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Research Article

Preliminary GC-MS Profiling and Anti-bacterial activity Investigation of *Chromolaena odorata* Linn. R.M. King and H. Robinson (Asteraceae)

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ABSTRACT
In this study, phytochemical analysis of the leaves and roots of *Chromolaena odorata* Linn. indicated the presence of alkaloids, flavonoids, glycosides, phenols, quinones, steroids, saponins, and tannins. In addition, the qualitative and quantitative profiles of the hexane and dichloromethane (DCM) extracts from the leaves and roots of *C. odorata* 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 32 compounds, 28 and 15 compounds from the leaves and roots, respectively; 11 compounds being common to the two plant parts. The most abundant compounds in the hexane and DCM leaf extracts were (-)-spathulenol (23.94%), caryophyllene (17.68%), α-cadinol (16.87%), copaene (14.62%), caryophyllene oxide (8.73%), and 1-tetracosanol (4.69%), and 2,6-dimethylnaphthalene (37.36%), 2-nonadecanone (24.51%), 2-pentacosanone (5.02%), and 4,8,12,16-tetramethylheptadecan-4-olide (3.07%); respectively. Likewise, the hexane and DCM root extracts consisted of mainly caryophyllene oxide (59.28%), methyl-(Z)-9-octadecenoate (18.22%), and pentadecanal (7,29%) for the hexane extract, and 2-nonadecanone (61,81%), methylhexadecanoate (5.16%), and 1-undecanol (3,19%) for 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 leaves hexane extract and the roots hexane and DCM extracts were the most active (exhibiting moderate activity, MIC ≈ 500 μg/mL) against *S. aureus* while the leaves DCM extract was inactive (MIC > 1000 μg/mL). Both extracts were inactive against *E. coli.*

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

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INTRODUCTION

Plants-derived natural products have a great prospect and promise in providing good and effective agents to manage or treat life threatening diseases. 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 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).

Chromolaena odorata (Linn) R.M. King & H. Robinson (Asteraceae) (syn. *Eupatorium odoratum* L. or *Osmia odorata* L.) is a shrub native to Central and Southern America but which has become established pantropically. It is a perennial, diffuse and scrambling shrub which grows to 3 to 7 m in height when growing in the open, and it goes by many common names including Siam weed, devil weed, French weed, communist weed, hagonoy, co hoy etc. (Mboukou-Kimbatsa et al., 2007; Ngozi et al., 2009; Chandrasekaran and Swamy, 2010; Vaisakh and Pandey, 2011; Kouamé et al., 2013; Otarigho and Morenikeji, 2013). Introduced to many places, either intentionally as an ornamental plant or accidentally, it is now regarded as one of the most harmful weeds present on earth due to its highly invasive and allelopathic nature (Goodall and Erasmus, 1996; Wollenweber and Roitman, 1996; Vaisakh and Pandey, 2011; Wollenweber et al., 1996; Otarigho and Morenikeji, 2013). In Central Africa, *C. odorata* is known as Matanga Mbala (the invader) in the Congo and Mighbe (the one that crashes all) in Cameroon, all these attributes reflecting the vigour with which this plant invades the local habitats (Kouamé et al., 2013). The plant can be poisonous to livestock as it has exceptionally high level of nitrate (5–6 times above the toxic level) in the leaves and young shoots; and the cattle feeding on these die of tissue anoxia (Rao et al., 2010).

Despite the negative sides to the plant, *C. odorata* has been found to be a highly efficacious medicinal herb across many traditional systems and cultures. This was proven and validated by its pharmacological evaluation performed by the scientific community throughout the world, the most established and discussed aspect of *C. odorata* having been its role in wound healing (Rao et al., 2010; Wafo et al., 2011; Vaisakh and Pandey, 2012).

Previous studies carried out in Benin (Kossouoh et al., 2011; Kouamé et al., 2013), Cameroon (Kouamé et al., 2013), Congo-Brazzaville (Mboukou-Kimbatsa et al., 2007), Ghana (Antwi-Boasiako and Damoah, 2010; Kouamé et al., 2013), India (Chandrasekaran and Swamy, 2010; Joshi, 2013; Onkaramurthy et al., 2013), Ivory Coast (Ngozi et al., 2009; Bedi et al., 2010; Kouamé et al., 2013; Tondoh et al., 2013), Malaysia (Jannah et al., 2006), Nigeria (Ikewuchi et al., 2013; Kouamé et al., 2013; Otarigho and Morenikeji, 2013; Yakubu, 2012), South Africa (Goodall and Erasmus, 1996), Togo (Koba et al., 2011), Thailand (Suksamrarn et al., 2004; Pisutthanan et al., 2005; Pisutthanan et al., 2006), Vietnam (Hanh et al., 2011; Wafo et al., 2011; Asomugha et al., 2013; Heiss et al., 2014), etc., showed that this plant contains alkaloids (Ikewuchi et al., 2013), amino acids (Ikewuchi et al., 2013), essential oil (Suksamrarn et al., 2004; Ikewuchi et al., 2013; Joshi, 2013), flavonoids (Suksamrarn et al., 2004; Ngozi et al., 2009; Suriyavathana et al., 2012; Ikewuchi et al., 2013), glycosides and phenols (Suriyavathana et al., 2012; Ikewuchi et al., 2013), saponins (Ngozi et al., 2009; Suriyavathana et al., 2012; Ikewuchi et al., 2013), sesquiterpene lactones and triterpenes (Suksamrarn et al., 2004; Ikewuchi et al., 2013), steroids

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(Suksamrarn et al., 2004; Ikewuchi et al., 2013), and tannins (Ngozi et al., 2009; Ikewuchi et al., 2013; Vijayaraghavan et al., 2013); and together with interesting and overlapping biological activities (Pisutthanan et al., 2006; Vaisakh and Pandey, 2012). It is used as antibacterial (Pisutthanan et al., 2005; Antwi-Boasiako and Damoah, 2010; Suriyavathana et al., 2012; Ikewuchi et al., 2013; Kouamé et al., 2013), anticholesterolemic (Ikewuchi and Ikewuchi, 2011), anticytotoxic (Suksamrarn et al., 2004), antidiabetic and anticataract (Antwi-Boasiako and Damoah, 2010; Vaisakh and Pandey, 2012; Onkaramurthy et al., 2013), antifungal (Ikewuchi et al., 2013; Kouamé et al., 2013), antihypertensive (Ikewuchi et al., 2013), antiinflammatory (Ngozi et al., 2009; Bedi et al., 2010; Hanh et al., 2011; Ikewuchi et al., 2013; Kouamé et al., 2013; Pandith et al., 2013; Heiss et al., 2014), antimycobacterial (Suksamrarn et al., 2004), antioxidant (Kouamé et al., 2013), antiplasmodial (Pisutthanan et al., 2005; Kouamé et al., 2013), antispasmodic, antiprotozoal, antitrypanosomal, astringent, diuretic and hepatotropic (Ngozi et al., 2009; Ikewuchi et al., 2013; Pandith et al., 2013), anticonvulsant (Amazu et al., 2014), cytoprotective (Jannah et al., 2006), insect deterrent (Antwi-Boasiako and Damoah, 2010), and molluscicidal (Otarigho and Morenikeji, 2013). In Vietnam, its fresh leaves or decoction of the leaves are used for treatment of leech bite, soft tissue wounds, burn wounds, skin infection and dento-alveolitis (Suriyavathana et al., 2012; Joshi, 2013; Otarigho and Morenikeji, 2013). Its crude alkaloid extract has also been found to increase the hormonal and spermatogenic indices in male rats (Yakubu, 2012).

Some specific compounds that have been reported from this plant include alkaloids (Ikewuchi et al., 2013), acids (Heiss et al., 2014), diterpenes (Wafo et al., 2011; Panda et al., 2012), fatty acids (Hanh et al., 2011), a fatty acid ester (Hanh et al., 2011), and fatty acid amides (Hanh et al., 2011), flavonoids (Wollenweber and Roitman, 1996; Suksamrarn et al., 2004; Panda et al., 2012;), steroids (Panda et al., 2012), and sesquiterpene lactones (Panda et al., 2012). Moreover, earlier workers had reported the use of this plant for phytoremediation of solutions as well as low level nuclear wastes for metals such as cadmium, lead, zinc, and cesium, and as nutrient sources and organic matter amendments for soil fertility maintenance (Tanhan et al., 2007; Tondoh et al., 2013).

As a consequence, these examples justify the importance to consider and evaluate this abundantly occurring weed species as potential source of medicines than as invasive flora (Vaisakh and Pandey, 2012).

However, whereas intensive work has been done on the phytochemical and biological activity investigations of *C. odorata* from a number of other African countries, plant sample from the Democratic Republic of Congo has not received comparable attention; yet the DR Congo ethno-pharmacological behavior differ from other countries, and the geographic location and ecological variability may lead to distinct phytochemical characteristics.

Thus, as part of our continued interest in 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 preliminarily GC-MS and bioactivity investigations of *Chromolaena odorata* L., an invasive weed occurring in DR Congo.

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Materials and Methods

Plant materials

The leaves and roots of *C. odorata* 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 (H. Breyne 2434 of May 2, 1975) 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 (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-

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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

About 60 compounds were separated and eluted from the extracts of this plant, the leaves displaying the highest number. A total of 28 compounds were identified from the leaf extracts (20 and 13 compounds from the hexane and DCM extracts, respectively; 3 compounds being common to these two extracts) (Table 1). The most abundant compounds were (-)-spathulenol (23,99%), caryophyllene (17,68%), α-cadinol (16,87%), copaene (14,62%), caryophyllene oxide (8.73%), 1-tetracosanol (4,69%), globulol (2,50%), and octadecanal (1.05%) for the hexane extract, and 3,7,11,15-tetramethyl-2-hexadecen-1-ol (37,36%), 2-nonadecanone (24,51%), 2-Pentacosanone (5,02%), 4,8,12,16-tetramethylheptadecan-4-olide (3,07%), 1-octadecanol (1.83%), 1-tridecane (1.58%), and 1-hexadecanol (1.10%) for the DCM extract.

Table 1. Chemical composition of *C. odorata* leaf extracts

Entry	Name	Retention Time (min)	Peak Area	Relative % Composition	Match Factor (%)
		I. Hexane extract			
1	Eugenol	8.6656	60129	0.13	95
2	Copaene	8.9477	6550368	14.62	92
3	Caryophyllene	9.6489	7924456	17.68	97
4	(-)-Spathulenol	11.9059	10729055	23.94	80
5	Caryophyllene oxide	11.9528	3912285	8.73	89
6	Globulol	12.0159	1118954	2.50	87
7	α-Cadinol	12.7509	7559379	16.87	80
8	**A**	15.4576	82073	0.18	91
9	**B**	17.0758	57262	0.13	91
10	**C**	17.1253	22843	0.05	94

11	**D**	17.3874	11542	0.03	91
12	1-Hexadecanol	18.7679	21558	0.05	97
13	Hexadecane	18.8317	9457	0.02	89
14	**E**	19.3859	628159	1.40	91
15	Tridecane	19.6549	3813	0.01	81
16	Tetracosane	20.4493	26081	0.06	82
17	Pentadecanal-	20.7208	151799	0.34	81
18	Octadecanal	21.4977	472556	1.05	88
19	1-Tetracosanol	21.9838	2102320	4.69	94
20	Eicosane	24.7073	53147	0.12	82
		II. Dichloromethane extract			
21	Naphthalene, 1,8-dimethyl-	9.6778	9456	0.10	93
22	Naphthalene, 2,6-dimethyl-	11.4511	28994	0.32	83
23	**F**	15.0336	3405587	37.36	92
24	**A**	15.4552	48520	0.53	89
25	**B**	17.0784	12852	0.14	88
26	**D**	17.3505	10156	0.11	93
27	1-Hexadecanol	18.7723	100655	1.10	90
28	**E**	19.381	279580	3.07	81
29	1-Octadecanol	21.9719	167229	1.83	87
30	2-Pentacosanone	22.1033	457285	5.02	87
31	2-Nonadecanone	23.5282	2234444	24.51	83
32	Pentadecane	24.7124	17086	0.19	89
33	1-Tridecene	24.7993	143785	1.58	81

A: Hexadecanoic acid, methyl ester; **B**: 9,12-Octadecadienoic acid (Z,Z)-, methyl ester; **C**: 9-Octadecenoic acid (Z)-, methyl ester

D: Octadecanoic acid, methyl ester; **E**: 4,8,12,16-Tetramethylheptadecan-4-olide; **F**: 3,7,11,15-Tetramethyl-2-hexadecen-1-ol

In addition, fifteen (15) compounds were identified from the root bark extracts (11 components from hexane and 5 from the DCM extract, 1 being common to these two extracts) (Table 2). The most abundant compounds were caryophyllene oxide (59,28%), methyl-(Z)-9-octadecenoate (18,22%), and pentadecanal (7,29%) for the hexane extract, and 2-nonadecanone (61,81%), methylhexadecanoate (5,16%), 1-undecanol (3,19%), 1-hexadecanol (2,52%) and tridecane (2,03%) from the DCM extract.

Table 2. Chemical composition of *C. odorata* root extracts

Entry	Name	Retention Time (min)	Peak Area	Relative % Composition	Match Factor (%)
	I. Hexane extract				
1	Caryophyllene oxide	11.8189	759887	59.28	92
2	Hexadecanoic acid, ethyl ester	16.1038	18419	1.44	89
3	1-Dodecanol	16.962	8872	0.69	94
4	**A**	17.0678	7815	0.61	86

5	**B**	17.3405	1688	0.13	84
6	**C**	17.6802	233600	18.22	82
7	Hexadecane	18.8249	5850	0.46	88
8	Eicosane	23.3387	16410	1.28	92
9	1-Hexadecanol	23.36	15836	1.24	94
10	Pentadecane	24.6886	9247	0.72	92
11	Pentadecanal-	25.9584	93458	7.29	88
	II. Dichloromethane extract				
12	**D**	15.4252	6066	5.16	81
13	1-Undecanol	16.9464	3749	3.19	93
14	Tridecane	21.1957	2384	2.03	90
15	1-Hexadecanol	25.8928	2963	2.52	83
16	2-Nonadecanone	26.6509	72706	61.81	84

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 only the hexane leaf extracts and the DCM root extract exhibited moderate activity (MIC ≈ 500 µg/mL) against *S. aureus* while the remaining extracts were inactive (MIC > 1000 µg/mL) against both 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).

Several similar studies (Suksamrarn et al., 2004; Pisutthanan et al., 2005; Owolabi et al., 2010), on the evaluation of both the chemical composition and antimicrobial activity of *C. odorata* L. have reported the activities as well as the occurrence of some of the compounds we found in *C. odorata* L. sampled (current study) in the DR Congo.

These, and additional studies on other plants species have established the antimicrobial activity as being related to the presence of, in particular, (-)-spathulenol (Rajeswari et al., 2011; Mazimba et al., 2012), caryophyllene, caryophyllene oxide (Owolabi et al., 2010; Rajeswari et al., 2011; Mazimba et al., 2012; Elisabeth and Arumugam, 2014), α-cadinol (Owolabi et al., 2010), copaene (Mazimba et al., 2012), and methylhexadecanoate (Saravanan et al., 2013; Shettima et al., 2013; Elisabeth and Arumugam, 2014). 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 (-)-spathulenol, caryophyllene, caryophyllene oxide, copaene, methyl-(Z)-octadecanoate, methylhexadecanoate, and 2-nonadecanone (current study) towards *C. odorata* L. activity may be particularly important since they were found to be the major compounds of the most active extracts. The remaining compounds have previously been reported from different plants species and have shown other activities (Mazimba et al., 2012; Saravanan et al., 2013; Elisabeth and Arumugam, 2014), etc. Although these are different activities, the presence the concerned compounds in the investigated extracts may be contributing to the synergistic effect in favour of the observed activities.

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 *C. odorata* L.

Conclusions

In this study, we have used gas chromatography and mass spectrometry (GC-MS) analysis, to determine the profile and relative composition of the non-polar extracts of the leaves and roots of *C. odorata* collected in Kinshasa / DR Congo. The present investigation has led to the identification of alkaloids, flavonoids, glycosides, phenols, quinones, steroids, saponins, and tannins. A total of 32 compounds were identified from the extracts (28 and 15 compounds from the leaf and root extracts, respectively; 11 compounds having been simultaneously identified from the leaves and roots extracts). The extracts were preliminarily evaluated for antibacterial activity against *Staphylococcus aureus* and *Escherichia coli.* The leaves hexane extract and the roots hexane and DCM extracts exhibited moderate activity (MIC=500 μg/mL) against *S. aureus* but the leaves DCM extract was inactive (MIC > 1000 μg/mL). 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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Conflict of interest :Nil

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