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Research Article

Preliminary GC-MS Profiling and Anti-bacterial activity Investigation of *Ageratum conyzoides* Linn. (Asteraceae)

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ABSTRACT

In this study, preliminary phytochemical analysis of the leaves and roots of *Ageratum conyzoides* Linn. indicated the presence of alkaloids, flavonoids, quinones, saponins, and triterpenes. In addition, the qualitative and quantitative profiles of the hexane and dichloromethane (DCM) extracts from the leaves and roots of *A. conyzoides* were determined by GC-MS. The individual constituents of the extracts (34 compounds) were identified by matching their data with those of similar compounds stored in the NIST 05L Library. The most abundant compounds in the hexane and DCM leaf extracts were caryophyllene (63.17%), and caryophyllene oxide (15.66%), and phytol (18.79%), caryophyllene oxide (17.12%), methyl-(Z,Z,Z)-9,12,15-octadecatrienoate (11.71%), 6,10,14-trimethyl-2-pentadecanone (7.41%), 2-nonadecanone (6.05%), and methylhexadecanoate (5.64%); respectively. Likewise, the root extracts consisted of mainly caryophyllene oxide (32,75%), 2-methylnonadecane (13,07%), hexadecanal (11,98%), and ethylhexadecanoate (11,33%) for the hexane extract, and oxacycloheptadec-8-en-2-one (63,76%) and caryophyllene oxide (31,57%) from the DCM extract. The extracts were subsequently evaluated for their antibacterial activity using a 96-well microdilution broth assay. They were tested against *Staphylococcus aureus* and *Escherichia coli* and the DCM extract exhibited moderate activity (MIC=500 µg/mL) against *S. aureus* while the hexane extract was inactive (MIC > 2000 µg/mL). Both extracts were inactive against *E. coli.*

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

INTRODUCTION

Natural products from many plants have been evaluated and used in the management and treatment of several diseases. The emphasis on searching in primary tropical forest habitats for new drugs has meant that plants growing in other habitats

have been ignored or overlooked. Yet, 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. In fact, 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 (Stepp, 2004).

On the other hand, secondary natural compounds in weeds are important for a variety of ecological functions, including the chemical defense against herbivores and other attacks such as those by fungi and bacteria; and could be a source of interesting antimicrobial compounds.

Thus, as part of our continued interest in invasive plants and weeds as potential sources of pharmacologically important compounds, and as previously reported (Mihigo et al., 2015), we undertook the GC-MS and bioactivity investigations of *Ageratum conyzoides* L., an invasive weed occurring in the Democratic Republic of Congo (DR Congo).

A. conyzoides Linn (Asteraceae) is an annual aromatic herb, up to 80 cm tall, stem terete, erect, hispidly hairy. The leaves are simple, ovate, petiolate; petioles, up to 4 cm long, hirsute; lamina, 2.0-6.5 x 1-4 cm, palmately 3-nerved, margin dentate, crenate or serrate, hirsute on both surfaces. The inflorescence is made of a terminal corymb, the flower pale blue in small head and the fruit is a narrowly oblong cypsela. It is propagated through seeds and it grows in the open fields, road sides, secondary forests, forest clearings, tea-gardens and hillocks; and the flowering and fruiting take place between November and June (Motaleb et al., 2013).

A. conyzoides is native to South America, and is now widely spread throughout the warm countries of the world, where it is sometimes found as common weed of waste places, especially in moist situations (Motaleb et al., 2013). This annual herb has a long history of traditional medicinal uses by various cultures worldwide. For example, its decoction or infusion is given in diarrhea, dysentery, intestinal colic, flatulence, rheumatism, fever and pain associated with navel in children; and it has been found to be bactericide, and anti-lithiatic in South America and Africa (Rahman et al., 2012). In Brazil, it is known as anti-inflammatory, analgesic, and healer of gynecological diseases; and is also used to treat colic, colds, fevers, diarrhea, reheumatism, spasms. The plant is also used elsewhere for the treatment of conjunctivitis, liver pain, opthalmia, pheumonia, sterility, skin diseases, wounds and cuts; and the root is used against harmful effects of perceived evil spirits in children (Kohli et al., 2006; Roy et al., 2008; Rahman et al., 2012; Motaleb et al., 2013). Other folk remedies include the antimicrobial, antitusive, antiitch, schistosomicide, insecticide, antidote to snake poison, antitetanus, and even the anti-HIV/AIDS property (Kohli et al., 2006; de Melo et al., 2011; Nathalya, 2011; Adebajo et al., 2012; Rahman et al., 2012). Prominent bioactive compounds have been isolated and identified from this plant, including essential oil components (Kohli et al., 2006; sfara et al., 2009; de Melo et al., 2011), flavonoids

(Gonzalez et al., 1991a; Horie et al., 1993; Kohli et al., 2006), chromenes (Gonzalez et al., 1991b; Adeleke, 2002; Kasali et al., 2002), coumarins, alkaloids, and some triterpenes such as friedelin, ß-sitosterol, stigmasterol and α-spinasterol (Kohli et al., 2006; Kaur et al., 2011). Besides, it also contains HCN, hexadecenoic acid, kaempferol and its glucosides, linoleic acid, nerolidal, and quercetin and its glucosides. Precocene I and II are the major constituents of the essential oils of *A. conyzoides* and are insecticidal, and possess anti-juvenile hormonal activity (Kohli et al., 2006).

Thus, these previous reports constitute a clear indication the *A. conyzoides* Linn. has a potential as source of interesting bioactive compounds.

However, it should be noted that no phytochemical work has been done on *A. conyzoides* 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 antibacterial study data on *A. conyzoides*.

Materials and Methods

Plant materials

The leaves and roots of *A. conyzoides* 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 specimen (A. Blomme 100, of February 8, 1958; and H. Breyne 4005 of June 10, 1980) 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 (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; Mihigo et al., 2015).

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; Mihigo 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; Mihigo et al., 2015). *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

From the Total Ion Chromatogram (TIC) profile, it was found that both the leaves and stem bark extracts of this plant are very rich in secondary metabolites, the most

abundant compounds eluting between 9-18 min. A total of 26 compounds were identified from the leaf extracts, the most abundant being caryophyllene (63.17%), caryophyllene oxide (15.66%), geranylgeranio (2.12%), 3,5,11,15-tetramethyl-1-hexadecen-3-ol (1.85%) and methyloctadecanoate (1.85%) from hexane, and phytol (18.79%), caryophyllene oxide (17.12%), methyl-(Z,Z,Z)-9,12,15-octadecatrienoate (11.71%), 6,10,14-trimethyl-2-pentadecanone (7.41%), 2-nonadecanone (6.05%), methylhexadecanoate (5.64%), methyl-18-methylnonadecanoate (1.82%), and methyl-(Z,Z)-9,12-octadecadienoate (1.34%) from the DCM extract.

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

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

Entry	Name	Retention Time (min)	Peak Area	Relative % Composition	Match Factor (%)
	I. Hexane extract				
1	Caryophyllene	9,694	17449923	63,17	96
2	Caryophyllene oxide	12,7679	4326533	15,66	83
3	1-Dodecanol	13,3909	19897	0,07	80
4	Hexadecanoic acid, methyl ester	15,452	252531	0,91	94
5	**A**	15,661	511074	1,85	91
6	Hexadecanoic acid, ethyl ester	16,0995	78248	0,28	91
7	**B**	17,069	104668	0,38	94
8	Octadecanoic acid, methyl ester	17,343	25164	0,09	94
9	**C**	19,3622	512135	1,85	90
10	1-Undecanol	20,4019	2717	0,01	81
11	Eicosane	20,4356	7400	0,03	90
12	1-Tetradecanol	21,9294	23964	0,09	83
13	1-Hexadecanol	23,3581	18127	0,07	94
14	Hexadecane	23,9974	13924	0,05	83
15	Geranylgeraniol	24,2026	584641	2,12	82
16	Tetracosane	24,6999	128013	0,46	83
17	Triacontane	24,6999	121473	0,44	86
18	Tridecane	25,4749	3581	0,01	87
19	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	29,5509	239761	0,87	84
	II. Dichloromethane extract				
20	Caryophyllene oxide	11,8228	635167	17,12	92
21	Methyl tetradecanoate	13,3209	12500	0,34	87
22	6,10,14-trimethyl-2-Pentadecanone	14,6258	274799	7,41	81
23	Hexadecanoic acid, methyl ester	15,4396	209082	5,64	96
24	**B**	17,066	49548	1,34	93
25	9-Octadecenoic acid (Z)-, methyl ester	17,1216	24891	0,67	91
26	**D**	17,1335	434403	11,71	86
27	Phytol	17,2449	696883	18,79	83
28	Octadecanoic acid, methyl ester	17,3387	28225	0,76	96

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29	2-Nonadecanone	18,9288	224417	6,05	85
30	Methyl 18-methylnonadecanoate	19,0833	67601	1,82	85
31	Tridecane	20,4426	1711	0,05	90
32	1-Hexadecanol	21,937	6203	0,17	92
33	Eicosane	24,6874	21855	0,59	91
34	1-Undecanol	24,74	3778	0,10	80

A: 3,5,11,15-Tetramethyl-1-hexadecen-3-ol, **B** : Methyl-(Z,Z)-9,12-octadecadienoate
C: 4,8,12,16-Tetramethylheptadecan-4-olide, **D**: Methyl-(Z,Z,Z)-9,12,15-octadecatrienoate

In addition, sixteen (16) compounds were identified from the root bark extracts (11 components from the hexane extract and 8 from the DCM extract, 3 being common to these two extracts). (Table 2). The most abundant compounds were caryophyllene oxide (32,75%), 2-methylnonadecane (13,07%), hexadecanal (11,98%), ethylhexadecanoate (11,33%), pentadecanal (3,64%), and methylhexadecanoate (3.30%) from hexane, and oxacycloheptadec-8-en-2-one (63,76%) and caryophyllene oxide (31,57%) from the DCM extract.

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

Entry	Name	Retention Time (min)	Peak Area	Relative % Composition	Match Factor (%)
	I. Hexane extract				
1	Caryophyllene oxide	11,8227	731150	32,75	92
2	Hexadecanoic acid, methyl ester	15,4332	73718	3,30	96
3	Hexadecanoic acid, ethyl ester	16,1107	252997	11,33	80
4	1-Hexadecanol	16,9733	9152	0,41	95
5	**A**	17,0765	55552	2,49	93
6	9-Octadecenoic acid (Z)-, methyl ester	17,1259	6942	0,31	87
7	Octadecanoic acid, methyl ester	17,3442	8449	0,38	91
8	Pentadecane	17,9791	5461	0,24	83
9	Nonadecane, 2-methyl-	21,9431	291732	13,07	89
10	Pentadecanal-	23,6352	81185	3,64	88
11	Hexadecanal	24,3145	267376	11,98	88
	II. Dichloromethane extract				
12	Caryophyllene oxide	11,8234	573332	31,57	86
13	Phenol, 2,4-bis(1,1-dimethylethyl)-	12,4395	5732	0,32	84
14	3-Pentanone, 2,2,4,4-tetramethyl-	15,1531	158	0,01	85
15	Hexadecanoic acid, methyl ester	15,4408	13036	0,72	93
16	1-Hexadecanol	16,9671	4649	0,26	95
17	Oxacycloheptadec-8-en-2-one	17,5245	1158040	63,76	83
18	Undecane	17,9811	1454	0,08	87
19	Tridecane	18,8393	2072	0,11	89

A: Methyl-(Z,Z)-9,12-octadecadienoate

The extracts were subsequently evaluated for their antibacterial activity using a 96-well microdilution broth assay. They were tested against *Staphylococcus aureus* and *Escherichia coli* and the DCM extract exhibited moderate activity (MIC=500 µg/mL) against *Staphylococcus aureus* while the hexane extract was inactive (MIC > 2000 µg/mL). Both extracts were found inactive against *Escherichia coli.* These results seem to corroborate earlier reports on the antimicrobial activities of plants extracts against gram positive and gram negative bacteria, indicating that the extracts were often found to be more active against gram positive than the gram negative bacteria (Adu et al., 2011; Edewor and Olajire, 2011, Mihigo et al., 2015).

Several similar studies (Kohli et al., 2006; Kalaiselvan et al., 2012; Sermakkani and Thangapandian, 2012; Ammal and Bai, 2013; Elisabeth and Arumugam, 2014), on the evaluation of both the chemical composition and antimicrobial activity of *A. conyzoides* L. have reported the activities as well as the occurrence of some of the compounds we found in *A. conyzoides* L. sampled in the DR Congo (current study). These, and additional studies on other plants species have established the antimicrobial activity as being related to the presence of, in particular, caryophyllene, caryophyllene oxide (Rajeswari et al., 2011; Mazimba et al., 2012; Elisabeth and Arumugam, 2014; Mihigo et al., 2015), methylhexadecanoate (Elekwa et al., 2011; Saravanan et al., 2013; Shettima et al., 2013; Elisabeth and Arumugam, 2014; Mihigo et al., 2015), phytol (Ammal and Bai, 2013; Kalaiselvan et al., 2012; Sermakkani and Thangapandian, 2012; Elisabeth and Arumugam, 2014; Mihigo et al., 2015), copaene (Mazimba et al., 2012), and (-)-spathulenol (Rajeswari et al., 2011; Mazimba et al., 2012; Mihigo et al., 2015). 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 phytol and oxacycloheptadec-8-en-2-one (current study) towards *A. conyzoides* activity may be particularly important since they were found to be the major (over 18 and 63%, respectively) compound of the most active extracts. The remaining compounds have previously been reported from different plants species and have shown other activities that may be contributing to the synergistic effect in favour of the observed activities (Uma et al., 2009; Elekwa et al., 2011; Mazimba et al., 2012; Saravanan et al., 2013; Elisabeth and Arumugam, 2014; Mihigo et al., 2015).

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 *A. conyzoides* L.

Conclusions

In the present study, a Congolese weedy plant (*Ageratum conyzoides* L.) has been submitted to preliminary phytochemical, GC-MS and antibacterial analyses for the first time. The present investigation has led to the identification of alkaloids, flavonoids, quinones, saponins, and triterpenes, and the determination of the qualitative and

quantitative profiles of the hexane and dichloromethane (DCM) extracts from the leaves and roots *A. conyzoides* L. A total of 34 compounds were identified from the extracts (26 and 16 compounds from the leaf and root extracts, respectively; 8 compounds having been simultaneously isolated from both extracts). We have also preliminarily determined the chemical percentage composition of the above extracts by GC-MS analyses. The extracts were evaluated for antibacterial activity against *Staphylococcus aureus* and *Escherichia coli.* Only the DCM extracts exhibited a moderate activity (MIC=500 µg/mL) against *S. aureus* both extracts were found inactive against *E. coli.*

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.

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