209 nm	0.283

Figure. 19: UV spectrum of beta sitosterol glucoside

Figures. 20 and 21 show IR spectrum of stigma sterol glucoside and beta sitosterol glucoside, respectively

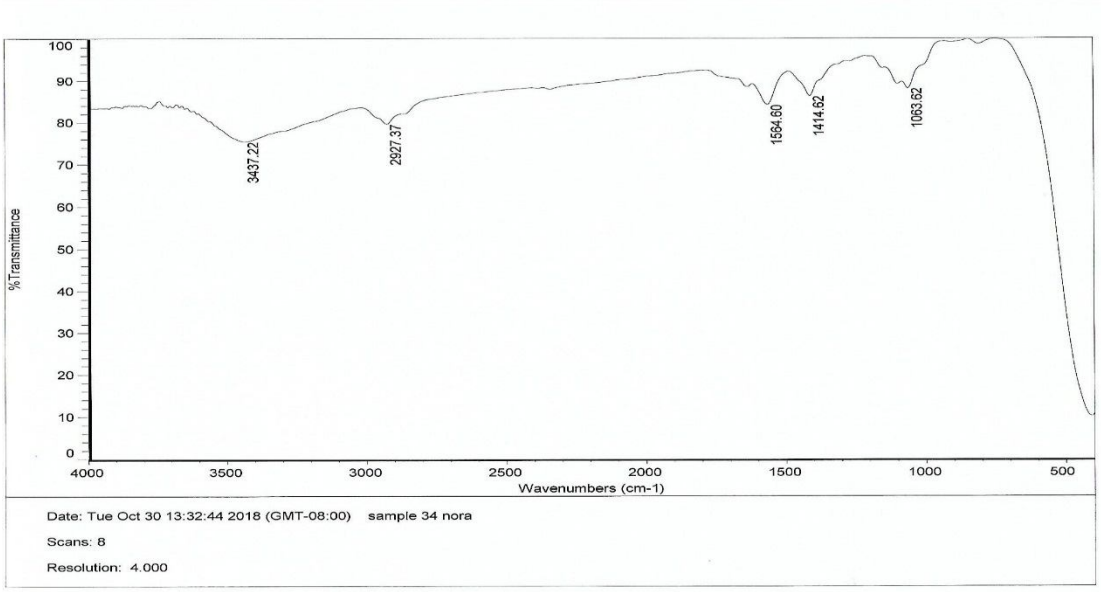


Figure. 20: IR spectrum of stigma sterol glucoside

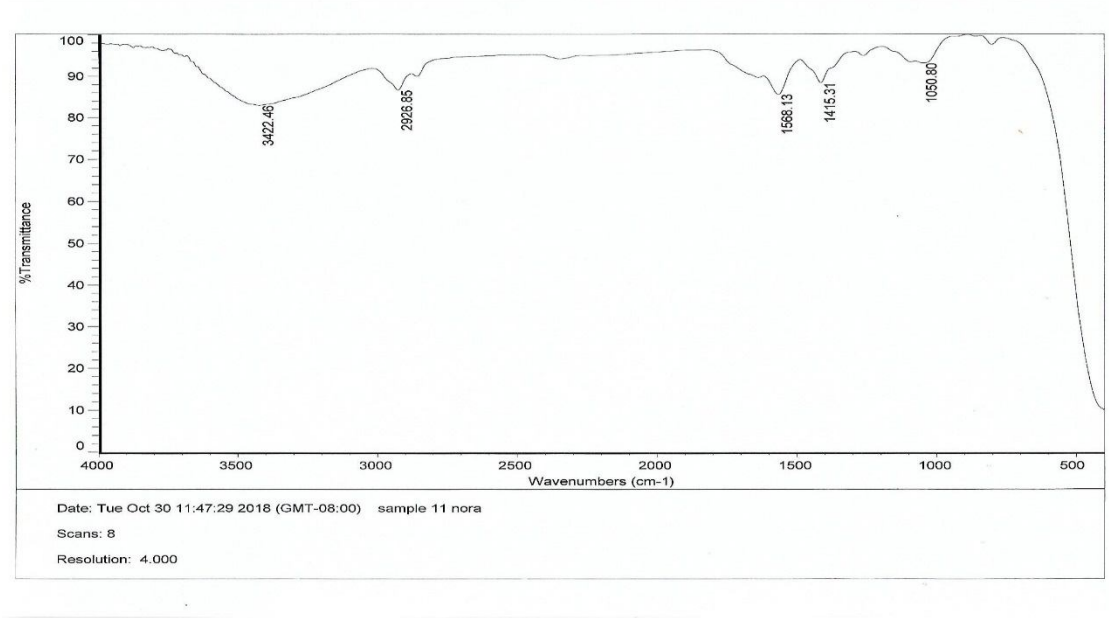


Figure. 21: IR spectrum of beta sitosterol glucoside

Figures. 22 and 23 show LC MS of stigma sterol glucoside and beta sitosterol glucoside, respectively

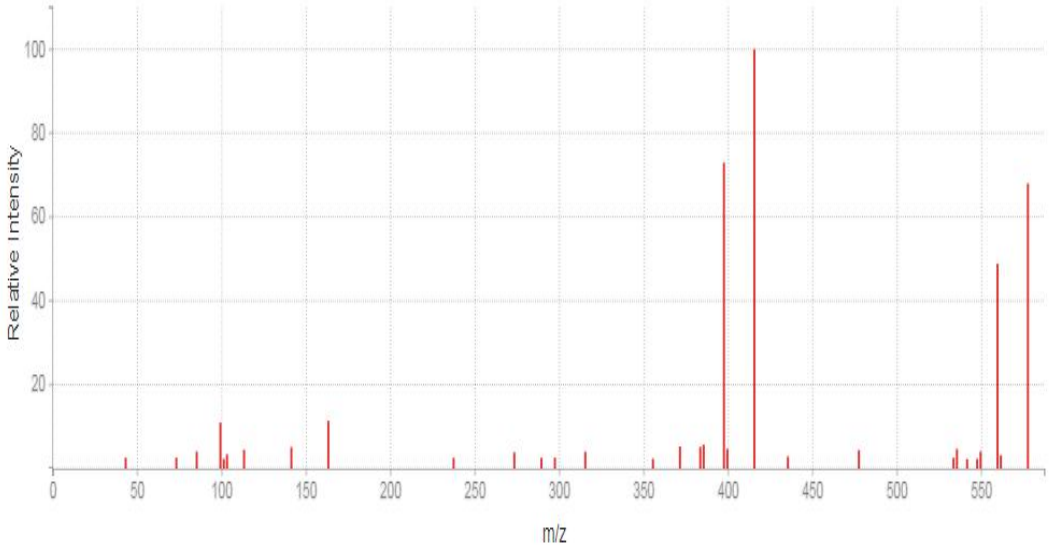


Figure. 22: LC MS of stigma sterol glucoside

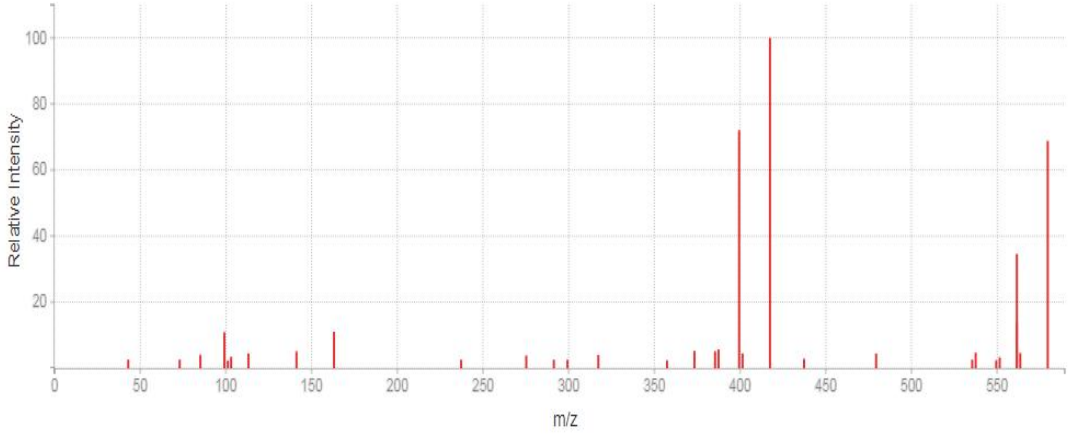


Figure.23: LC MS of beta sitosterol glucoside

Figures. 24, 25, 26 … and 36 show NMR spectrum of stigma sterol glucoside (fra. 9.1) and beta sitosterol glucoside (fra. 9.2), respectively

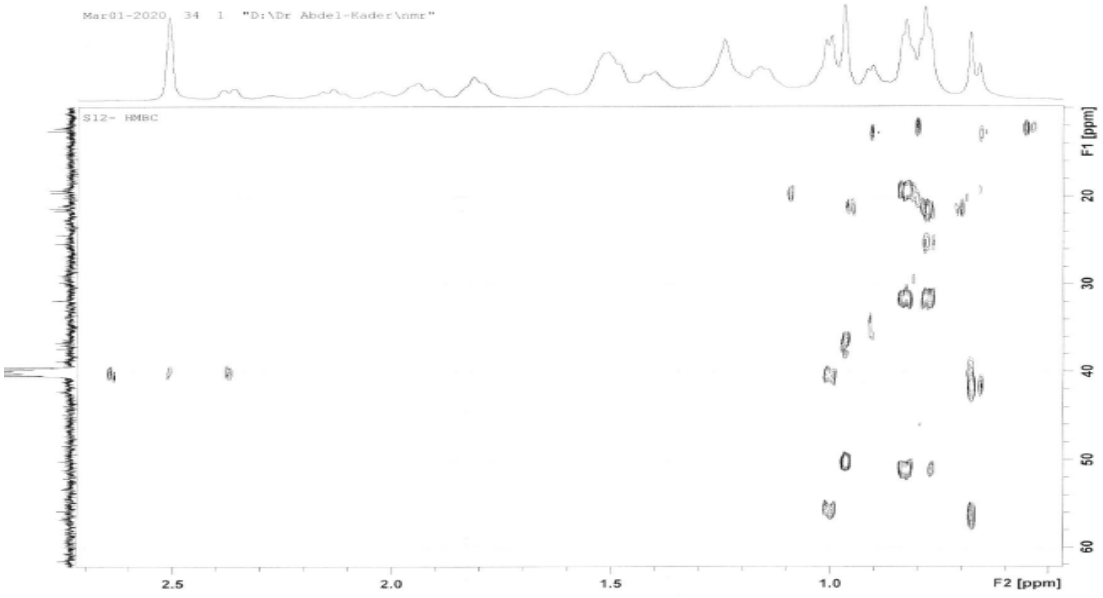


Figure. 24: Partial HMPC spectrum of fraction 9. 1 in DMSO

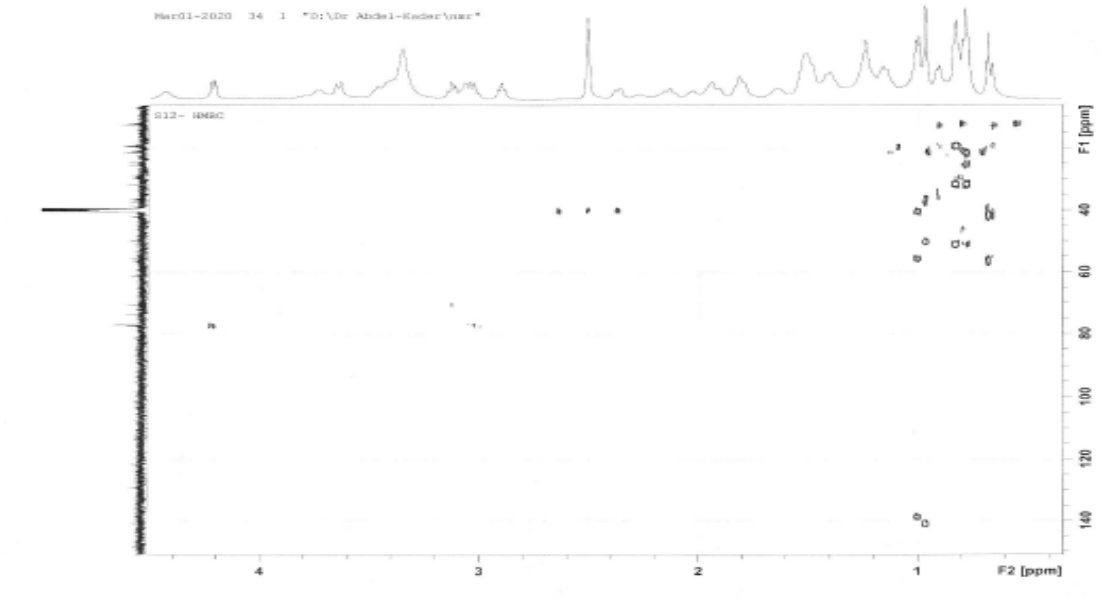


Figure. 25: Partial HMPC spectrum of fraction 9. 2 in DMSO

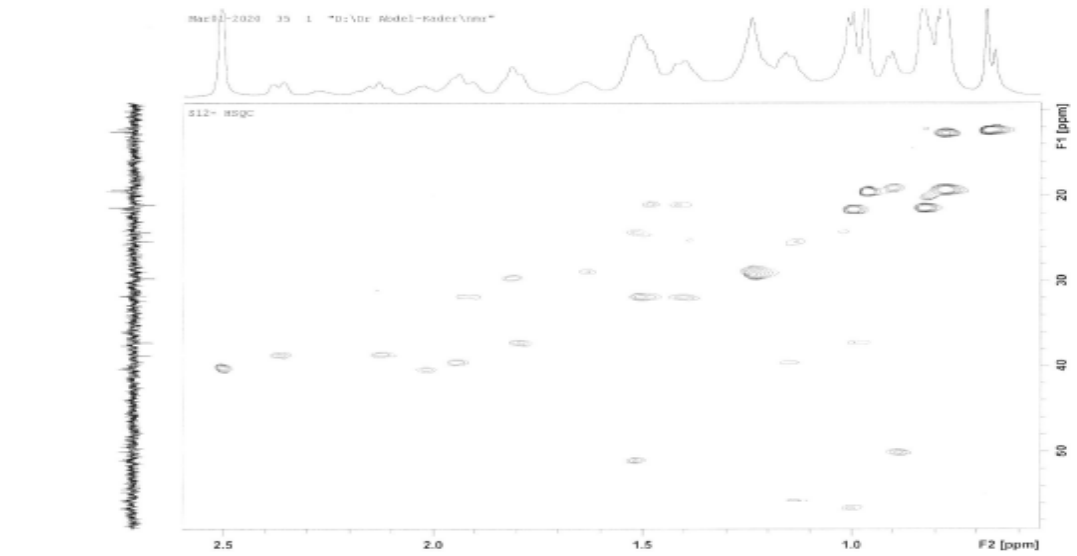


Figure. 26: Partial HSQC spectrum of fraction 9. 1 in DMSO

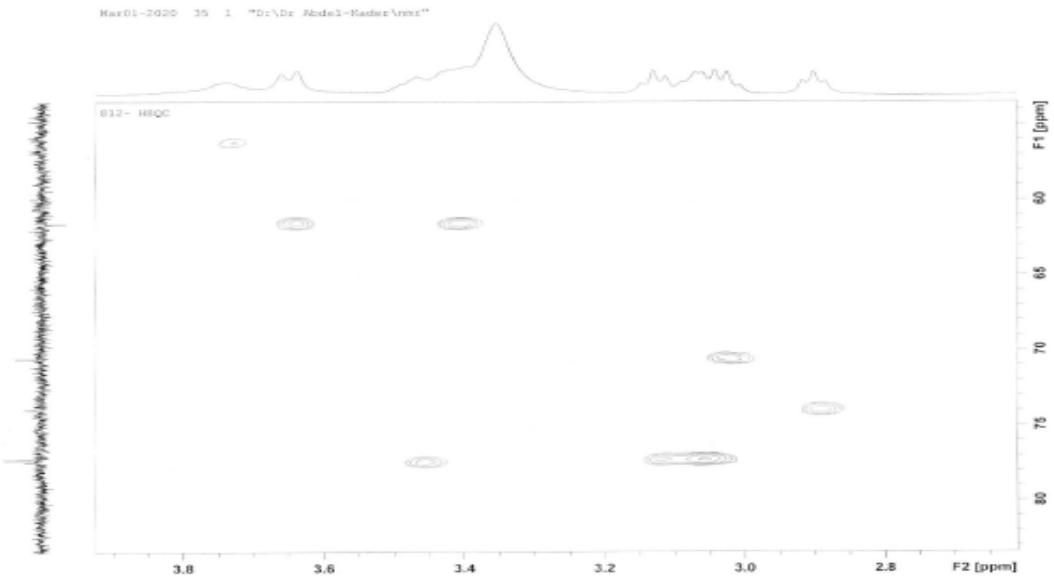


Figure. 27: Partial HSQC spectrum of fraction 9. 1 in DMSO

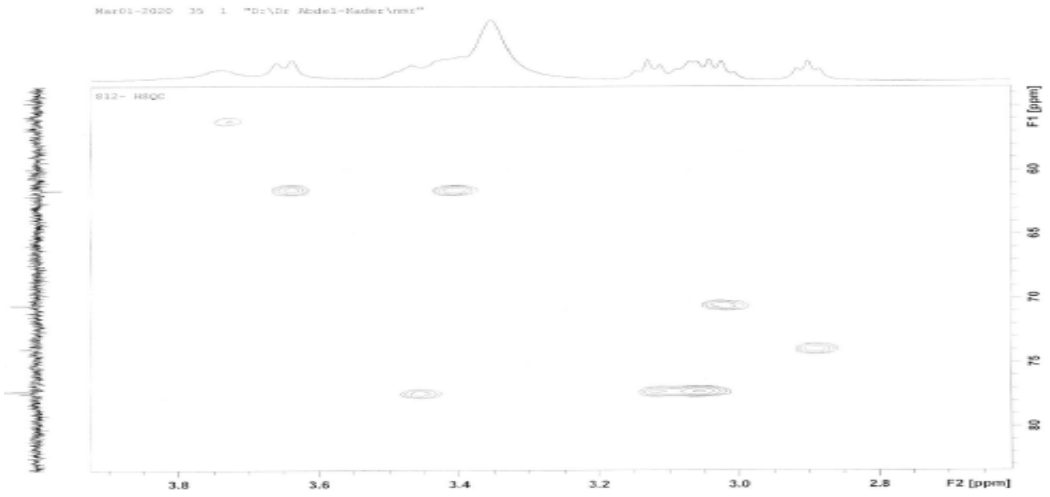


Figure. 28: Partial HSQC spectrum of fraction 9. 2 in DMSO

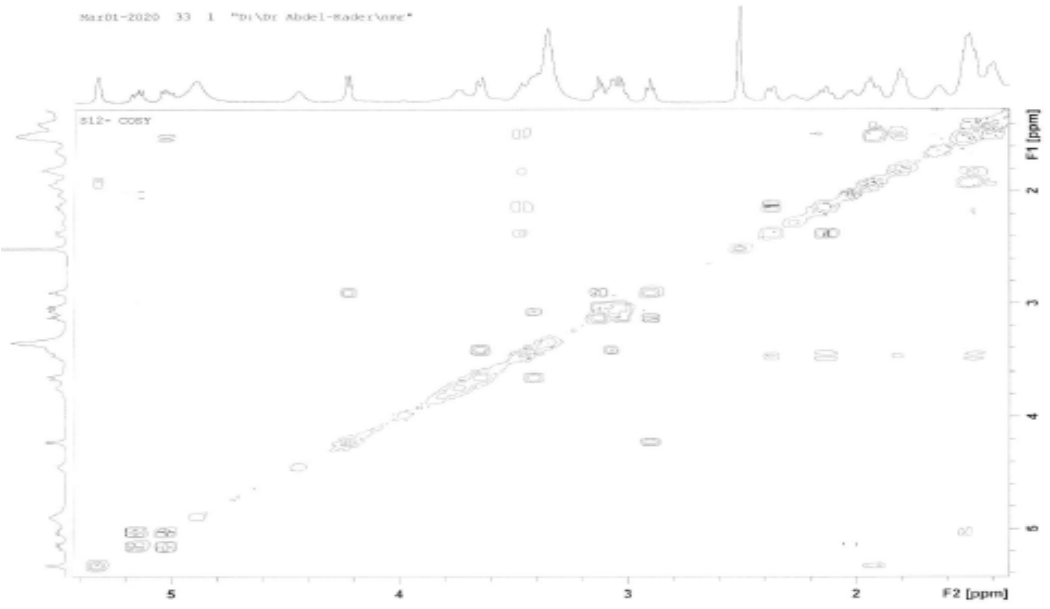


Figure. 29: Partial COSY spectrum of fraction 9. 1 in DMSO

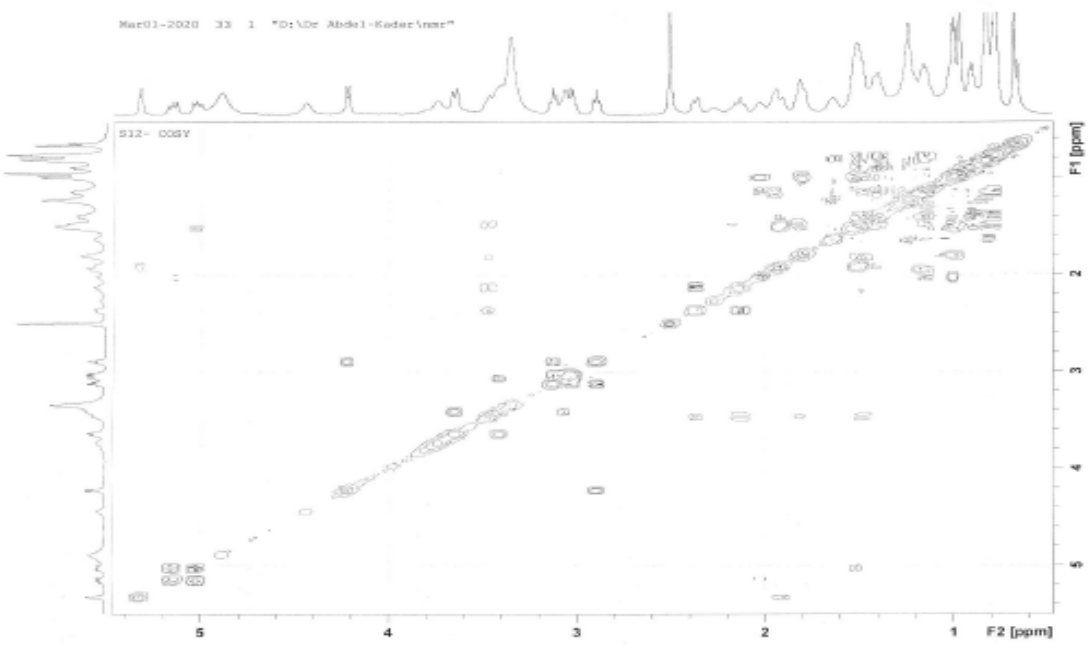


Figure. 30: Partial COSY spectrum of fraction 9. 2 in DMSO

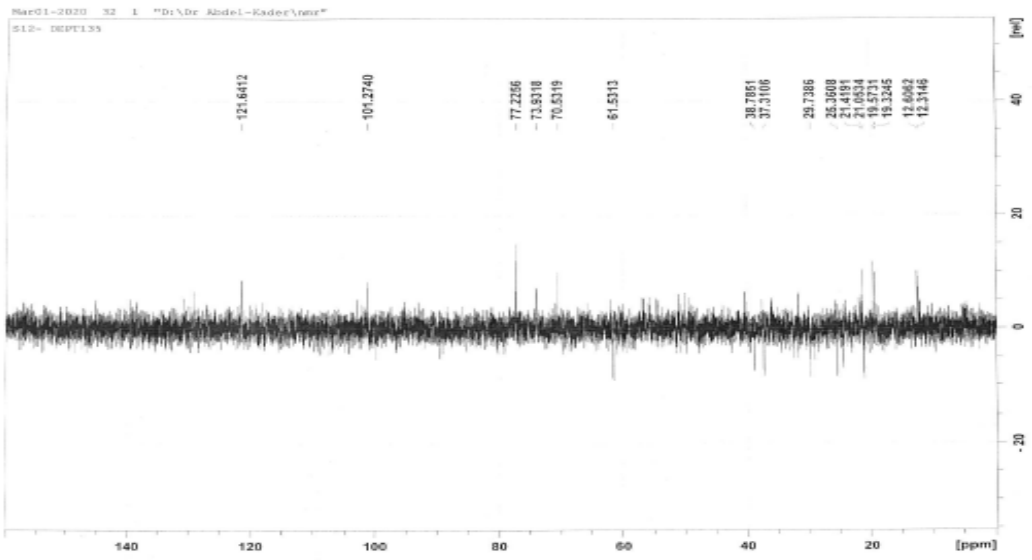


Figure. 31: ^{13}CNMR and DEPT 135 spectra of fraction 9. 1 in DMSO

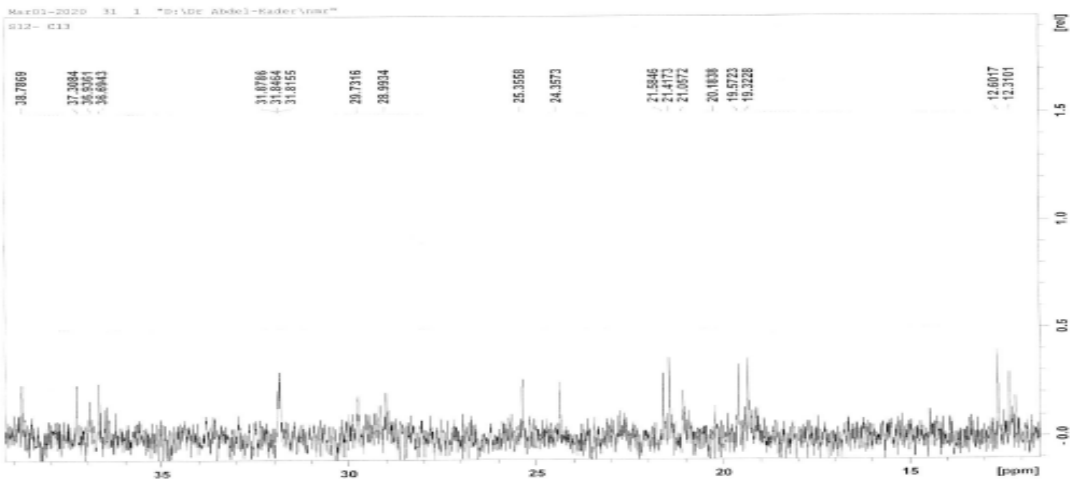


Figure. 32: ^{13}CNMR spectrum of fra. 9. 1 in DMSO

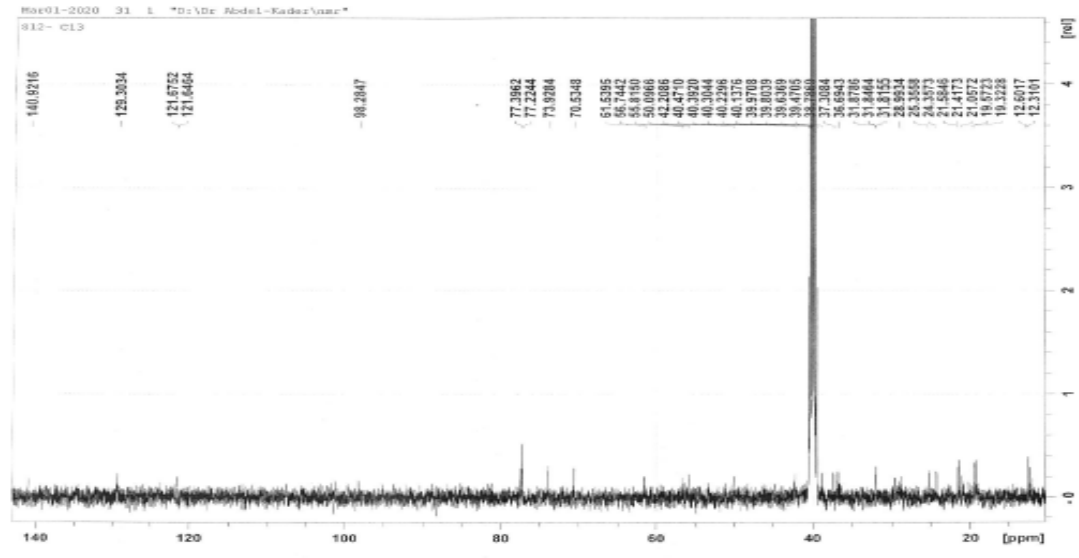


Figure. 33: ^{13}CNMR spectrum of fra. 9. 2 in DMSO

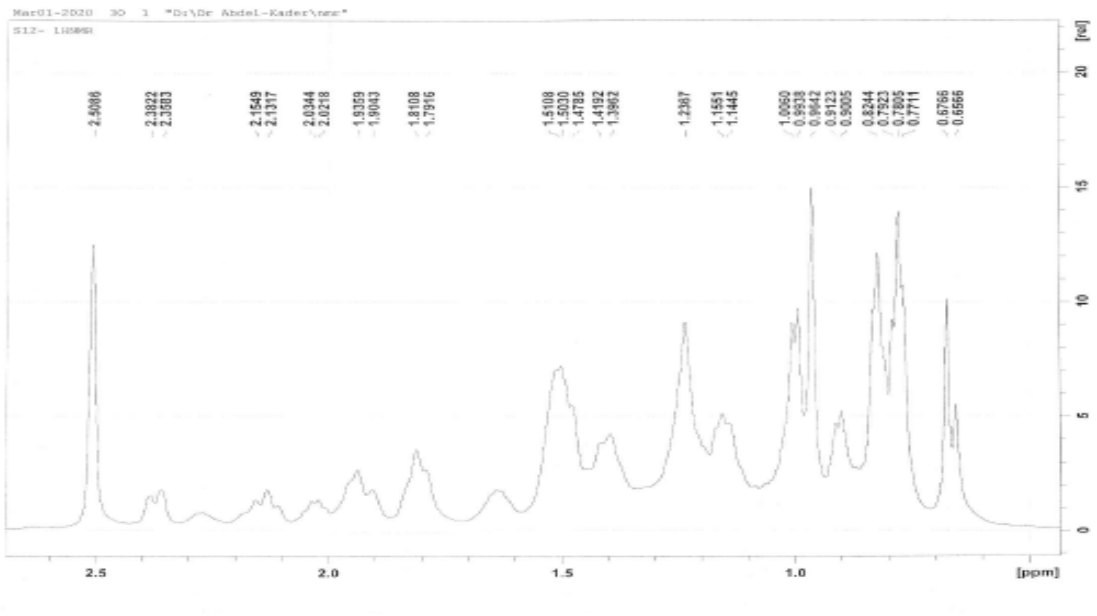


Figure. 34: ^{1}HNMR spectrum of fra. 9. 1 in DMSO

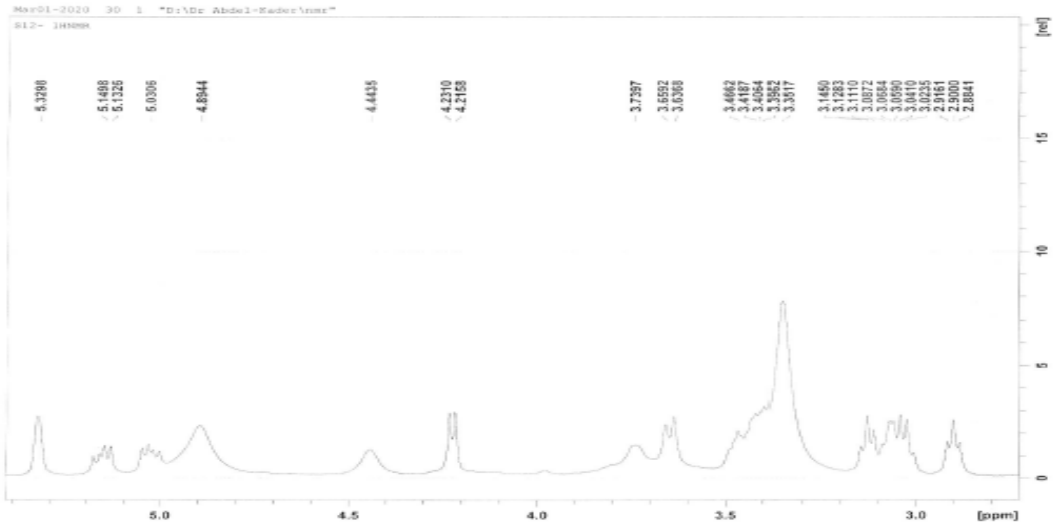


Figure. 35: ^{1}HNMR spectrum of fra. 9. 1 in DMSO

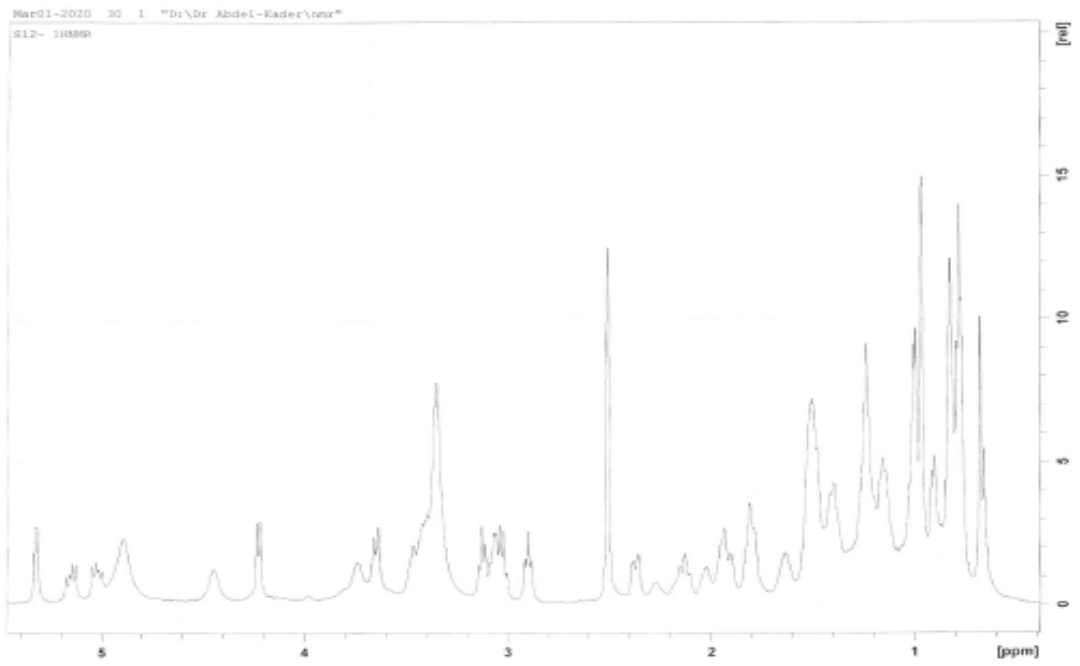


Figure. 36: ^{1}HNMR spectrum of fra. 9. 2 in DMSO

3.1.8.3 Spectral data of compound C

Compound C obtained having a White solid, melting at 197 C^0–198 C^0, and giving a positive Liebermann-Burchard test proved to be of triterpenoids. base, was elucidated by ((3S,4aR,6aR,6bS,8aR,12aR,14aR,14bR)-4,4,6a,6b,8a,11,11,14b-octamethyl-1,2,3,4a,5,6,7,8,9,10,12,12a,14,14a-tetradecahydropicen-3-ol), , to be beta amyrin.

UV spectrum

The UV spectrum of compound (C) recorded in methanol shows an absorption peak at 231 nm.

IR spectrum

The IR spectrum of compound (C) shows broadband at 3411 cm^{-1} that clearly indicated the O-H str. vib. ; The spectrum shows aromatic C=C str. vib. of carbon at 1455 & 1636 cm^{-1}. Bending band appearing at

2927 cm^{-1} indicating the presence of aliphatic C-H str.vib. and CO phenolic str. vib. appearing at 1033 cm^{-1}.

Mass spectrum: Chemical Formula $C_{30}H_{50}O$, molecular weight: 426.72 g/mol.

NMR spectrum

^{1}H NMR (500 MHz, DMSO-d6, δ, ppm)

0.69 (2 CH_3); 0.72 (2 CH_3); 0.76 (CH_3) 0.87 (CH_3) 0.89 (2 CH_3) 1.39-1.14 (m, 2H, CH_2); 1.56-1.31 (dd, 4H, 2CH_2); 2.28- 2.3 (m, 6H, 3CH_2); 3.34-3.14 (m, 2H, 2CH_2); 3.55-3.58 (t, 2H, 2CH_2); 4.49 (bs, 2H, CH_3); 4.61 (bs, 1H, OH).

^{13}C NMR (500 MHz, DMSO-d6, δ, ppm)

15.39, 15.99, 16.13, 18.01, 18.33, 19.32, 20.94, 22.72 (8 C, CH_3); 25.14, 25.76, 27.04, 27.45, 27.99, 29.38, 29.45, 29.63, 29.72, 29.85, 31.94, (10C, CH_2); 32.08, 34.28, 35.59, 37.17, 38.05, (5C, CH); 38.71, 38.86, 40.01, 40.83, 42.83, 43.01, 79.02 (7C, C-tert).

The NMR data measured for compound C (Table. 18) were compared with those reported in the literature, Stanley**, (**2007). Compound was identified as beta amyrin. Figure. 37 shows the elucidated structure of compound C (beta amyrin)

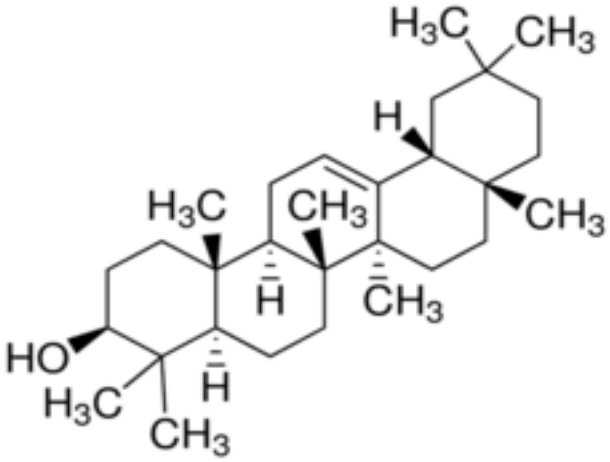
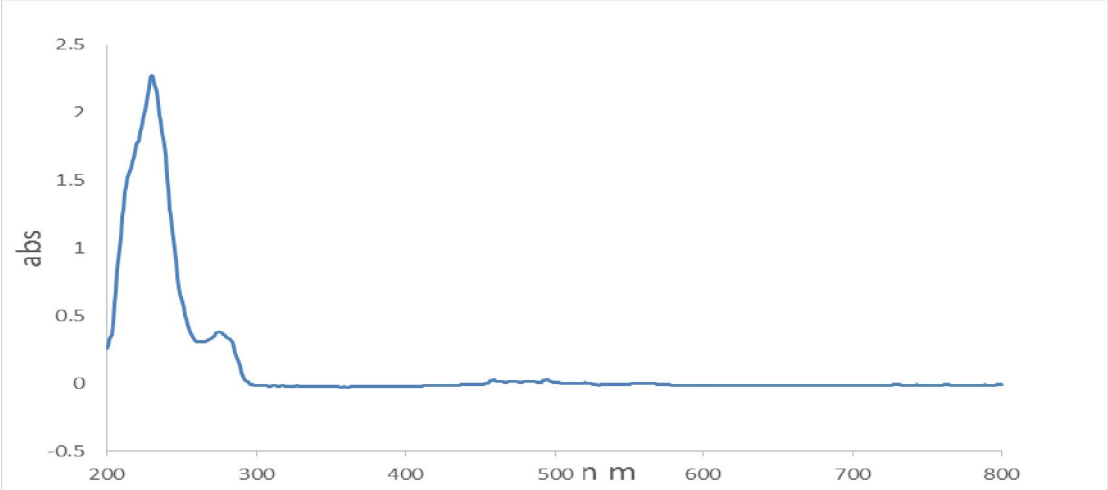


Figure. 37: beta amyrin

Figure. 38, 39 and 40 show UV, IR and LCMS spectrum of beta amyrin, respectively:



Sa B	length	Abs
	231 nm	2.268

Figure. 38: UV spectrum of beta amyrin

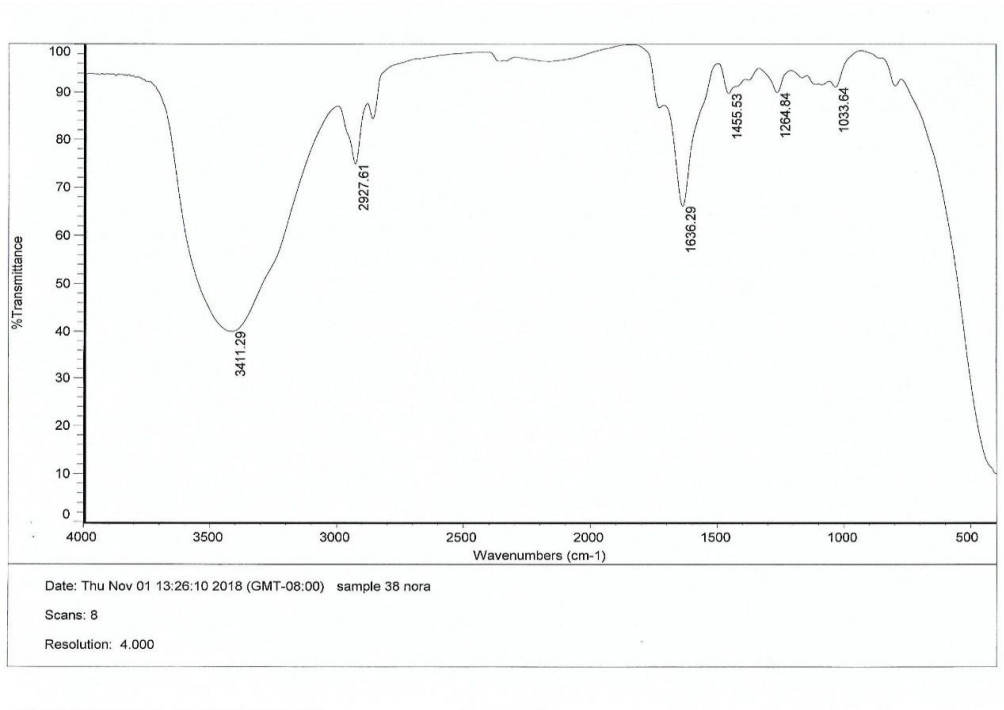


Figure. 39: IR spectrum of beta amyrin

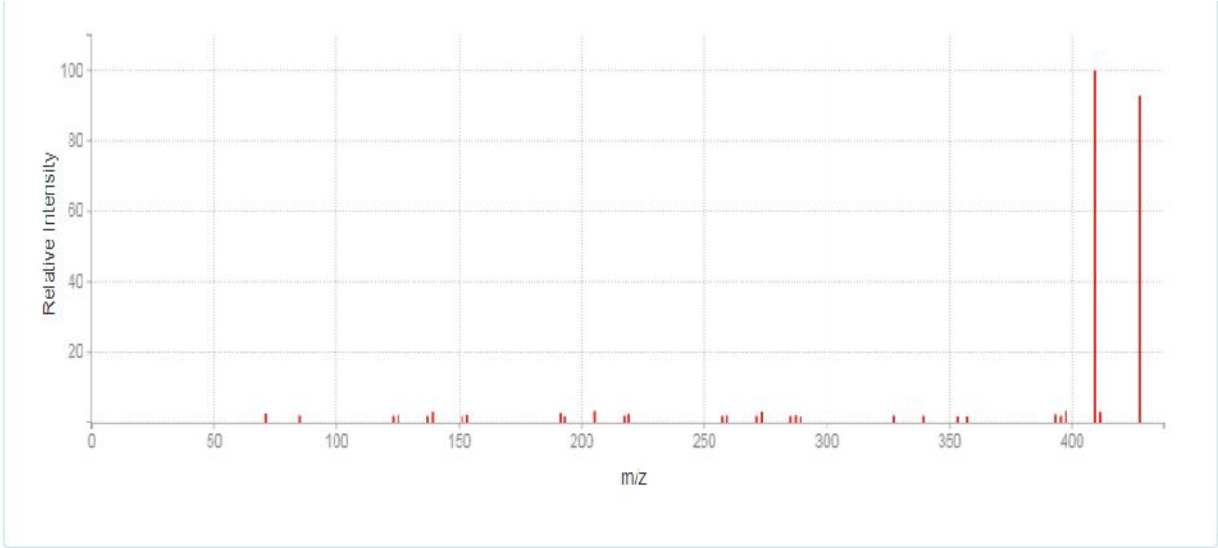


Figure. 40: LC MS of beta amyrin

Table (18): NMR spectral data of isolated beta amyrin in DMSO

Position	Parameter δ H (ppm); multiplicity; J (Hz)	δ C (ppm)
C-1	1.56; 1.31	25.14
C-2	1.55; 1,47	25.76
C-3	3.34 (dd, j = 4.4; 10.8)	79.30
C-4	-	38.71
C-5	0.94 (d, j = 11.0)	34.28
C-6	1.63; 1.38	27.04
C-7	1.56; 1.31	27.45
C-8	-	38.63
C-9	0.94	35.59
C-10	-	40.01
C-11	1.63; 1.38	27.99
C-12	5.12 (t, j = 3.2	109.35
C-13	-	150.9
C-14	-	42.83
C-15	1.56 (td, j = 4.0)	29.38
C-16	1.56 (td, j = 4.3)	29.45
C-17	1.04	43.01
C-18	1.04	38.05
C-19	1.93 (dd, j = 4.0)	29.63
C-20	-	79.02
C-21	1.56; 1.31	29.72
C-22	1.56; 1.31 m	29.85
C-23	0.89 (s)	25.76
C-24	0.89 (s)	25.14
C-25	0.84 (s)	25.40
C-26	0.89 (s)	18.01
C-27	0.89 (s)	18.33
C-28	0.70 (s)	19.32
C-29	0.87 (s)	20.94
C-30	0.87 (s)	22.72
1’	4.77 (b s)	171.40
2’	4.49 (b s)	21.49

Figures. 41, 42, 43 … and 58 show NMR spectrum of beta amyrin (fra. 21), respectively

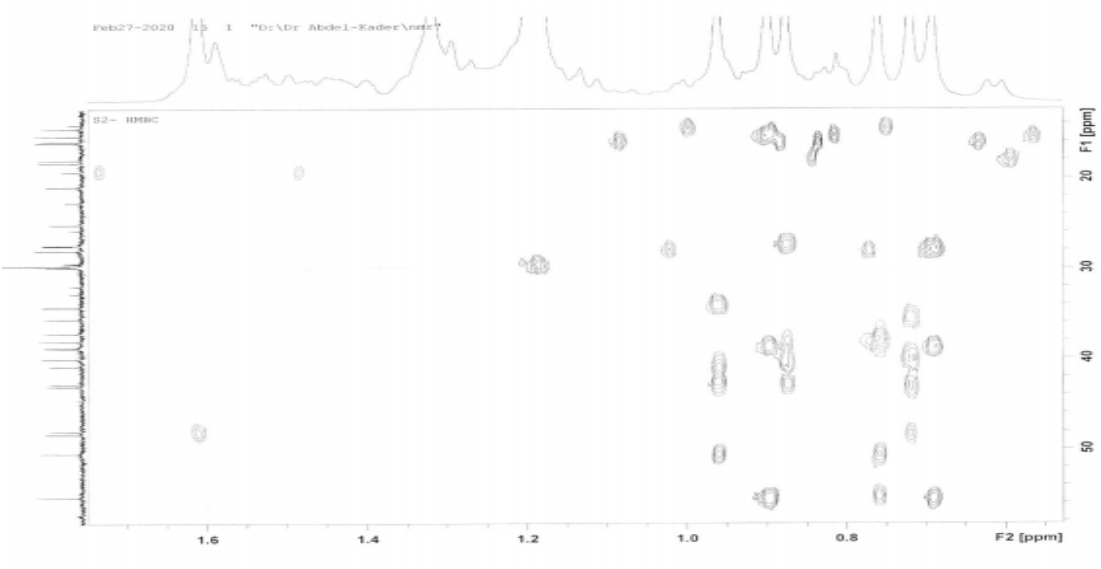


Figure. 41: Partial HMPC spectrum of fraction 21 in DMSO

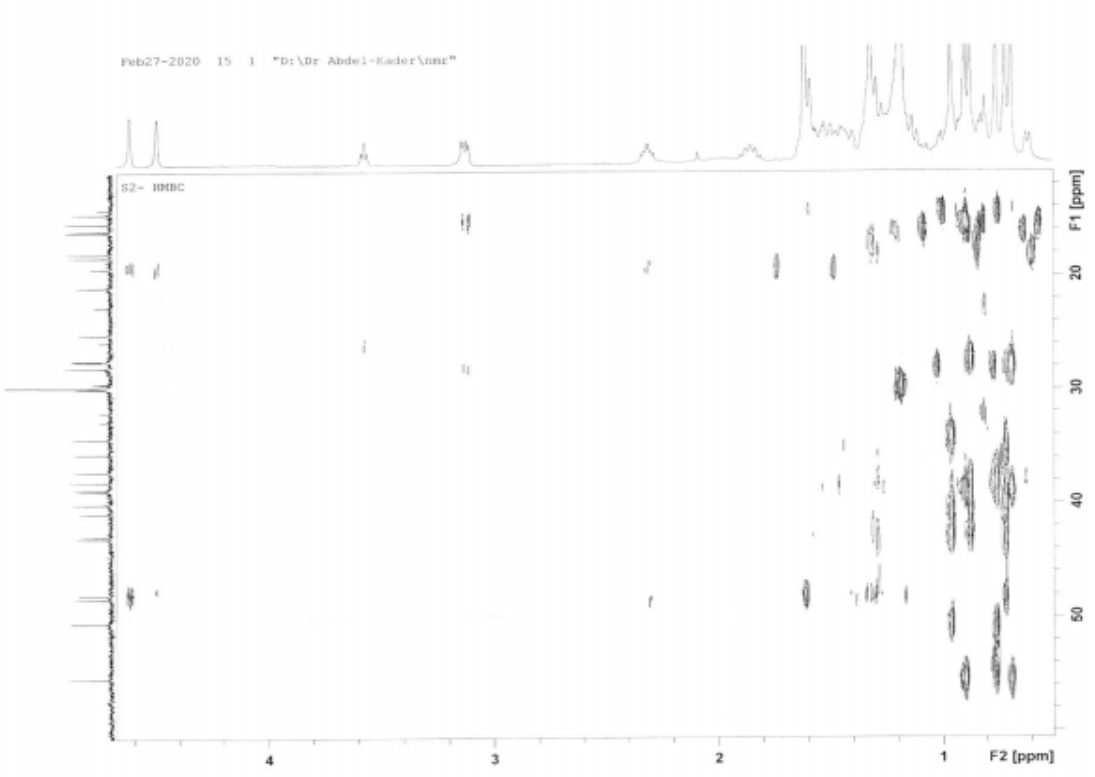


Figure. 42: Partial HMPC spectrum of fraction 21 in DMSO

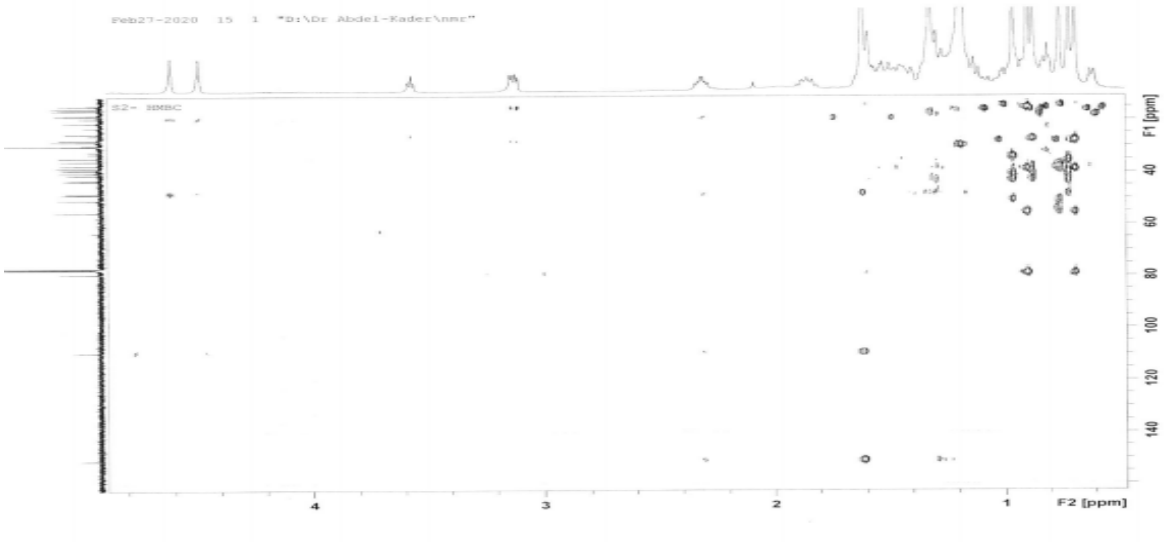


Figure. 43: Partial HMPC spectrum of fraction 21 in DMSO

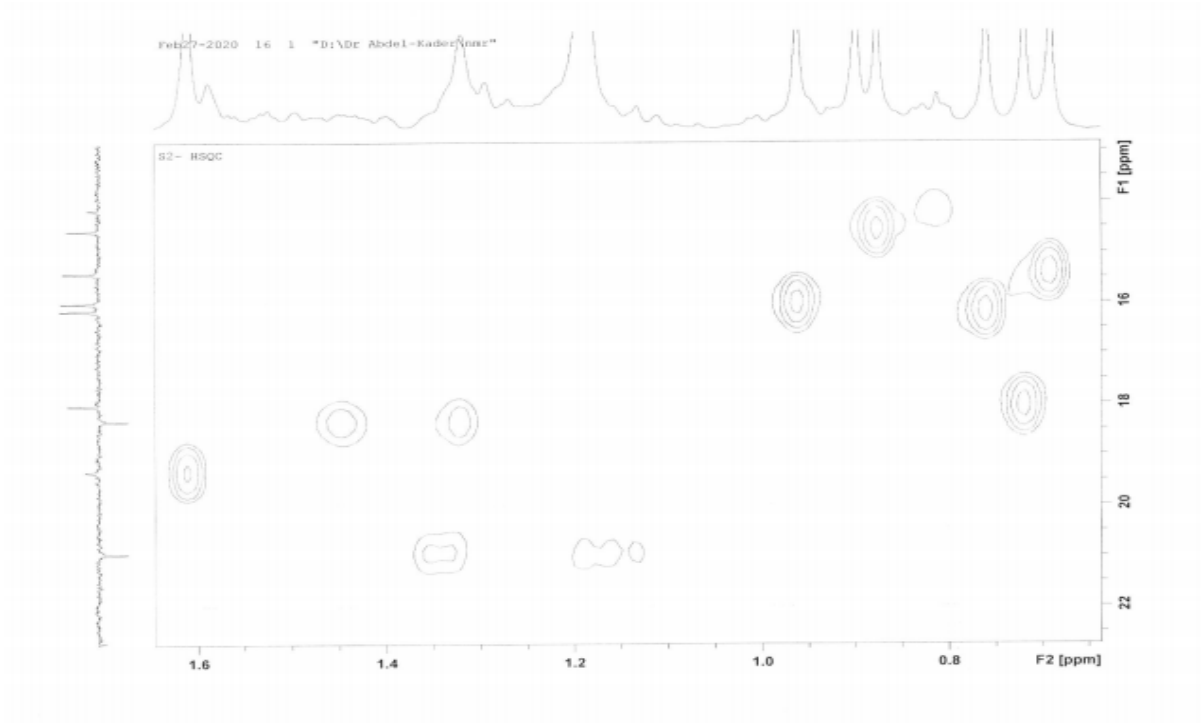


Figure. 44: Partial HSQC spectrum of fraction 21 in DMSO

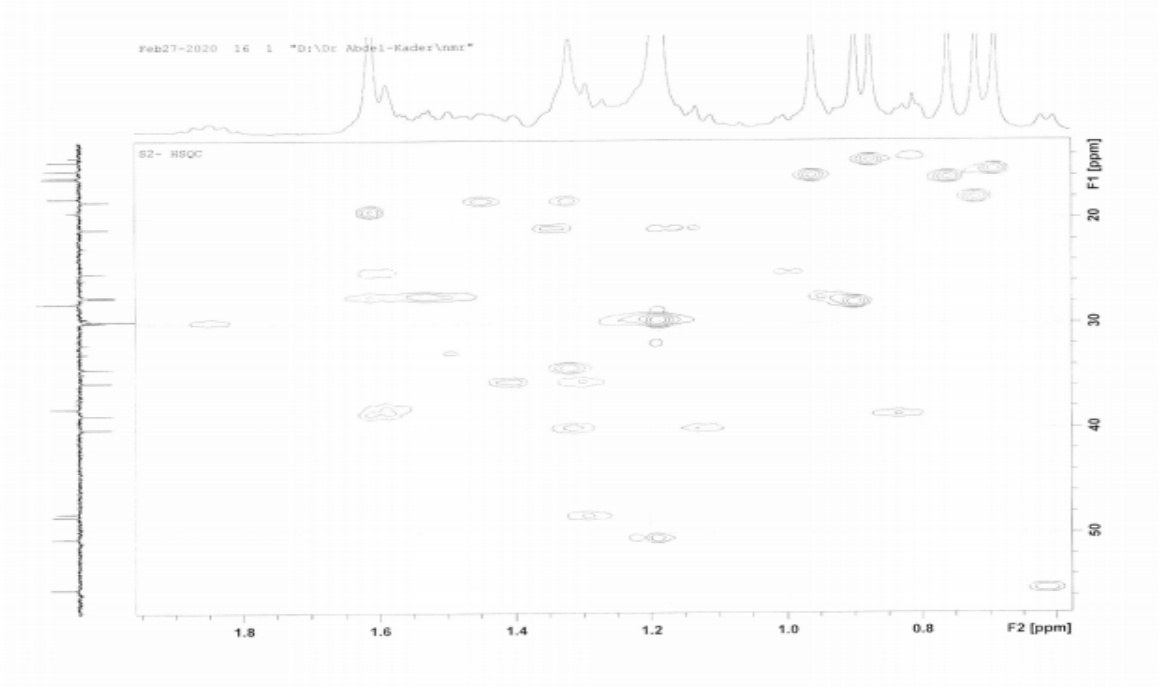


Figure. 45: Partial HSQC spectrum of fraction 21 in DMSO

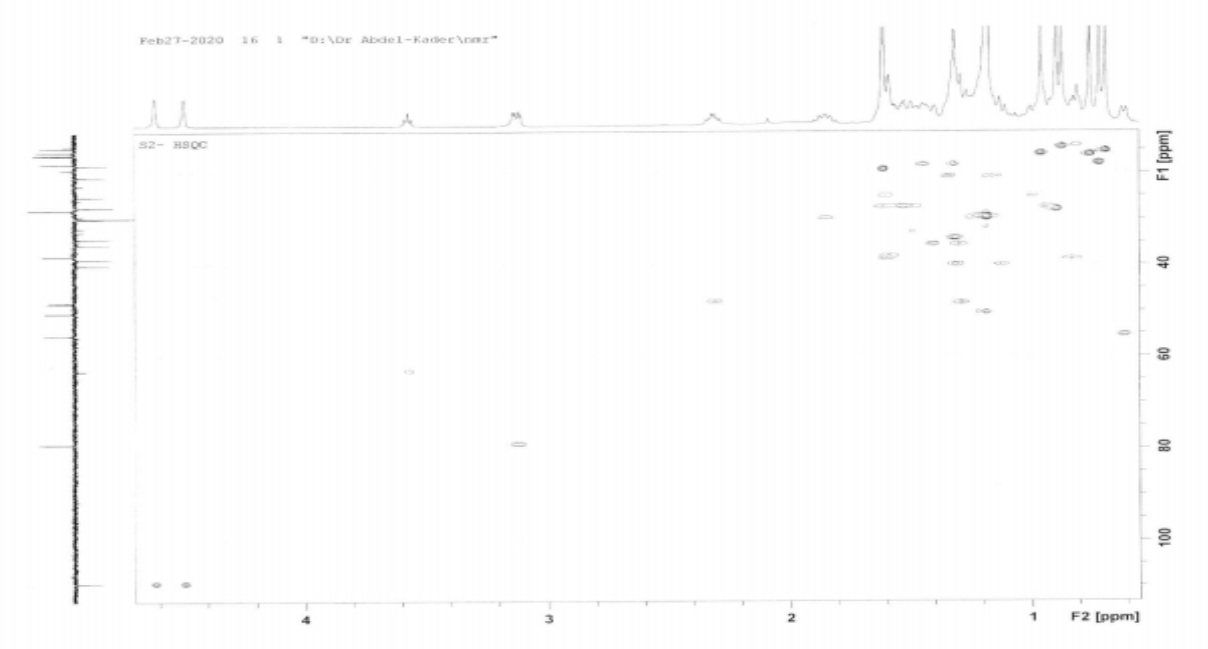


Figure. 46: Partial HSQC spectrum of fraction 21 in DMSO

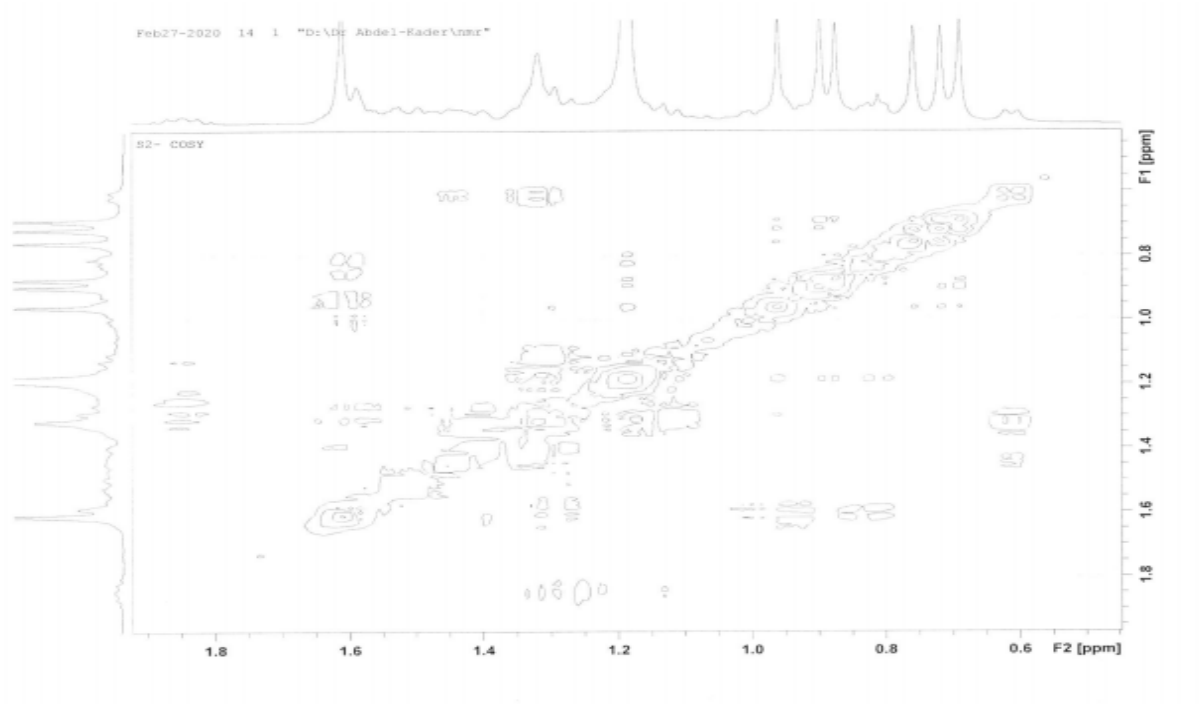


Figure. 47: Partial COSY spectrum of fraction 21 in DMSO

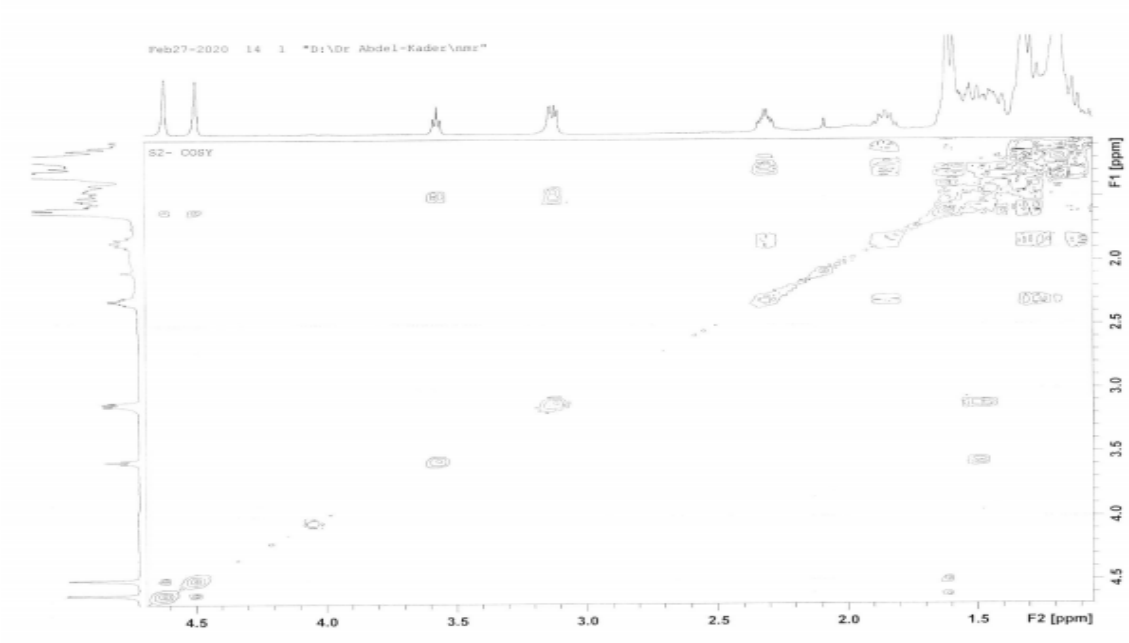


Figure. 48: Partial COSY spectrum of fraction 21 in DMSO

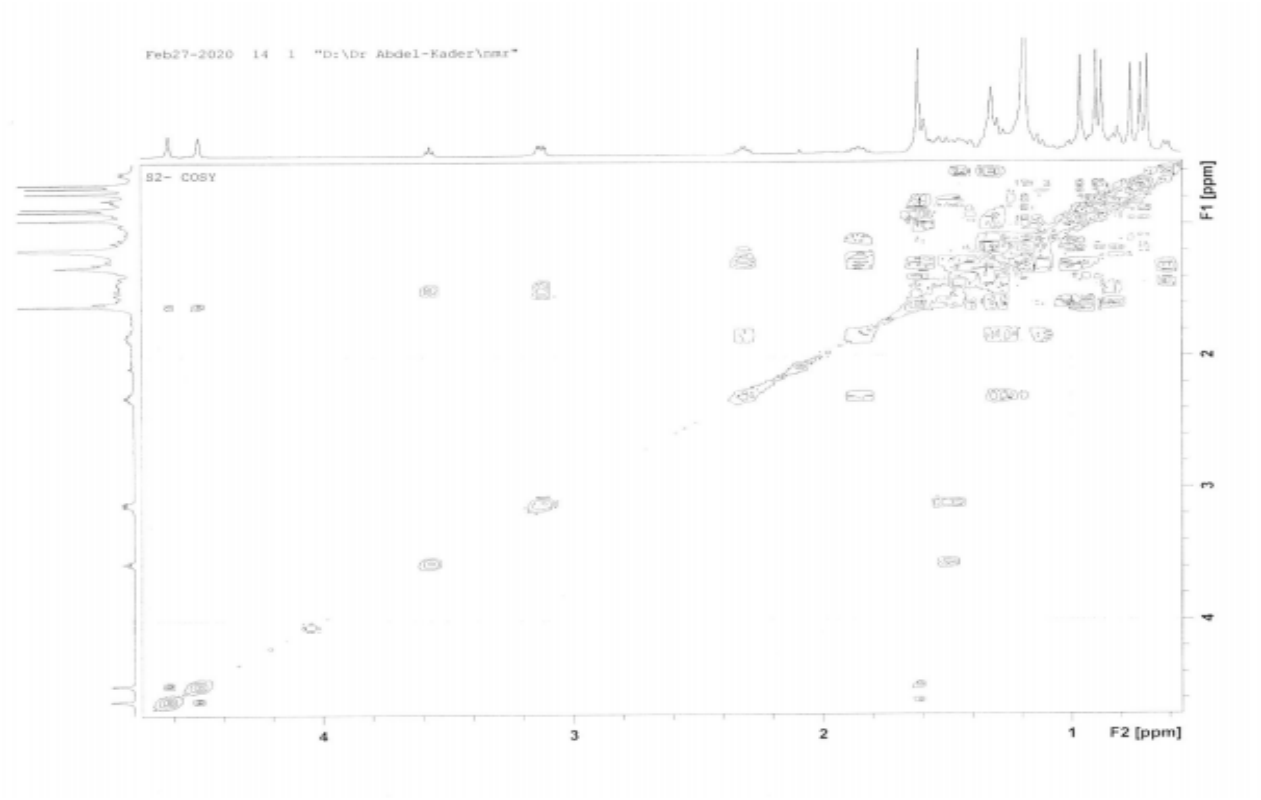


Figure. 49: Partial COSY spectrum of fraction 21 in DMSO

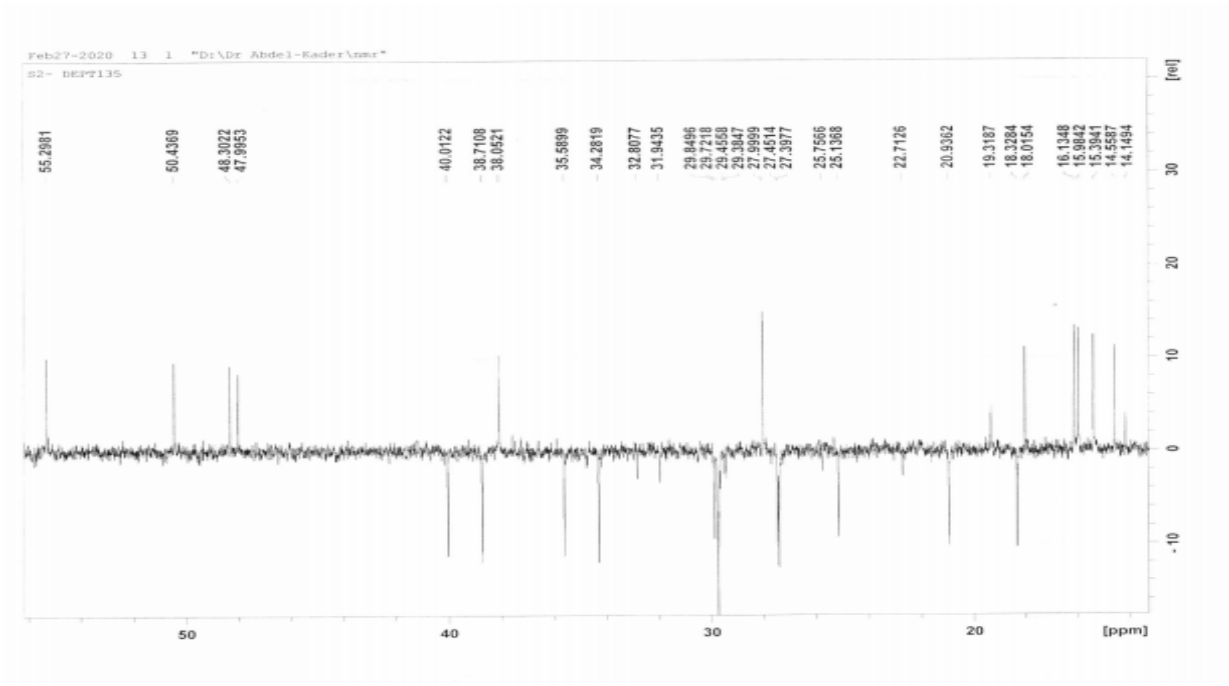


Figure. 50: ^{13}CNMR and DEPT 135 spectra of fra. 21 in DMSO

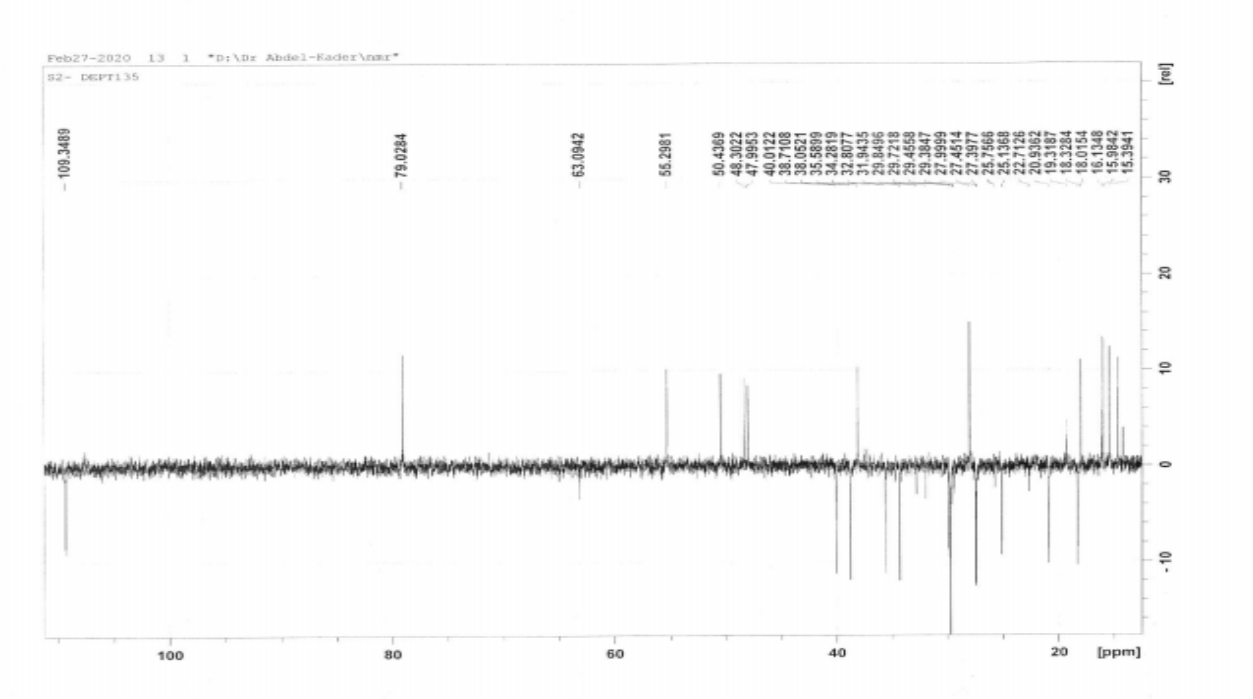


Figure. 51: ^{13}CNMR and DEPT 135 spectra of fra. 21 in DMSO

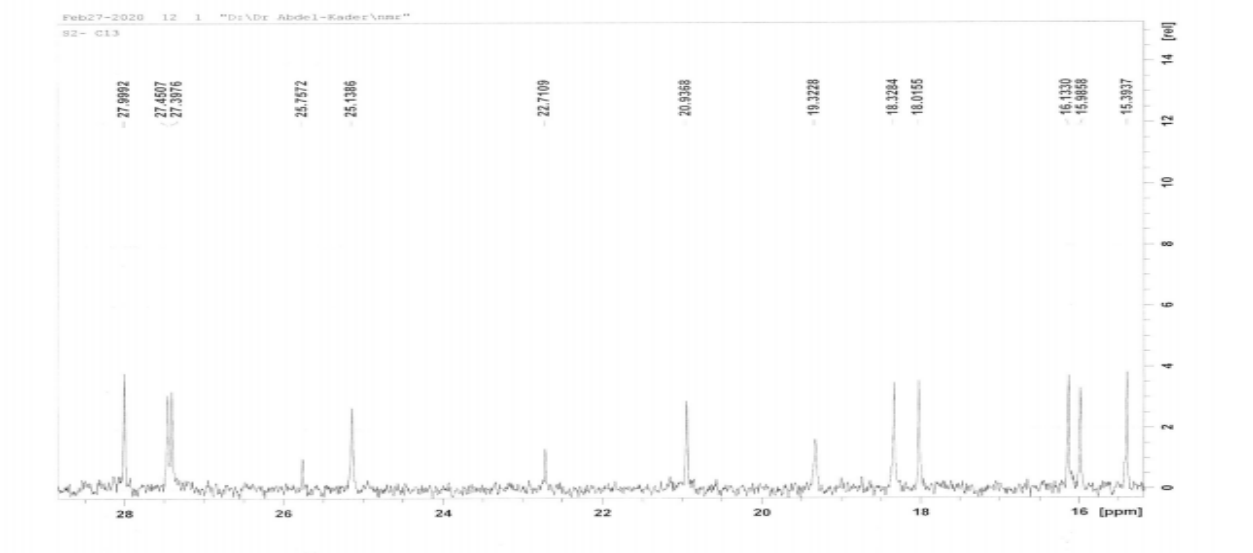


Figure. 52: ^{13}CNMR spectrum of fra. 21 in DMSO

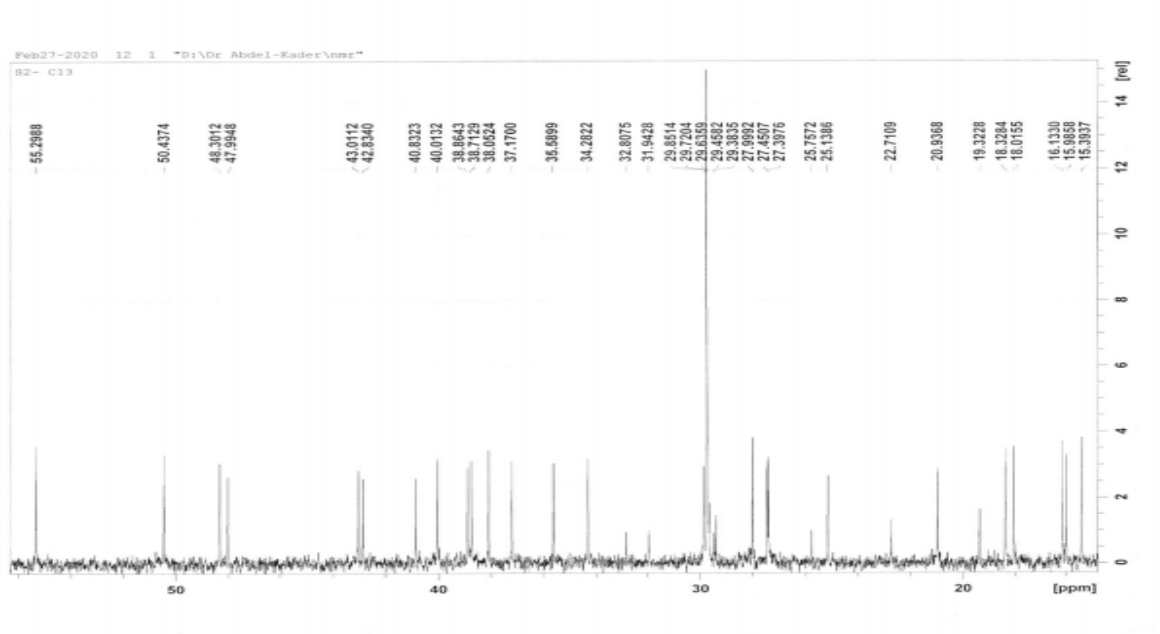


Figure. 53: ^{13}CNMR spectrum of fra. 21 in DMSO

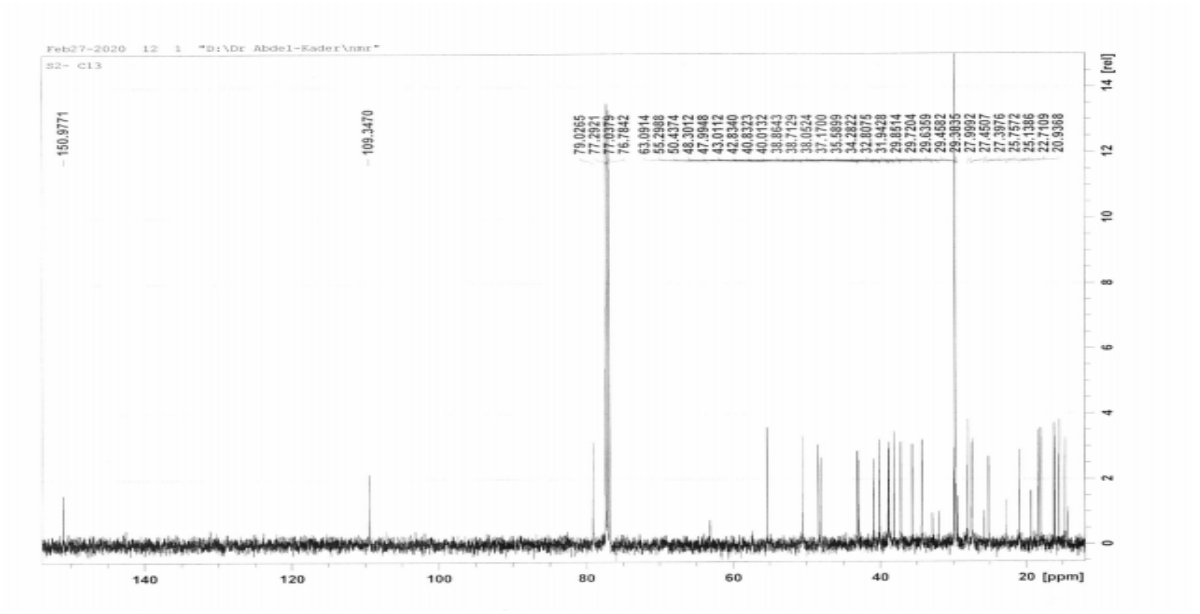


Figure. 54: ^{13}CNMR spectrum of fra. 21 in DMSO

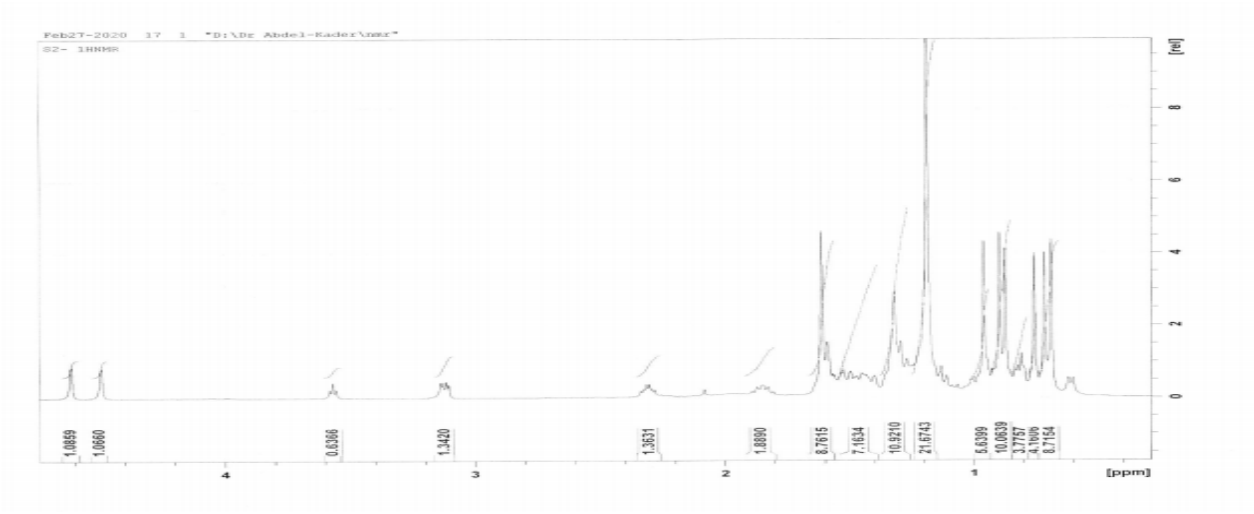


Figure. 55: ^{1}HNMR spectrum of fra. 21 in DMSO

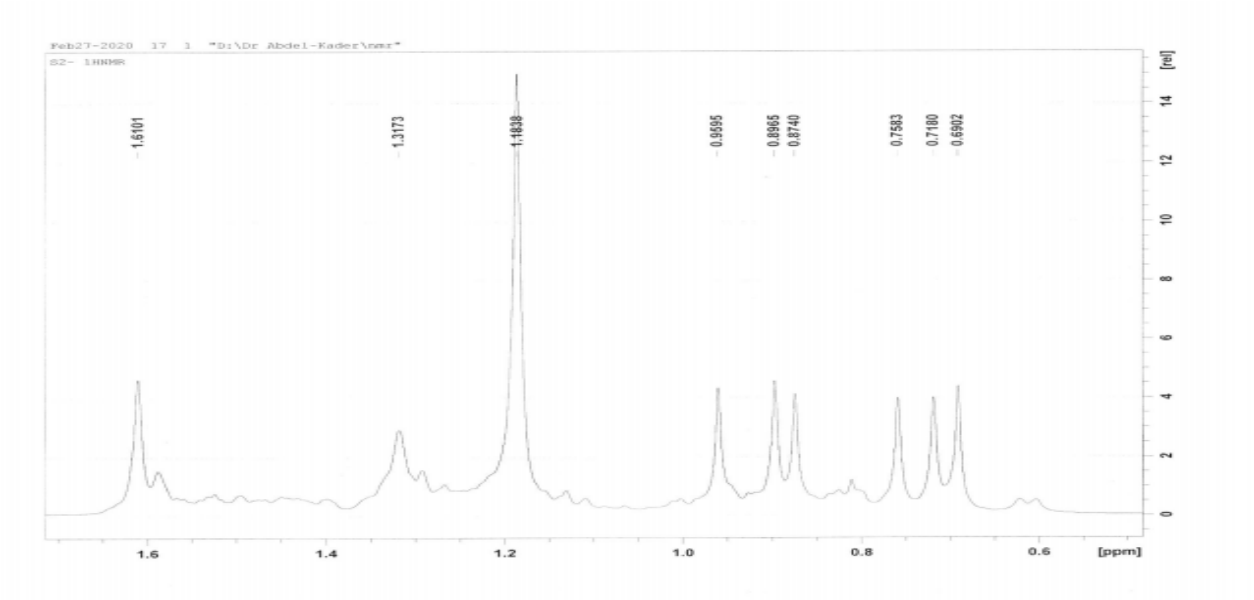


Figure. 56: ^{1}HNMR spectrum of fra. 21 in DMSO

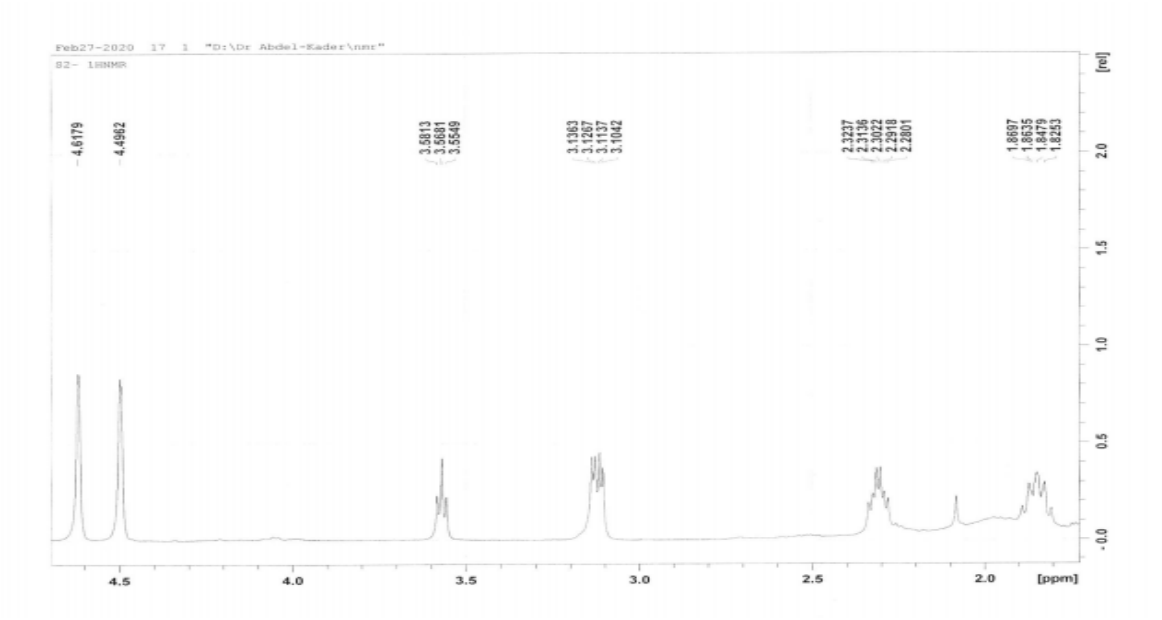


Figure. 57: ^{1}HNMR spectrum of fra. 21 in DMSO

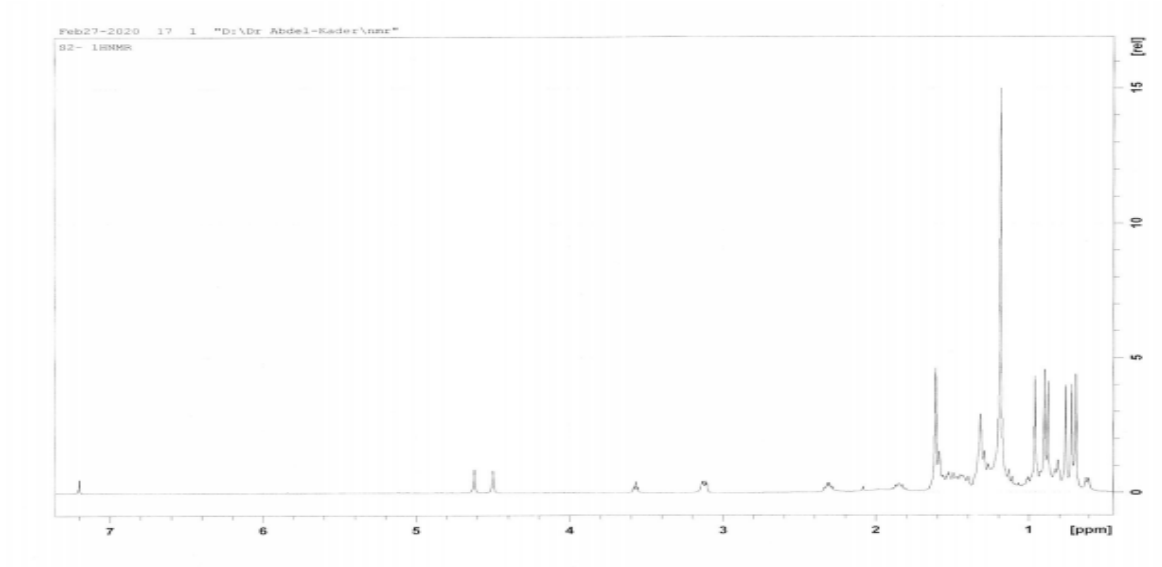


Figure. 58: ^{1}HNMR spectrum of fra. 21 in DMSO

3.2 Discussion

Nature is a source of bioactive compounds with great chemical diversity, and the use of natural products in the field of drug development has achieved remarkable success. The aim of this work was to isolate and characterize the structure of the bioactive compound of *Solenostemma argel* after various bioactivity assays. The parts of the stems were extracted from the *S. Argel* plant with water in different proportions, resulting in the production of five fractions. The yield ratios of the extract mainly depend on the sample fraction used and the amount of extractable substance present, and the yield ration was fractionated sequentially with n-hexane, chloroform, ethyle acetate and n-butanol. After bioactivity testing, it was found that the chloroform extract was more effective.

Various types of secondary metabolites of *Solenostemma argel* stems were found in medium concentration in crude extract, saponins and coumarin, and steroids and triterpenoids in high concentration in chloroform extract and ethyl acetate extract in medium concentration in normal hexane and normal butanol. The heavy precipitate with Mayer's reagents and Dragendefts indicated a high concentration of alkaloids in the chloroform and ethyl acetate extracts. The flavonoid test showed a good appearance of flavonoids in the crude extract and their molecules, but glycosides were only absent in the natural extracts of butanol and water. The test for anthraquinones gave a negative result indicating the absence of anthraquinones in the crude extract.

Absorption of other minerals are a major regulatory step in mineral balance (for example, P, Cu, Zn, Na, and K). Phosphorous are generally absorbed in the upper parts of the small intestine. This essential component enters the epithelial cells via an antagonistic mathematical

mechanism with Na. Vitamin D can significantly stimulate transporter expression of this total mineral (Prasad and Bhadauria, 2013). Copper absorption can be explained by two processes that are rapid (low-capacity system) and gentle (high-capacity system) that can be compared with the two processes described in calcium absorption. In Table (7), the results indicated that Calcium, Magnesium, Copper, Iron, Manganese, and Lead for the studied *S. argel* stems were 21.10, 29.95, 0.014, 1.055, 0.059 and 0.052%, respectively.

Several studies have been conducted in Sudan to test the effectiveness of antimicrobials some medicinal plants. Ahmed (2004) tested the extracts of 10 Sudanese medicinal plants against Gram-positive and Gram-negative bacteria as well as *Candida albicans.* A marked antibacterial effect was found, against the Gram-positive bacteria, *Staph aureus*, followed by *Escherichia coli* and *Candida albicans*. Fenugreek oil was also found to inhibit *Salmoenlla typhimurium*, and the inhibition zone diameters were 15 mm when the oil concentrations were 100%. No reports have been found in the literature on the effects of fenugreek oil against these bacteria; however, the effectiveness of ginger oil and clove oil against these bacteria has been reported (James *et al*., 1999; Elboshra, 2005 and Badreldin, 2006). In this study, the crude extracts and their particles were used for four bacteria (*Ec*, *Ps*, *Sa*, *Bs*) and the area of inhibition is shown in Table 4. The results indicated low activity of the crude extract, n-hexane and aqueous extract against the four bacteria. Ethyl acetate and chloroform extract showed high efficacy with *B.s & S.a*. Here again n-hexane and aqueous extract showed low activity against the standard fungi used. An increase in the zone of inhibition was observed with chloroform extract.

Because of eco-friendly to the environment and safety to the human health, botanical insecticides considered as safe alternatives compared to chemical insecticides, Isman, (2005) and Idris *et al*., (2011) reported that the farmers in Kassala State, Sudan used *S. argel* shoots as pesticide to control pests on tomatoes (aphids, and white flies), and on okra (Egyptian bull worm). Several studies explored the potential of *S argel* plant application as botanical insecticide to control, mosquito species, the causative agent of malaria in Sudan, (Awad, *et al*., 2012, Feiha, *et al*., 2009; Stngeland, *et al*., 2011, Sameh and Abdelhalim, 2011). In the current investigation on various powders of the stems of *Solenostemma argel* which were tested against the 3rd larval instars of the *Tribolium castaneum* to count the mortality of the 3rd larval *Tribolium castaneum*, during 24 hours; the result indicated that this part studied had insecticidal activity against the test insect compared with that of control. The response varied with plant part and exposure time, this might be due to the fact that *S. argel* was found to contain phytochemicals which included, Saponins, Cumarines, Alkaloids, Tannins, Flavonoids, Steroids and triterpenes. This study showed that chloroform extract gave highly significant toxicity.

The first compound obtained (A) was a pale white powder; melted at 278-280 ° C gave an indication of the steroid base in the Lieberman-Burchard test and had Rf 0.544. According to the following results, Compound A demonstrated the structure ((E) -2 - ((17- (5-ethyl-6-methylhept-3-en-2-yl) -10,13-dimethyl) 2, 3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta [a] phenanthren-3-yl (oxy) -6- (hydroxy-methyl) tetrahydro -2H-pyran -3,4,5-triol), stigmasterol glucoside. The UV spectrum of compound (A) recorded in methanol showed an absorbance peak at 210 nm. The infrared

spectrum showed the absorption bands for hydroxyl (3437), aliphatic groups (2927), C = C (1564), C-O (1258, 1165, 1071 and 1026) while the strong absorption band was distinguished at 3437 and 1071 as a glycoside compound.

The ^{1}H NMR spectrum of compound A (Table 10) shows methyl class III at 1.01 (Me-18) and 0.65 (Me-19), and methyl secondary at 0.91 (Me-21), 0.84 (Me-26) and 0.80. (Me-27), one elemental methyl at 0.79 (Me-29), one proton with olefin substitution at 5.32 (H-6), two protons with substituted olefin at 5.18 (H-22) and 5.04 (H-23) an anomalous proton at δ 4.23.

The ^{13}C NMR spectrum of compound A (Table 10) shows the presence of 35 carbon atoms in the molecule. An anomalous carbon signal at 100.77 indicated a monosaccharide fraction. The four-methine resonances were at 70.14, 76.75, 73.43 and 76.63 in addition to the methylene resonance at 61.10 due to C-2 ', C-3', C-4 ', C-5' and C-6, respectively, from Dglucopyranoside. The olefinic resonance at 121.04, 137.85, and 128.79 corresponds to methene carbonate C-6, C-22 and C-23, and a signal at 140.43 corresponds to the C-5 quaternary carbon of the sterol cleavage. A value of J = 7.8 over 1 '(anomalous proton) reflected that the proton is the axial axis of the C-2' proton, which means that the glucopyranoside fraction binds to the sterol moiety (Hassan *et al*., 2008). Based on the description of the above data and comparison with the physical and spectral data of the previously known compound, the molecular structure of compound A is given in Fig.16 as stigmast-5,22-dien-3-O-β-D-glucopyranoside.

The relationships in the correlation structure were demonstrated by the long-term correlation between ^{1}HNMR and ^{13} CNMRs of the HMBC spectrum (Table 10). The presence of long-term associations of protons at

4.23 (H-1 ') with carbon at 76.95 (C-3) and 76.63 (C-5') indicates that the glucose group is bound to C-3 (oxy carbon sp3). Long-term correlations of protons at 5.32 (H-6) with methylene carbon at 31.30 (C-7), methane carbon at 31.38 (C-8), and quaternary carbon at 36.15 (C-10) reinforce this. C-6 is an olefinic bond in the second ring of the sterol skeleton. Long-term correlations of protons at 0.65 (H-18) with methylene carbon at 41.62 (C-12), quaternary carbon at 41.80 (C-13), methane carbon at 56.20 (C-14), and methane carbon at 56.11 (C- 17) confirm that methyl carbon (C-18) binds to the quaternary carbon (C-13). Then the long-term relationships between protons at 0.99 (H-19) with methylene carbon at 38.32 (C-1), quaternary carbon at 36.15 (C-10), quaternary carbon at 140.43 (C-5), and methane boosting carbon at 49.57 (C-9) that methyl carbon (C-19) binds to the quaternary carbon (C-10).

The distance relationship between the proton multiplier at 0.91 (H-21) with carbon methane at 56.11 (C-17), and carbon methane 35.37 (C-20) enhances the bonding of methyl carbon (C-21) with carbon methane (C). -20). The distance relationship between the proton multiplier at 0.84 (H-26) with methyl carbon at 31.20 (C-25), methane carbon at 45.14 (C-24), and methyl carbon at 18.89 (C-27) reinforces that methyl carbon (C -26) binds to methene carbon (C-25), as well as long-term correlations between a proton doubling at δ 0.80 (H-27) with carbon (C-24) and (C-25) and (C-26) strengthens that methyl carbon (C-27) binds to methane (C-25) carbon. In addition, long-term correlations between proton doubling at 0.79 (H-29) with methylene carbon at 45.12 (C-24) and methylene carbon at 23.76 (C-28) enhance the binding of methyl carbon (C-29) to methylene carbon (C-28).

These compounds belong to the class of organic compounds known as stigmastanes and derivatives. These are sterol lipids with a skeleton-

based structure of stigmastine, which consists of a colistan fraction carrying an ethyl group in the C24 carbon atom. Schottenol 3-glucoside is a very weak (mainly neutral) (pKa based) compound.

In addition, the compound (B) obtained had, White crystalline solid, 144-146 °C, m. p., 0.499 Rf gave an indication of steroid base on Liebermann-Burchard test. The structure of compound was elucidated and identified as beta Sitosterol Glucoside according the following spectroscopic analyses. The UV spectrum of compound (B) recorded in methanol shows an absorption peak at 210 nm. The IR spectrum of compound (B) shows broadband at 3422 cm^{-1} that clearly indicated the 0-H str. vib. . The spectrum shows aromatic C=C str. vib. of carbon at 1415 & 1568 cm^{-1}. Bending band appeared at 2926 cm^{-1} indicated the presence of aliphatic C-H str.vib. and CO phenolic str. vib. Appearing at 1050 cm^{1}.

NMR data of compound B were compared to those reported in the literature. The compound was identified as 2-{[14-(5-ethyl-6-methylheptan-2-yl)-2, 15-dimethyltetracyclo [8.7.0.0^{2}, ^{7}.0^{11}, ^{15}] heptadecan-5-yl] oxy}-6-(hydroxymethyl) oxane-3, 4, 5-triol (Beta-Sitosterol-3-O-Glucoside as be seen in Figure (18))

The ^{1}H NMR spectrum of the compound varied between 0.74 to 5.38 ppm, this spectrum shows the presence of six high intensity peaks indicating the presence of six methyl groups at δ 0.74, 0.84, 0.967, 1.04, 1.20 and 1.53 ppm. The proton corresponding to the H-3 of a sterol moiety appeared as a triplet of doublet at δ 3.53 ppm. At δ 5.19 ppm and at δ 5.37 ppm corresponding to a single peak in the region of the ethylene protons suggesting the presence of three protons. ^{13}CNMR spectrum of the compound gives signals at 140.9 and 121.3ppm for C5=C6 double bond respectively, 71.9 for C3 β-hydroxyl group 19.1 and 12.1 for angular methyl carbon atoms for C19 and C18, respectively. 138.4 ppm

for C-22 and 129.3 ppm for C-23. The C5, C6, C22 and C23 appeared to be alkene carbons. Sitosterol beta-d-glucoside belongs to stigmastanes and derivatives class of compounds. Those are sterol lipids with a structure based on the stigmastane skeleton, which consists of a cholestane moiety bearing an ethyl group at the carbon atom C24. Sitosterol beta-d-glucoside is practically insoluble (in water) and a very weak acidic compound (based on its pKa). sitosterol beta-d-glucoside can be found in pomegranate, which makes sitosterol beta-d-glucoside a potential biomarker for the consumption of this food product.

A third compound was obtained, compound C, containing pentacyclic triterpene alcohol, which was isolated from the stems of the *Solenostemma argel*, Beta amyrin. The component structure was clarified by comparing the physical data (melting point) and the spectral data with respect to the pure components. It gave a positive color reaction with Lieberman - Borchard reagent for triterpenoids. The structure of the component was clarified by comparing its physical (melting point: 197-198 ^{o}C) and spectral data with respect to the pure components.

The UV spectrum of compound recorded in methanol shows an absorption peak at 231 nm. Mass spectrum shows its chemical formula to be $C_{30}H_{50}O$ having two distinct peaks at 426 and 218. The IR spectrum of compound shows broadband centered at 3510 cm^{-1}, which indicated the presence of O– H group in the molecule. A strong peak was observed at 2904 cm^{-1} that was assigned to a strong presence of CH_3 groups. The peak obtained at 1639 cm^{-1} revealed the presence of a cyclic C=C. For further elucidation of the structure of the compound, ^{1}H NMR spectrum of the compound was recorded. Peak assignments were made by peak integration and multiplicity. The methylene groups at the apex were at the same environment and they appeared as a singlet range 1.09–1.67.

Comparing its spectral data with those reported in the literature, the compound was identified as beta amyrin. The ^{1}H NMR of component produced singlet peaks at d: 0.79, 0.83, 0.87, 0.93, 0.96, 0.99 and 1.13 ppm. The peak at 0.87 was the strongest, probably representing two singlets from the compound. The methylene group below the plane gave triplet in the range 0.67–0.64 ppm. The ^{1}HNMR spectrum gave a doublet at 1.44 and 1.46 characteristic of the methylene group, which is above the plane. The other protons showed dimethylene, which exist in the same environment as triplet at 198 ° C; the physical data were consistent with those reported in the literature (Vesterberg *et al*. 2010). All proton and carbon signals were mapped based on 1HCOSY, DEPT, HMQC and HMBC analysis as shown in Table (11). The compound was demonstrated as 3b-hydroxylolean-12-ene beta-amerine (Fig.40). Beta-amyrin is one of the most important triterpens. This compound is more powerful than aspirin, which inhibits platelet aggregation induced by collagen. In addition, b-amyrin includes skin care, anti-irritation and anti-inflammatory applications as well as an enhanced sun protection factor for organic sunscreens and emollient effects (Kweifio-Okai *et al*., 1995 and Ching *et al*., 2010). Beta-amyrin, also known as amyrin or (3beta) -olean-12-en-3-ol, is a member of the class of compounds known as triterpenoids. Triterpenoids are terpenes that contain six isoprene units. Thus, beta-amerine is considered the isoprenoid lipid molecule. Beta amyrin is practically insoluble (in water) and a very weak acidic compound (based on pKa). Beta amyrin can be made from oleanins. Beta-amyrin is also a major compound for other conversion products, including but not limited to erythrodiol, glycyrrhetaldehyde, and 24-hydroxy-beta-amyrin.

Beta-amyrin can be found in a number of nutrients such as thistle, pepper (*C. baccatum*), wakame, and endive, making beta-amyrin a potential biomarker for consuming these food products. Amyrins are three naturally occurring chemical compounds closely related to the triterpene class. They are designated α-amyrin (ursane skeleton), β-amyrin (oleian skeleton) and δ-amyrin. Each of them is penta cyclic triterpenol with the chemical formula $C_{30}H_{50}O$. It is widely distributed in nature and has been isolated from a variety of botanical sources such as wax. In plant biosynthesis, α-amyrin is a precursor to ursolic acid and am-amyrin is a precursor to oleanolic acid. All three amyrins occur in the surface wax of tomato fruit. A-Amyrin is found in dandelion coffee.

3.3 Conclusion

In this study, a phytochemical screening of the leaves of *Solenostemma argel* was performed in order to determine its constituents, which could have important roles in the protective activities that the plant maintains. Various ingredients were detected such as alkalis, flavonoids, terpenoids, saponins, tannins, steroids and anthraquinones in the extract and fraction extract. Owing to its widespread use against various diseases especially infectious legs, its antimicrobial activity and insecticide effect were of great help. The crude extract and solvent extract fractions were investigated for inhibition efficacy against bacteria (*Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruinosa* and *Escherichia coli)* fungi (*Aspergillus niger* and *Candida albicans*) and *Tribolium castaneum* third insect larvae and showed high efficacy in inhibiting growth and larvae. Mortality rate, respectively.

Solenostemma argel can be used in therapeutic procedures as an antibiotic for infectious disease or as an anti-fungal and anti-insecticide in agriculture to preserve and protect agricultural plants. It is rich in organic ingredients and the fact that it is natural and widely spread makes it a good and potential candidate for the pharmaceutical industry. According to the above results, the chloroform extract was very effective against insecticides and microbes. The current study gave a new picture of the existence of some basic and rare elements in plants. It contained two types of new phytosterols; new stigmasterol glucoside, beta-sitosterol glucoside and beta-amyrine triterpenoids that had been identified by comparing their recorded spectroscopic spectra and determined phytochemical properties with those of identical chemical formula and structure reported in the literature.

3.4 Recommendations

1. Following the success of isolation, purification and elucidation of active antimicrobial compounds from *S. argel* lure, similar research work models to produce, from the different parts of the plant, on an industrial scale, pesticides, fungicides wound healing agents and diabetes type II drugs to reduce glucose level in blood circulation. Pharmacological, toxicological and clinical studies should be carried out on the selected medicinal plants (especially *Solenostemma argel*) to assess their safety, therapeutic efficacy and potential for commercial utilization.

2. Considering bio-assay-guided fractionation and purification may lead to the isolation of the active compounds. The chemical structures of these compounds can then be elucidated. This can then help in:

I. The standardization of the active ingredients.

II. The study of the structure activity relationship for the production of compounds with improved characteristics.

III. The study of the pharmacokinetics of the pure active compounds and it helps in the formulation procedures.

3. That there is at present steadily increasing research work by Sudanese scientists in the field of medicinal plants demands strongly wide chemical screening of active ingredients in wide and cultivated Sudanese plants and indigenous food to improve nutrition and develop traditional medicine.

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Appendix