International Journal of Chemistry and Aquatic Sciences
http://chemistryjournal.kypublications.com/
Vol.1.Issue.1(June-July).,2015
ISSN:2455-040X

Research Article

Preliminary GC-MS Profiling and Anti-bacterial activity Investigation of *Acanthospermum hispidum* DC (Asteraceae)

Shetonde O. Mihigo[1,*], Luvenga Ndombele[1], Ishmael B. Masesane[2], K. Sichilongo[2]
[1]Department of Chemistry, University of Kinshasa, P.O. Box: 190 Kinshasa XI, Democratic Republic of Congo. [2]Department of Chemistry, University of Botswana, P/Bag 00704 Gaborone, Republic of Botswana.
***Corresponding Author**: shetonde.mihigo@unikin.ac.cd; smihigo@yahoo.com
Phone number: +243-0-821587299 (SOM)

Shetonde O. Mihigo

Article Received : 02-06-2015
Accepted for publications: 12-07-2015
Published Online: 31-7-2015

ABSTRACT
In this study, the qualitative and quantitative profiles of the hexane and dichloromethane (DCM) extracts from the leaves and roots of *Acanthospermum hispidum* DC were determined by GC-MS. The individual constituents of the extracts were identified by matching their data with those of similar compounds stored in the NIST 05L Library. AMDIS analyses of the Total Ion Chromatograms lead to the identification of 22 and 11 compounds from the leaves and roots, respectively. The hexane and DCM leaf extracts consisted of alcohols (60.26%), hydrocarbons (9.71%), fatty acid esters (3.43%), one ketone (5.82%), and one ether (2.36%); and alcohols (73.79%), one ketone (2.14%), and one aldehyde (5.81%), respectively. The most abundant compounds in the hexane and DCM leaf extracts were (-)-spathulenol (23.22%) and 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (73.36%), respectively. Likewise, the hexane and DCM root extracts consisted of hydrocarbons (17.47%), fatty acid esters (8.48%), and one ketone (4.81%); and fatty acid esters (49.31%) and alcohols (34.53%), respectively. Major compounds in the root hexane and DCM extracts were isopropylmyristate (7.18%) and isopropyltetradecanoate (45.24%), respectively. The leaf extracts were subsequently evaluated for their antibacterial activity using a 96-well microdilution broth assay. They exhibited moderate activity against *Staphylococcus aureus* but were found inactive against *Escherichia coli.*

Keywords: *Acanthospermum*, Antibacterial, Chromatography, GC-MS.

INTRODUCTION

Over the past few decades, great attention has been focused on plants natural products for their potential as active principles in the management and treatment of diseases. Knowledge

of the chemical constituents of plants is desirable not only for the discovery of therapeutic agents, but also because they can serve as templates for the synthesis of complex chemical substances and for discovering the actual significance of folkloric remedies (Prabhu et al., 2013).

Gas chromatography, coupled with Mass spectrometry (GC-MS) constitutes a simple, direct, reliable, and a valuable analytical technique that has been increasingly applied for the detection and analysis of various samples such as non polar components and volatile essential oil, fatty acids and lipids. For example, through extensive GC-MS investigations, traditional medicines and medicinal plants have been found to possess a high number of phytochemicals that display various and sometimes overlapping biological activities. In Natural Products Chemistry, 'plant' includes trees, shrubs, weeds, bushes, grasses, etc., as well as what one normally associates with the term plant; and all parts of a plant can be explored for their phytochemistry and bioactivity potentials (Thomas, 2007).

Thus, we hypothesized that the chemical and biological investigations of invasive plants and weeds' extracts, for their antimicrobial activities might be a potential source of pharmacologically important compounds. Consequently, instead of a blind screening of many plant species for antimicrobial activity, we will investigate whether invasive and weedy species may be used as a source of useful extracts.

The genus *Acanthospermum* (Asteraceae) comprises annual herbaceous plants that are either erect or prostrate. The leaves are simple, opposite, with serrate or entire margins, and the infl orescence is axial or terminal, with yellow flowers. The fruit is an achene, oblong, with rigid and persistent hairs (Araújo et al., 2008).

Acanthospermum hispidum DC, also called Bristly Starbur or Goathead (English), Herbe tricorne (French), Carapichno (Spanish), is an upright annual plant with dichotomous (Y-shaped) branching. The Y-shaped form of branching gives the plant one of its common names, Slingshot Weed. The scientific name of the genus, *Acanthospermum*, is from the Greek words *acantha* (thorn) and *sperma* (seed) and refers to the prickly fruit. *Hispidum* is Latin, and means rough, shaggy, prickly or bristly. The stem is shaggy, prickly or bristly and is densely covered with hairs. Some leaves can be up to 11.5 cm long. The margins of the leaves can have irregular teeth or they may be entire and smooth. The flowers are typical of the Aster or Daisy Family. Each head has 5-9 ray flowers. The petals (corollas) of the ray flowers are pale yellow and are about 1.5 mm long. The fruits are flattened, triangular in shape, covered with stiff and hooked hairs; and have either a straight or curved pair of spines at the top (Araújo et al., 2008; Adepiti et al., 2014).

Although *A. hispidum* has long been principally considered an invasive weed of agricultural plantations, it has recently become sought after as raw material to manufacture syrup produced by public health services in a number of Brazilian municipalities to treat asthma. As a consequence, a high demand for this plant in Brazil has led to its cultivation as it occurs spontaneously only during the rainy season. Popularly known as "Espinho-de-cigano" ("Gypsy-Thorn"), *A. hispidum*, has been traditionally used in northeastern Brazil for treating bronchitis, dysentery, fevers and as expectorant, as vermifuge and against intestinal pains (Araújo et al., 2008). It is also used elsewhere in the treatment of yellow fever, malaria, stomach disorder. Other medicinal properties include the following: antichromonal (Adepiti et al., 2014), antiplasmodial (Chakraborty et al., 2012; Koussounda et al., 2013), antibacterial, antiviral,

antifungal, anticancer, antidiarrhoeal, abortive, antifeedant, immunostimulant, antitrypanosomal, and antileishmanial (Araújo et al., 2008; Adu et al., 2011; Faleye et al., 2012; Chakraborty et al., 2012; Koussounda et al., 2013). In addition, plant parts of *A. hispidum* DC from different places have proven to be sources of pharmacologically interesting natural products. For example, sesquiterpene lactones such as acanthospermal B, acanthospermal B epoxide, hispidunolide A and B; glycosides, flavonoids (Roy et al., 2010; Edewor and Olajire, 2011; Chakraborty et al., 2012; Faleye et a., 2012; Adepiti et al., 2014), saponins, steroids, fatty acid esters, amino acids, alkaloids, tannins, and polyphenols (Araújo et al., 2008; Roy et al., 2010; Faleye et al., 2012; Temidayo, 2013), fatty alcohols and hydrocarbons (Chakraborty et al., 2012), minerals (K, Na, Ca, Mg, Mn, P, Fe, and Zn), and vitamins such as ascorbic acid, riboflavin, thiamin, and niacin (Faleye et al., 2012; Roy et al., 2010) have been reported to occur in this plant. In the Democratic Republic of Congo, *A. hispidum* DC is used against a number of diseases, including microbial infections and snake bites. Thus, these previous reports constitute a clear indication the *A. hispidum* DC has a potential as source of interesting bioactive compounds.

However, it should be noted that no phytochemical work has been done on *A. hispidum* DC plant material sampled in the DR Congo; hence the current investigation that has proposed to carry out a preliminary phytochemical study in order to provide a GC-MS profile of, and antibacterial study data on *A. hispidum* DC.

Materials and Methods

Plant materials

The leaves and roots of *A. hispidum* were collected on February 06, 2014, from their natural habitats in Kimwenza/Kinshasa, DR Congo. The collected plant materials were authenticated by Mr Boniface Nlandu of the INERA (Institut National d'Etudes et Recherches Agronomiques) Herbarium located at the Faculty of Science/University of Kinshasa. A voucher specimen (R. Germain 4538, of December 8, 1940) is on deposit at the INERA herbarium. The plant materials were separately dried under shade at room temperature, then ground into a powder that was used for extractions.

Extractions

The dried and powdered materials were successively macerated and extracted by shaking at room temperature using hexane (2 x 48h), and dichloromethane (DCM, 48h) giving the required organic fractions. The extracting solvent was about 15 mL/g. The fractions were filtered using appropriate Whatman filter paper to obtain particle-free fractions from which the solvents were evaporated under reduced pressure (using a rotary evaporator). The resulting extracts (4 in total) were flashed with nitrogen and stored for GC-MS and antibacterial analyses.

GC-MS analyses

The hexane and DCM extracts (1% w/v solution) were submitted to GC-MS analyses and were found to contain a high number of metabolites. The Gas chromatography (GC) analysis was carried out on a 7890A GC chromatograph fitted with HP-5 MS column (30 m x 0.25 mm, 0.25 µm) and interfaced with a mass spectrometer 5975C (both Agilent Technologies). The GC analytical conditions were as follows: carrier gas He (99.999% purity; 1 mL/min), injector temperature 280°C, column temperature programmed from 100 °C (4 min hold) to 300 °C (16 min hold) at 10 °C/min. Samples were injected by splitless mode. The volume injected and the inlet pressure were 1.0 µL and 72.553 kPa, respectively; and the total running time was 46

minutes. The MS conditions were as follows: ionisation voltage 70 eV; emission current 34 mA; acquisitions scan mass range of 50 – 600 amu at a sampling rate of 2.0 scan/s.

Identification and quantification of constituents

The identification of constituents of the extracts was conducted based on GC retention times on an HP-5MS capillary column and by matching their corresponding names, molecular formulae, molecular weights, and the acquired mass spectra (and the fragmentation patterns) with those of similar compounds stored on commercial libraries, in this case the NIST 05L Mass Spectral Library. The relative quantification (percent composition) of the extracts constituents were determined by computerized peak area measurements using the internal normalization method. AMDIS (Automated Mass spectral Deconvolution and Identification System) software was used as a tool to collect and compare the chromatographic profiles (*fingerprint*) of each extract with those stored in the libraries. Match Factors above 80 % (very good to perfect agreement /match) of the spectra were considered for identification of individual components of the extracts (Clement, 1991).

Antibacterial activity

The antibacterial activity was evaluated by determining the minimum inhibitory concentration (MIC), using the broth micro-dilution method (De Martino et al., 2009; Okusa, 2012; Mazimba et al., 2015). The stock solution of the extract (4000 µg/mL) was used for the preparation of dilutions. Serial dilutions of the extracts were made in a sterile 96-well micro plate filled with Mueller-Hinton broth. In this way, concentrations ranging from 4000 µg/mL to 62.5 µg/mL were obtained. The sample was first sterilized, then stirred, and inoculated with 100 µL of physiological solution containing appropriate microbial strains, and incubated at 37 °C. Cultures, containing only sterilized physiologic solution and Mueller-Hinton broth, instead of the extract sample, were used as positive control and were found not toxic to the microorganisms. A 2% solution (20 µL) of 2,3,5-Triphenyltetrazolium chloride was added to each well before observation of bacterial growth and the subsequent estimation of the MIC value; the principle of this method being based on the ability of living cells to reduce the tetrazolium salt in a red precipitate or formazan (Okusa, 2012). The MIC was determined as the lowest concentration of the sample that did not permit any visible growth of the tested microorganism after incubation (37 °C, 24-48 h). Whenever the germs did not grow in a certain well, this denoted a bactericidal action of the extract (De Martino et al., 2009; Okusa, 2012). *Staphylococcus aureus* and *Escherichia coli* clinic strains from the hospital of the Faculty of medicine / University of Kinshasa (DR Congo), were used in this study.

Results and Discussion

The Total Ion Chromatogram (TIC) profile suggested that over 40 compounds were separated and eluted from the extracts of this plant, the leaves displaying the highest number. The relatively low number of compounds observed in the root bark extracts may be due to a small quantity (only 2g) of plant material/powder used for the extractions.

A total of 22 compounds were identified from the leaf extracts (19 and 6 compounds from the hexane and DCM extracts, respectively; 3 being common to the two extracts) (Table 1). Of these, the most abundant components were (-)-spathulenol (23,22%), α-bisabolol (11,42%), 1-heptacosanol (9,10%), phytol (8,80%), 6,10,14-trimethyl-2-pentadecanone (5,82%), eicosane (4,74%), 1-octadecanol (4,57%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (2,99%), methyloctacosanoate (2,87%), 2-methyleicosane (2.50%), copaene (2,47%) and caryophyllene

oxide (2,36%) in hexane and 3,7,11,15-tetramethyl-2-hexadecen-1-ol (73,36%), hexadecanal (5,81%) and 2-nonadecanone (2,14%) in the DCM extract.

Table 1. Chemical composition of *A. hispidum* Leaf extracts

Entry	Name	Retention Time (min)	Peak Area	Relative % Composition	Match Factor (%)
	I. Hexane extract				
1	Copaene	8.9282	394129	2.47	89
2	1-Undecanol	10.47	14948	0.09	89
3	Caryophyllene oxide	11.429	376552	2.36	87
4	(-)-Spathulenol	11.815	3702445	23.22	85
5	α-Bisabolol	12.992	1821767	11.42	92
6	**A**	14.649	928668	5.82	93
7	**B**	15.439	40473	0.25	85
8	Hexadecanoic acid, ethyl ester	16.117	47432	0.30	87
9	**C**	17.079	6723	0.04	86
10	Phytol	17.284	1402717	8.80	86
11	Octadecanoic acid, methyl ester	17.359	6996	0.04	91
12	Pentadecanal-	18.251	122735	0.77	83
13	Eicosane	21.965	755391	4.74	88
14	Eicosane, 2-methyl-	23.78	398140	2.50	85
15	1-Heptacosanol	24.069	1450436	9.10	80
16	1-Octadecanol	24.839	728289	4.57	81
17	Methyl octacosanoate	25.011	456957	2.87	88
18	1-Hexadecanol	27.644	10366	0.07	89
19	**D**	29.596	477164	2.99	83
	II. Dichloromethane extract				
20	Hexadecanal	14.3344	293979	5.81	89
21	**D**	14.5877	3711765	73.36	90
22	1-Dodecanol	15.0863	19093	0.38	92
23	**B**	15.4297	5232	0.10	84
24	2-Nonadecanone	17.1593	108316	2.14	87
25	1-Hexadecanol	17.32	2755	0.05	81

A: 6,10,14-trimethyl-2-Pentadecanone **B**: methylhexadecanoate, **C**: methyl-(Z,Z)-9,12-octadecadienate, **D**: 3,7,11,15-Tetramethyl-2-hexadecen-1-ol

In addition, eleven (11) compounds were identified from the root bark extracts (7 components from hexane and 6 from the DCM extract, 2 being common to these two extracts) (Table 2). The most abundant compounds were isopropyltetradecanoate (7,18%), 2-methylundecane (7,08%), 2-methyldodecane (6,70%), 6,10,14-trimethyl-2-pentadecanone (4,81%) and 2,7,10-trimethyldodecane (2,47%) in hexane extract, and isopropyltetradecanoate (45,24%), 2-methyl-5-(1-methylethyl)-phenol (29,75%), 1-hexadecanol (3,66%) and ethylhexadecanoate (2,74%) in the DCM extract.

International Journal of Chemistry and Aquatic Sciences
http://chemistryjournal.kypublications.com/
Vol.1.Issue.1(June-July).,2015
ISSN:2455-040X

Table 2. Chemical composition of *A. hispidum* Root extracts

Entry	Name	Retention Time (min)	Peak Area	Relative % Composition	Match Factor (%)
	I. Hexane extract				
1	Isopropyltetradecanoate	14.4082	28396	7.18	87
2	2-Pentadecanone, 6,10,14-trimethyl-	14.6303	19017	4.81	86
3	Hexadecanoic acid, ethyl ester	16.1003	5140	1.30	89
4	Dodecane, 2,7,10-trimethyl-	19.6489	9759	2.47	84
5	Hexadecane	20.4427	4838	1.22	89
6	Dodecane, 2-methyl-	21.2059	26514	6.70	83
7	Undecane, 2-methyl-	21.9415	28021	7.08	83
	II. Dichloromethane extract				
8	Phenol, 2-methyl-5-(1-methylethyl)-	7.454	56118	29.75	97
9	Isopropyltetradecanoate	14.3942	85331	45.24	80
10	1-Hexadecanol	14.9847	6894	3.66	92
11	Hexadecanoic acid, methyl ester	15.4314	2523	1.34	90
12	1-Undecanol	15.6709	2109	1.12	80
13	Hexadecanoic acid, ethyl ester	16.1003	5140	2.73	89

The leaf extracts of *A. hispidum* DC were preliminarily studied for their antibacterial activity. The DCM extract was found to be more active (125 µg/mL) than the hexane extract which showed a moderate activity against *S. aureus* (500 µg/mL). All the extracts of *A. hispidum* were inactive against *E. coli* (MIC > 2000 µg/mL) and this seems to corroborate previous comparative reports on the antimicrobial activities of plants extracts against gram positive and gram negative bacteria, indicating that these extracts were often found more active against gram positive than the gram negative bacteria (Adu et al., 2011; Edewor and Olajire, 2011).

Similar studies, on the evaluation of both the chemical composition and antimicrobial activity of *A. hispidum* DC from Brazil (Araújo et al., 2008), Congo Brazzaville (Koussounda et al., 2013), Ghana (Adu et al., 2011), Nigeria (Edewor and Olajire, 2011; Faleye et a., 2012; Temidayo, 2013), and India (Uma et al., 2009; Chakraborty et al., 2012; Jegadeeswari et al., 2012; Kalaiselvan et al., 2012; Selvamangai and Bhaskar, 2012; Saravanan et al., 2013) have reported the activities as well as the occurrence of some of the compounds we found in *A. hispidum* DC sampled (current study) in the DR Congo.

These, and additional studies on other plants species have established their antimicrobial activity as being related to the presence of, in particular, caryophyllene oxide (Rajeswari et al., 2011; Mazimba et al., 2012; Elisabeth and Arumugam, 2014), methylhexadecanoate (Elekwa et al., 2011; Saravanan et al., 2013; Shettima et al., 2013; Elisabeth and Arumugam, 2014), phytol (Ammal and Bai, 2013; Kalaiselvan et al., 2012;

Sermakkani and Thangapandian, 2012; Elisabeth and Arumugam, 2014), copaene (Mazimba et al., 2012), (-)-spathulenol (Rajeswari et al., 2011; Mazimba et al., 2012), and 3,7,11,15-tetramethyl-2-hexadecen-1-ol (Jegadeeswari et al., 2012; Kalaivani et al., 2012; Sermakkani and Thangapandian, 2012; Ammal and Bai, 2013). Thus, these compounds seem to be responsible of the observed antibacterial activity. Moreover, although all these compounds are known to have antibacterial activity, the contribution of 3,7,11,15-tetramethyl-2-hexadecen-1-ol towards *A. hispidum* DC activity may be particularly important since it was found to be the major (over 73%) compound of the most active DCM extract.

Additional compounds previously reported from different plants species and that have shown other activities include ethylhexadecanoate (antioxidant, flavor, hypocholesterolemic, nematicide, pesticide, lubricant, antiandrogenic, hemolytic, and 5-α-reductase inhibitor) (Elekwa et al., 2011; Gopalakrishnan et al., 2011; Saravanan et al., 2013; Sermakkani and Thangapandian, 2012; Elisabeth and Arumugam, 2014), methylhexadecanoate (antioxidant, flavor, hypocholesterolemic, and 5-α-reductase inhibitor) (Saravanan et al., 2013), hexadecanal (antianemic, antiviral, diuretic, insecticide) (Sujatha et al., 2014), 1-undecanol (used as flavor) (Selvamangai and Bhaskar, 2012), phytol (anticancer, antioxidant, anti-inflammatory, diuretic) (Jegadeeswari et al., 2012), copaene, α-caryophyllene, caryophyllene oxide, and α-bisabolol (Uma et al., 2009; Mazimba et al., 2012), etc. Although these are different activities, the presence the concerned compounds in the investigated extracts may just be a contribution to synergistic effect in favour of the observed activities.

Additional and detailed phytochemical investigations are needed and will provide a sound basis for the biochemical and pharmacological properties that may be associated with any compounds that have been and could be identified and/or isolated from *Acanthospermum hispidum* DC.

The root extracts were not tested for antibacterial activity due to limited and insufficient quantities of sample.

Conclusions

In the present study, a Congolese weedy plant (*Acanthospermum hispidum* DC) has been submitted to GC-MS and antibacterial analyses for the first time. The present investigation has led to the determination of the qualitative and quantitative profiles of the hexane and dichloromethane (DCM) extracts from the leaves and roots *A. hispidum* DC. A total of 29 compounds were identified from the extracts (22 and 11 compounds from the leaf and root extracts, respectively; 4 compounds having been simultaneously isolated from the leaves and roots extracts). We have also preliminarily determined the chemical percentage composition of the above extracts by GC-MS analyses. The leaf extracts were evaluated for antibacterial activity against *Staphylococcus aureus* and *Escherichia coli.* They exhibited a moderate activity against the former but were found inactive against the later.

Further studies for the isolation, purification, identification and/or characterisation of individual chemical compounds, and their biological evaluation against a larger number of bacteria and fungi are envisaged and could lead to significant results.

Acknowledgements

The authors thank the Department of Chemistry of the University of Botswana for providing part of the laboratory facilities. SOM is very grateful to the Organization for the Prohibition of Chemical Weapons (OPCW) for an internship support. The authors also thank Mr D. Mosimanethebe for gaz chromatography-mass spectrometry (GC-MS) experiments.

References

[1]. Adepiti A.O., Adewunmi C.O. and Agbedahunsi J.M. (2014) Antichromonal activity of *Acanthospermum hispidum* DC (Asteraceae). African Journal of Biotechnology, 13(11): 1303-1307.

[2]. Adu F., Gbedema S.Y., Akanwariwiak W. G., Annan K. and Boamah V.E. (2011) The effects of *Acanthospermum hispidum* extract on the antibacterial activity of amoxicillin and ciprofloxacin. Hygeia Journal for Drugs and Medicines, 3(1): 58-63.

[3]. Ammal R.M. and Bai G.V.S. (2013) GC-MS Determination of bioactive constituents of *Heliotropium indicum* leaf. Journal of Medicinal Plants Studies, 1(6): 30-33.

[4]. Araújo E.L., Karina P., Randau K.P., Sena-Filho J.G., Pimentel R.M.M. and Xavier H.S. (2008) *Acanthospermum hispidum* DC (Asteraceae): perspectives for a phytotherapeutic product. Brazilian Journal of Pharmacognosy, 18 (Supl.): 777-784.

[5]. Chakraborty A.K., Gaikwad A.V. and Singh K.B. (2012) Phytopharmacological review on *Acanthospermum hispidum*. Journal of Applied Pharmaceutical Science, 2 (1): 144-148.

[6]. Clement R. 1991. Techniques for the Gaz Chromatography-Mass Spectrometry identification of organic compounds in effluents. ISBN, 0-7729-59834, Queen's Prenter for Ontario.

[7]. De Martino L., De Feo V., Formisano C., Mignola E. and Senatore F. (2009) Chemical composition and antimicrobial activity of the essential oils from three chemotypes of *Origanum vulgare* L. ssp. *hirtum* (Link) Ietswaart growing wild in Campania (Southern Italy). Molecules, 14: 2735-2746.

[8]. Edewor T.I. and Olajire A.A. (2011) Two flavones from *Acanthospermum hispidum* DC and their antibacterial activity. International Journal of Organic Chemistry, 1: 132-141.

[9]. Elekwa I., Okereke S.C. and Chukwudorwo C.S. (2011) Phytochemical screening and GC-MS analysis of the essential oil of *Dennettia tripetala* (pepper fruit) seeds. ABSU Journal of Environment, Science and Technology, 1: 93-98.

[10]. Elezabeth V.D. and Arumugam S. (2014) GC-MS Analysis of ethanol extract of *Cyperus rotundus* leaves. International Journal of Current Biotechnology, 2(1): 19-23.

[11]. Faleye F.J., Odeyemi A.T. and Aderogba A.A. (2012) Evaluation of the chemical composition and antimicrobial activities of three Nigerian medicinal plants. Elixir Applied Biology, 45: 7652-7656.

[12]. Gopalakrishnan S. and Vadivel E. (2011) GC-MS analysis of some bioactive constituents of *Mussaenda frondosa* Linn. International Journal of Pharma and Bio-Sciences, 2(1): 313-320.

[13]. Jegadeeswari P., Nishanthini A., Muthukumarasamy S. and Mohan V.R. (2012) GC-MS Analysis of bioactive components of *Aristolochia krysagathra* (Aristolochiaceae). Journal of Current Pharmaceutical Sciences, 2(4): 226-232.

[14]. Kalaiselvan A., Gokulakrishnan K. and Anand T. (2012) Gas chromatography-Mass spectrum analysis of bioactive components of the ethanol extract of *Andrographis paniculata*. Journal of Pharmaceutical and Biomedical Sciences, 20(15): 1-3.

[15]. Kalaivani C.S., Sathish S.S., Janakiraman N. and Johnson M. (2012) GC-MS studies on *Andrographis paniculata* (Burm.f.) Wall. ex Nees-A medicinally important plant. International Journal of Medicinal and Aromatic Plants, 2(1): 69-74.

[16]. Koussounda F.K., Abena A.-A., Nzoungani A., Mombouli J.-V., Ouamba J.-M., Kun J. and Ntoumi F. (2013) In vitro evaluation of antiplasmodial activity of extracts of *Acanthospermum hispidum* DC (Asteraceae) and *Ficus thonningii* Blume (Moraceae), two plants used in traditional medicine in the Republic of Congo. African Journal of Traditional, Complementary, and Alternative Medicine, 10(2): 270-276.

[17]. Mazimba O., Masesane I.B., Majinda R.R.T. and Muzila A. (2012) GC-MS analysis and antimicrobial activities of the non-polar extracts of *Mundulea sericea*. South African Journal of Chemistry, 65: 50–52.

[18]. Mazimba O., Wale K., Kwape T.E., Mihigo S.O. and Bokolo M.K. (2015) *Cinnamomum verum*: ethylacetate and methanol extracts antioxidant and antimicrobial activity. Journal of Medicinal Plants Studies, 3(3): 28-32.

[19]. Okusa P, 2012. Etude phytochimique et activité antimicrobienne directe et indirecte de *Cordia gilleti* de Wild (Boraginaceae). PhD Thesis. Université Libre de Bruxelles, Belgium.

[20]. Prabhu P.T., Panneerselvam P., Suresh R., Atlee C.W. and Balasubramanian S. (2013) GC-MS analysis of ethanolic extract of *Canthium parviflorum* Lamk leaf. Journal of Applied Pharmaceutical Science, 3 (2): 166-168.

[21]. Rajeswari R.N., Lakshmi R.S. and Muthuchelian K. GC-MS analysis of bioactive components from ethanolic extract of *Canthium dicoccum* (Gaertn.) Teijsm & Binn. Journal of Chemical and Pharmaceutical Research, 3(3): 792-798.

[22]. Roy H., Chakraborty A., Bhanja S., Nayak B.S., Mishra S.R. and P. Ellaiah. (2010) Preliminary phytochemical investigation and anthelmintic activity of *Acanthospermum hispidum* DC. Journal of Pharmaceutical Science and Technology, 2 (5): 217-221.

[23]. Saravanan D., Kasisankar V. and Asharani I.V. (2013) GC-MS analysis of phytocomponents in the leaves of *Actinodophne madraspatana* Bedd. International Journal of Research in Pharmaceutical Sciences, 4(3): 469-473.

[24]. Selvamangai G. and Bhaskar A. (2012) GC-MS analysis of phytocomponents in the methanolic extract of *Eupatorium triplinerve*. Asian Pacific Journal of Tropical Biomedicine, S1329-S1332.

[25]. Sermakkani M. and Thangapandian V. (2012) GC-MS analysis of *Cassia italica* leaf methanol extract. Asian Journal of Pharmaceutical and Clinical Research, 5(2): 90-94.

[26]. Shettima A.Y., Karumi Y., Sodipo O.A., Usman H. and Tijjani M.A. (2013) Gas Chromatography-Mass Spectrometry (GC-MS) analysis of bioactive components of ethyl acetate root extract of *Guiera senegalensis* J.F. Gmel. Journal of Applied Pharmaceutical Science, 3(3): 146-150.

[27]. Sujatha M., Karthika K., Sivakamasungari S., Mariajancyrani J. and Chandramohan G. (2014) GC-MS analysis of phytocomponents and total antioxidant activity of hexane extract of *Sinapis alba*. International Journal of Pharmaceutical, Chemical, and Biological Sciences, 4(1): 112-117.

[28]. Temidayo A.R. (2013) Extraction and isolation of flavonoids present in the methanol extract of leaves of *Acanthospermum hispidum* DC. Global Journal of Medicinal Plant Research, 1(1): 111-123.

[29]. Thomas G. (2007) Medicinal Chemistry, an Introduction. 2nd ed., John Wiley & Sons, Chichester.

[30]. Uma B., Prabhakar K., Rajendran S. and Sarayu L.Y. (2009) Studies on GC/MS spectroscopic analysis of some bioactive antimicrobial compounds from *Cinnamomum zeylanicum*. Journal of Medicinal Plants, 8(31): 125-131.

Conflict of Interest : Nil

Financial Support: Not Mentioned