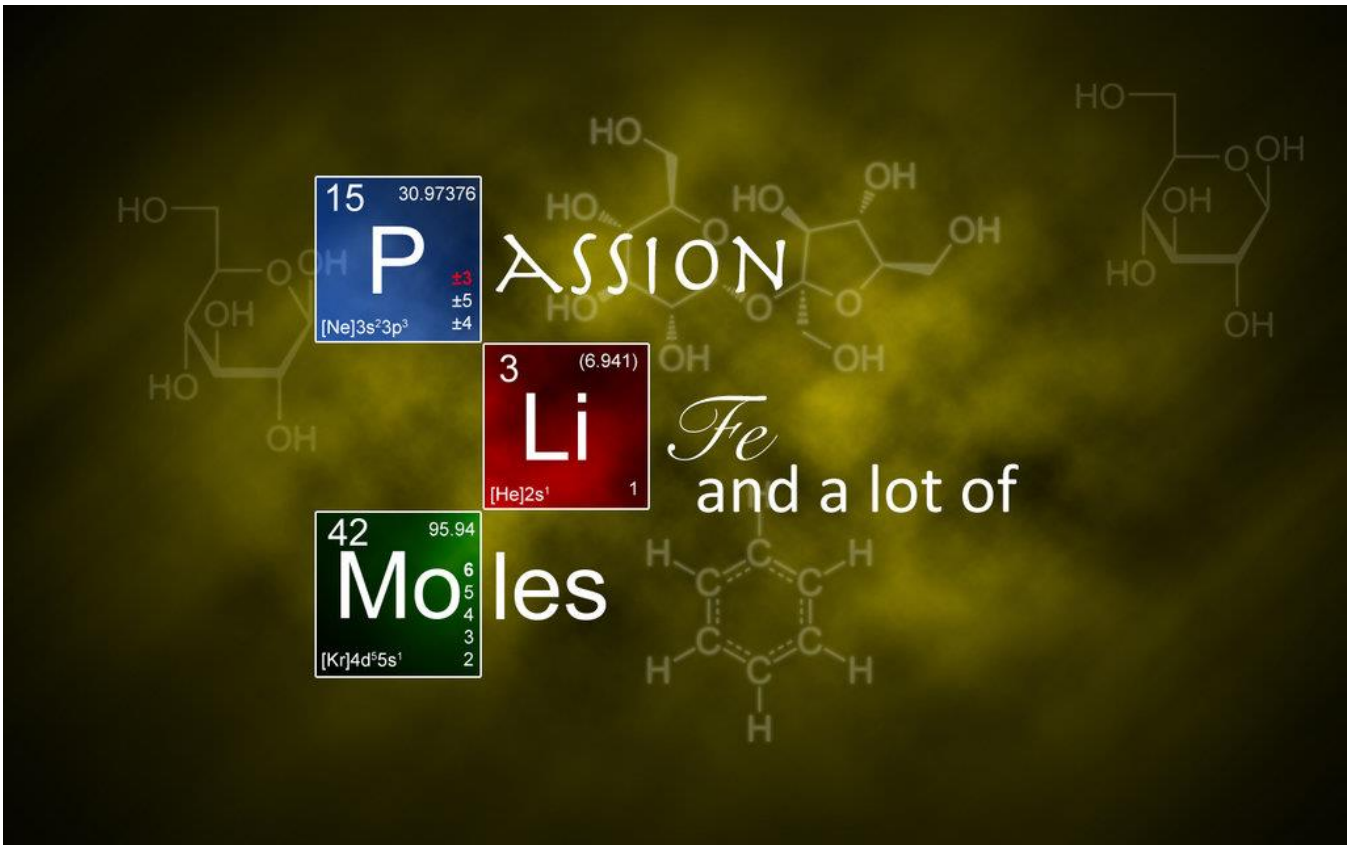

Volume 2 Issue.1 :2016

INTERNATIONAL JOURNAL OF CHEMISTRY AND AQUATIC SCIENCES (IJCA)

WWW.CHEMISTRYJOURNAL.KYPUBLICATIONS.COM

ISSN:2455-040X

www.kypublications.com

PRELIMINARY GC-MS PROFILING AND ANTI-BACTERIAL POTENTIAL OF TWO CONGOLESE INVASIVE WEEDS, *SYNEDRELLA NODIFLORA* (L.) GAERTN. AND *ASPILIA KOTSCHYI* (SCHULTZ BIP.) OLIV. (ASTERACEAE)

RESEARCH ARTICLE

SHETONDE O. MIHIGO*[1], MAVONGA TEKAYELA[1], ISHMAEL B. MASESANE[2], KWENGA 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*

Shetonde O. Mihigo

ABSTRACT

In this study, phyptochemical analyses of the leaves and root barks of Synedrella nodiflora indicated the presence of alkaloids, flavonoids, coumarins, quinones, saponins, steroids, and terpenoids. In addition, the qualitative and quantitative profiles of the non-polar extracts of Synedrella nodiflora and Aspilia kotschyii were determined by GC-MS. Twenty-six (26) and 25 compounds were identified from S. nodiflora and A. kotschyii, respectively. The most abundant compounds in S. nodiflora included caryophyllene oxide (32.36%), 6,10,14-trimethyl-2-pentadecanone (23-25.96%), methyl-(Z)-9-octadecenoate (23.74%), caryophyllene (17.64%), triacontane (8.01%), 3,5,11,15-tetramethyl-1-hexadecen-3-ol (7.13%), pentadecanal (6.63%), and geranylgeraniol (4.23%). Likewise, the most abundant compounds in A. kotschyii were caryophyllene (56-72.90%), octadecanal (42.24%), methyl-(Z)-9-octadecenoate (22.14%), isopropylmyristate (14.39%), 1-hexadecanol (12.90%), 3,5,11,15-tetramethyl-1-hexadecen-3-ol (7.86%), pentadecanal (9.44%), cedrene (6.23%), 1-octadecanol (4.40%), and eicosane (3.13%). S. nodiflora extracts were evaluated for antibacterial activity against Staphylococcus aureus and Escherichia coli using a 96-well microdilution broth assay. The hexane and DCM leaf extracts, and DCM root extract exhibited moderate activity (MIC = 500-1000 µg/mL) but the remaining extracts were found inactive (MIC > 1000 µg/mL) against both test bacteria. A. kotschyii was not analysed for both phytochemical composition and antibacterial activity due to limited and insufficient quantities of sample.

Keywords: Synedrella nodiflora, Aspilia kotschyii, Antibacterial, GC-MS.

INTRODUCTION

Medicinal plants of various types and from different settings/habitats are of great importance to human as well as veterinary medicine. The screening of plants extracts represents continuous efforts to find compounds with potential to act against life-threatening diseases such as new and/or re-emerging microbial infections (Alka and Padma, 2013). Antimicrobials of plant origin have enormous therapeutic potential and have been used since time immemorial. They have been proved effective in the treatment of infectious diseases and simultaneously mitigating many of the side effects which are often associated with synthetic antibiotics (Christudas et al., 2012). On the other hand, while it is likely that important new drugs remain hidden in plants in primary tropical rainforest, they may also lie hidden in those belonging to other

ecosystems. In addition, and in relation to other types of plants, there has been significant evidence that weeds are relatively high in bioactive secondary compounds and are thus likely to hold promise for drug discovery (Stepp, 2004).

S. nodiflora (L.) Gaertn. (Asteraceae) is an annual herb that grows up to about 60-120 cm high. It is a native tropical American weed but now dispersed pan-tropically, and occurring throughout the India, the Andamans, and the African regions (Amoateng et al., 2011; Nahar et al., 2012; Zahan et al., 2012). This plant has been in use in various traditional systems of medicine. For instance, in Ghana, the foliage is readily eaten by livestocks whereas in Indonesia the foliage is eaten as a vegetable by some indigenous tribes. In Ghanaian traditional medicine, the whole plant is boiled and the aqueous extract drunk for the treatment of epilepsy. The leaves are used for the management of hiccup and threatened abortion. The plant has also been extensively used in Nigeria for cardiac problems, wounds and for stopping bleeding. In Malaysia and Indonesia, the plant has also been reported in the treatment of headaches, earaches, stomachaches, and embroacation for rheumatism (Amoateng et al., 2011). Additional properties associated with *S. nodiflora* include anti-bacterial, anti-nociceptive, anti-inflammatory, anti-convulsant, free radical scavenging and anti-lipid peroxidative activity (Amoateng et al., 2011), analgesic and CNS depressant effects (Nahar et al., 2012), anti-diarrhoeal and hypoglycemic effects (Zahan et al., 2012), and phytotoxic effects (Ghayal et al., 2013), and its use for phytoextraction of heavy metals (Akoto et al., 2012). Previous studies have shown that *S. nodiflora* contains phenols, reducing sugars, flavonoids, steroids, triterpenoids, tannins, and saponins (Kafundu et al., 1988; Nahar et al., 2012); and some specific compounds that have been reported from this plant include caryophyllene oxide, 2-pentadecanone, hexadecanois acid, phytol, diisooctylphthalate, and neophytidiene (Ghayal et al., 2013).

On the other hand, *Aspilia kotschyi* (Schultz Bip.) Oliv. is an annual herb which grow up to 70 cm; its leaves are sessile and opposite, with a ring around the stem at base, and 5-7 lateral nerves. Two offshoot stems separate from main stem at each ring and white hairs with violet bases cover rough stems (giving appearance of violet dots). Unlike *S. nodiflora*, literature review reveal that the phytochemical work on *A. kotschyi* has not been extensive. Only saponins seem to have been reported from its leaves (Kafundu et al., 1988).

As a consequence, it is important to consider and evaluate these abundantly occurring weed species as potential sources of bioactive compounds than as invasive flora; especially that their plant materials sampled in the Democratic Republic of Congo have not been subjected to detailed phytochemical investigations. Thus, as part of our continued interest in the invasive and weedy plants as potential sources of pharmacologically important compounds, and as a contribution to a wider study of the phytochemistry of the Congolese flora used in traditional medicine (Mazimba et al., 2015), we undertook the current study, consisting of the anti-bacterial investigations and preliminarily GC-MS analyses of *S. nodiflora* and *A. kotschyi*.

Materials and Methods

Plant materials

The leaves and root barks of *S. nodiflora* and *A. kotschyii* 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. Voucher specimens (Diankenda 114 of July 24, 1973; and R. Devred 3375 of April 21, 1947; respectively for *Synedrella nodiflora* and *Aspilia kotschyii*) are 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 (8 in total) were flashed with nitrogen and stored for antibacterial and/or GC-MS analyses.

GC-MS analyses

The hexane and DCM extracts (1% w/v solutions) 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 and Taguchi, 1991; Stein, 1999).

Antibacterial activity

The antibacterial activity was evaluated by determining the minimum inhibitory concentration (MIC), using the broth micro-dilution method (Eloff, 1998; 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* clinical strains from the hospital of the Faculty of medicine / University of Kinshasa (DR Congo) were used in this study.

Results and Discussion

A good separation of the constituents of all extracts from these plants was achieved. Twenty-six (26) compounds were identified from *S. nodiflora*, 19 compounds from the leaves (14 and 5 compounds from the hexane and DCM extracts, respectively (Table 1) and 10 compounds from the root bark extracts (9 components from hexane and 2 from the DCM extract, 1 being common to these two extracts) (Table 2). Three (3) compounds were found common to these two plant parts. The most abundant components in the leaves extracts were 6,10,14-trimethyl-2-pentadecanone (25,96%), caryophyllene (17,64%), triacontane (8,01%), 3,5,11,15-tetramethyl-1-hexadecen-3-ol (7,13%), pentadecanal (6,63%) and geranylgeraniol (4,23%) from hexane and 3,7,11,15-tetramethyl-2-hexadecen-1-ol (27,43%), oxacycloheptadec-8-en-2-one (26,03%), and 7-methoxy-2,2-dimethyl-2*H*-1-benzopyran (7.59%) from the DCM extract.

Table 1. Chemical composition of *S. nodiflora* Leaf extracts

Entry	Name	Retention Time (min)	Peak Area	Relative % Composition	Match Factor (%)
	I. Hexane extract				
1	Caryophyllene	10.0864	1125371	17.637	89
2	Pentadecane	10.5912	14849	0.233	94
3	Heptadecane	13.0308	17532	0.275	89
4	Pentadecanal-	13.2329	422928	6.628	86
5	1-Dodecanol	13.931	7068	0.111	93
6	**A**	14.6685	1656356	25.958	93
7	**B**	15.6705	454610	7.125	90
8	Hexadecanoic acid, ethyl ester	16.1134	100643	1.577	89
9	**C**	17.0793	71238	1.116	93
10	**D**	17.3933	16328	0.256	80
11	Hexadecane	19.6558	13912	0.218	88
12	1-Hexadecanol	23.9982	34145	0.535	88
13	Geranylgeraniol	24.154	269858	4.229	82
14	Triacontane	24.7514	511105	8.010	87
	II. Dichloromethane extract				
15	**E**	10.1952	466888	7.59	84
16	**F**	14.8472	1686516	27.43	92
17	**C**	17.0841	6895	0.11	85
18	**D**	17.3562	2178	0.04	80
19	Oxacycloheptadec-8-en-2-one	17.5445	1600333	26.03	85

A: 2-Pentadecanone, 6,10,14-trimethyl- ; **B**: 1-Hexadecen-3-ol, 3,5,11,15-tetramethyl-; **C**: 9,12-Octadecadienoic acid (Z,Z)-, methyl ester **D**: Octadecanoic acid, methyl ester; **E**: 2*H*-1-Benzopyran, 7-methoxy-2,2-dimethyl-; **F**: 3,7,11,15-Tetramethyl-2-hexadecen-1-ol

Likewise, the most abundant compounds in the root bark hexane extract were caryophyllene oxide (32,36%), methyl-(Z)-9-octadecenoate (23,74%) and ethylhexadecanoate (5,71%). Only two compounds (1-undecanol (9,85%) and tridecane (4,18%)), accounting for about 14% of the total extract), were identified from the DCM extract (Table 2).

Table 2. Chemical composition of S*. nodiflora* Root extracts

Entry	Name	Retention Time (min)	Peak Area	Relative % Composition	Match Factor (%)
	I. Hexane extract				
1	1-Dodecanol	10.4715	11175	1.31	95
2	Tridecane	10.5766	1795	0.21	90
3	Caryophyllene oxide	11.8158	276384	32.36	88
4	Menthol	14.5412	14143	1.66	81
5	Hexadecanoic acid, methyl ester	15.4332	13716	1.61	93
6	Hexadecanoic acid, ethyl ester	16.1019	48752	5.71	95
7	Methyl-(Z)-9-octadecenoate	17.6269	202761	23.74	85
8	Hexadecane	21.2056	3038	0.36	93
9	Eicosane	24.6967	6403	0.75	93
	II. Dichloromethane extract				
10	Tridecane	15.9618	1011	4.18	87
11	1-Undecanol	16.4114	2385	9.85	94

In addition, a total of 25 compounds were identified from *A. kotschyi*. Sixteen (16) compounds were identified from the leaves (15 and 2 compounds from the hexane and DCM extracts, respectively; 1 compound being common to these two extracts) (Table 3) and 14 compounds were identified from the root bark extracts (9 components from hexane and 7 from the DCM extracts, 2 compounds being common to these two extracts) (Table 4). Five (5) compounds were found common to these two plant parts. The most abundant compounds in the leaves hexane extract were caryophyllene (56,41%), cedrene (6,23%), and 1-octadecanol (4,40%). Only two compounds (caryophyllene (72.90%) and 2-(1,3-butadienyl)-1,3,5-trimethyl-benzene (8,79%)) were identified from the DCM extract and accounted for about 81% of the total extract.

Table 3. Chemical composition of *A. kotschyii* Leaf extracts

Entry	Name	Retention Time (%)	Peak Area	Relative % Composition	Match Factor (%)
	I. Hexane extract				
1	Caryophyllene	9.636	6577620	56.41	97
2	trans-α-Bergamotene	9.7555	143508	1.23	85
3	Pentadecane	10.5705	9324	0.08	97
4	Cedrene	10.9909	725962	6.23	84
5	Caryophyllene oxide	11.4169	174409	1.50	83
6	2,5-Heptadien-4-one, 2,6-dimethyl-	13.032	24054	0.21	80
7	2,6,10,14-Tetramethyl-pentadecane	13.0764	3128	0.03	86
8	Hexadecanoic acid, methyl ester	15.434	7209	0.06	84
9	Hexadecanoic acid, ethyl ester	16.0909	5635	0.05	90
10	1-Hexadecanol	16.9622	22067	0.19	88
11	Eicosane	17.9718	13200	0.11	87
12	Phenol, 2-ethyl-	19.4356	725	0.01	81
13	Tetracosane	21.9565	198206	1.70	81
14	1-Octadecanol	23.4214	512670	4.40	87
15	Triacontane	24.72	117474	1.01	84
	II. Dichloromethane extract				
16	2-(1,3-butadienyl)-1,3,5-Trimethyl-benzene	9.099	115954	8.79	84
17	Caryophyllene	9.7549	961385	72.90	97

Likewise, the most abundant compounds in the root barks extract were octadecanal (42,24%), and 3,5,11,15-tetramethyl-1-hexadecen-3-ol (7,86%) from hexane, and methyl-(Z)-9-octadecenoate (22,14%), isopropylmyristate (14,39%), 1-hexadecanol (12,90%), pentadecanal (9,44%) and eicosane (3,13%) from the DCM extract.

Table 4. Chemical composition of A. *kotschyii* Root extracts

Entry	Name	Retention Time (min)	Peak Area	Relative % Composition	Match Factor (%)
	I. Hexane extract				
1	1-Undecanol	13.3585	1584	0.25	87
2	Hexadecanoic acid, methyl ester	15.429	9452	1.52	93
3	3,5,11,15-tetramethyl-1-hexadecen-3-ol,	15.6498	48823	7.86	84
4	Hexadecanoic acid, ethyl ester	16.0946	3301	0.53	85
5	1-Hexadecanol	16.9541	7100	1.14	96
6	Hexadecane	19.6445	7484	1.20	92
7	Octadecanal	22.941	262545	42.24	89
8	Benzene, tert-butyl-	23.6979	317	0.05	82
9	Pentadecane	25.4857	3215	0.52	93

	II. Dichloromethane extract				
10	Isopropylmyristate	14.3994	103522	14.39	86
11	Hexadecanoic acid, methyl ester	15.4322	3341	0.46	87
12	Methyl-(Z,Z)-9,12-octadecadienoate	16.6157	5102	0.71	84
13	Methyl-(Z)-9-octadecenoate	17.3406	159256	22.14	81
14	Eicosane	21.9289	22524	3.13	82
15	1-Hexadecanol	23.3614	92824	12.90	92
16	Pentadecanal-	25.9067	67894	9.44	89

S. nodiflora extracts were subsequently evaluated for their antibacterial activity using a 96-well microdilution broth assay. They were tested against *Staphylococcus aureus* and *Escherichia coli*. The hexane and DCM leaf extracts and DCM root extract exhibited moderate activity (MIC = 500-1000 µg/mL) but the remaining extracts were inactive (MIC > 1000 µg/mL) against the test bacteria. These results seem to corroborate earlier reports on the antimicrobial activities of plants components against gram positive and gram negative bacteria, indicating that these extracts were often found to be more active against gram positive than the gram negative bacteria (Adu et al., 2011; Edewor and Olajire, 2011).

Interestingly, these and several other and similar studies (Okiei et al., 2009; Faleye et al., 2012; Chaddha et al., 2013; Erhabor et al., 2013; Mensah et al., 2013), on the evaluation of plants extracts for of both the chemical composition and antimicrobial activity have reported the activities as well as the occurrence of some of the compounds we found in the plant species under study. These and many other plants studies have established the antimicrobial activity as being related to the presence of, in particular, caryophyllene and caryophyllene oxide (Mazimba et al., 2012; Elisabeth and Arumugam, 2014), methylhexadecanoate (Saravanan et al., 2013; Shettima et al., 2013; Elisabeth and Arumugam, 2014), and 3,7,11,15-tetramethyl-2-hexadecen-1-ol (Ammal and Bai, 2013; Musa et al., 2015). Thus, these compounds seem to be responsible of the observed antibacterial activity. Moreover, although these compounds are known to have antibacterial activity, the contribution of 6,10,14-trimethyl-2-pentadecanone, 3,5,11,15-tetramethyl-1-hexadecen-3-ol, 7-methoxy-2,2-dimethyl-2*H*-1-benzopyran, pentadecanal, triacontane, geranylgeraniol, oxacycloheptadec-8-en-2-one, tridecane, and 1-undecanol towards *S. nodiflora* activity may be particularly important since they were also found in good quantities in the most active extracts. The remaining compounds have previously been reported from different other plants species and have shown similar activities (Mazimba et al., 2012; Saravanan et al., 2013; Elisabeth and Arumugam, 2014) that may be contributing to the synergistic effect in favour of the observed activities. However, *A. kotschyi* was not analysed for both secondary metabolites classes (phytochemical screening) and anti-bacterial activity due limited and insufficient quantities of sample. Additional and detailed phytochemical investigations are therefore 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 *S. nodiflora* and *A. kotschyi*.

Conclusions

In this study, we have used gas chromatography and mass spectrometry (GC-MS) analysis, to determine the relative profile and composition of the non-polar extracts of the leaves and root barks of *S. nodiflora* and *A. kotschyi* collected in Kinshasa / DR Congo. The present investigation has led to the identification of a total of 26 and 25 compounds from *S. nodiflora* and *A. kotschyi*, respectively. In general, the leaves had a higher number of metabolites. The extracts of *S. nodiflora* were preliminarily evaluated for antibacterial activity against *Staphylococcus aureus* and *Escherichia coli.* The hexane and DCM leaf extracts and DCM root extract exhibited moderate activity (MIC = 500-1000 µg/mL) but the remaining extracts were inactive (MIC > 1000 µg/mL) against the test bacteria. *A. kotschyi* was not analysed for anti-bacterial activity due limited and insufficient quantities of sample.

Further studies on 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 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]. Adu F., Gbedema SY., Akanwariwiak WG., Annan K. and Boamah VE. (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.

[2]. Akoto O., Lawson BA. And Marfo JT. (2012) Potentials of *Synedrella nodiflora* and *Sida fallax* growing naturally around an abandoned railway station for phytoextraction of heavy metals. Journal of Environmental Chemistry and Ecotoxicology, 4(10): 188-194.

[3]. Alka J. and Padma K. (2013) Antimicrobial potential of flavonoids of *Tridax procumbens* L. against pathogenic microorganisms. International Research Journal of Pharmacy, 4(2): 119-121.

[4]. Ammal RM. and Bai GVS. (2013) GC-MS Determination of bioactive constituents of *Heliotropium indicum* leaf. Journal of Medicinal Plants Studies, 1(6): 30-33.

[5]. Amoateng P., Koffuor, GA., Sarpong K. and Agyapong KO. (2011) Free radical scavenging and anti-lipid peroxidative effects of a hydro-ethanol extract of the whole plant of *Synedrella nodiflora* (L.) Gaertn (Asteraceae). Free Radicals and Antioxidants, 1(3): 70-78.

[6]. Chaddha V., Singh Kushwah A. and Shrivastava V. (2013) An importance of herbal drugs as anti diarrheal: A review. International Journal of Research in Applied Natural and Social Sciences, 1(7): 25-28.

[7]. Christudas S., Kulathivel TM. and Agastian P. (2012) Phytochemical and antibacterial studies of leaves of *Tridax procumbens* L. Asian Pacific Journal of Tropical Biomedicine, S159-S161

[8]. Clement RE. and Taguchi VY. 1991. Techniques for the Gaz Chromatography-Mass Spectrometry identification of organic compounds in effluents. ISBN, 0-7729-59834, Queen's Printer for Ontario.

[9]. 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.

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

[11]. Elizabeth VD. and Arumugam S. (2014) GC-MS analysis of ethanol extract of *Cyperus rotundus* leaves. International Journal of Current Biotechnology, 2(1): 19-23.

[12]. Ellof JN. (1998) A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Medica, 64: 711-713.

[13]. Erhabor JO., Oshomoh EO., Timothy O., Osazuwa ES. and Idu M. (2013) Antimicrobial activity of the methanol and aqueous leaf extracts of *Emilia coccinea* (Sims) G. Don. Nigerian Journal of Biotechnology, 25: 37-45.

[14]. Faleye FJ., Odeyemi AT. and Aderogba AA. (2012) Evaluation of the chemical composition and antimicrobial activities of three Nigerian medicinal plants. Applied Biology, 45: 7652-7656.

[15]. Ghayal N., Dhumal K., Deshpande N., Ruikar A. And Phalgune U. (2013) Phytotoxic Effects Of Leaf Leachates Of An Invasive Weed *Synedrella Nodiflora* And Characterisation Of Its Allelochemicals. International Journal Of Pharmaceutical Sciences Review And Research, 19(1): 79-86.

[16]. Kapundu M., Penders A., Warin R., Delaude C. And Huls R. (1988) Structure Of Kotschyioside, A New Saponin Isolated From *Aspilia Kotschyi* (Sch. Bip.) Oliv. Bulletin Des Sociétés Chimiques Belges, 97(5): 329–342.

[17]. Mazimba O., Masesane IB., Majinda RRT. 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 TE. Mihigo SO. and Bokolo MK. (2015) *Cinnamomum verum*: ethylacetate and methanol extracts antioxidant and antimicrobial activity. Journal of Medicinal Plants Studies, 3(3): 28-32.

[19]. Mensah JK., Ihenyen J. and Iyamu M. (2013) Phytochemical and antimicrobial properties of *Emilia coccinea* (CASS.). Asian Journal of Contemporary Sciences, 2(2): 26-31.

[20]. Musa AM., Ibrahim MA., Aliyu AB., Abdullahi MS., Tajuddeen N., Ibrahim H. and Oyewale AO. (2015) Chemical composition and antimicrobial activity of hexane leaf extract of *Anisopus mannii* (Asclepiadaceae). Journal of Intercultural Ethnopharmacology, 4(2): 129-133.

[21]. Nahar L., Zahan R., Morshed MTI., Haque A., Alam Z. and Mosaddik A. (2012) Antioxidant, analgesic and CNS depressant effects of *Synedrella nodiflora*. Pharmacology Journal, 4(31): 29-36.

[22]. Okiei W., Ogunlesi M. and Ademoye MA. (2009) An assessment of antimicrobial properties of extracts of various polarities from *Chasmanthera dependens*, *Emilia coccinea* and *Cuscuta australis*, herbal medications for eye diseases. Journal of Applied Sciences, 9(22): 4076-4080.

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

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

[25]. 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.

[26]. Stepp JR. (2004) The role of weeds as sources of pharmaceuticals. Journal of Ethnopharmacology, 92: 163–166.

[27]. Stein SE. (1999) An integrated method for spectrum extraction and compound identification from GC/MS data. Journal of the American Society for Mass Spectrometry, 10(8): 770-781.

[28]. Zahan R., Nahar L., Haque A., Mosaddik A., Fazal A., Alam Z. and Haque ME. (2012) Antidiarrhoeal and hypoglycemic effects of Synedrella nodiflora. Phytopharmacolgy, 2(2): 257-264.