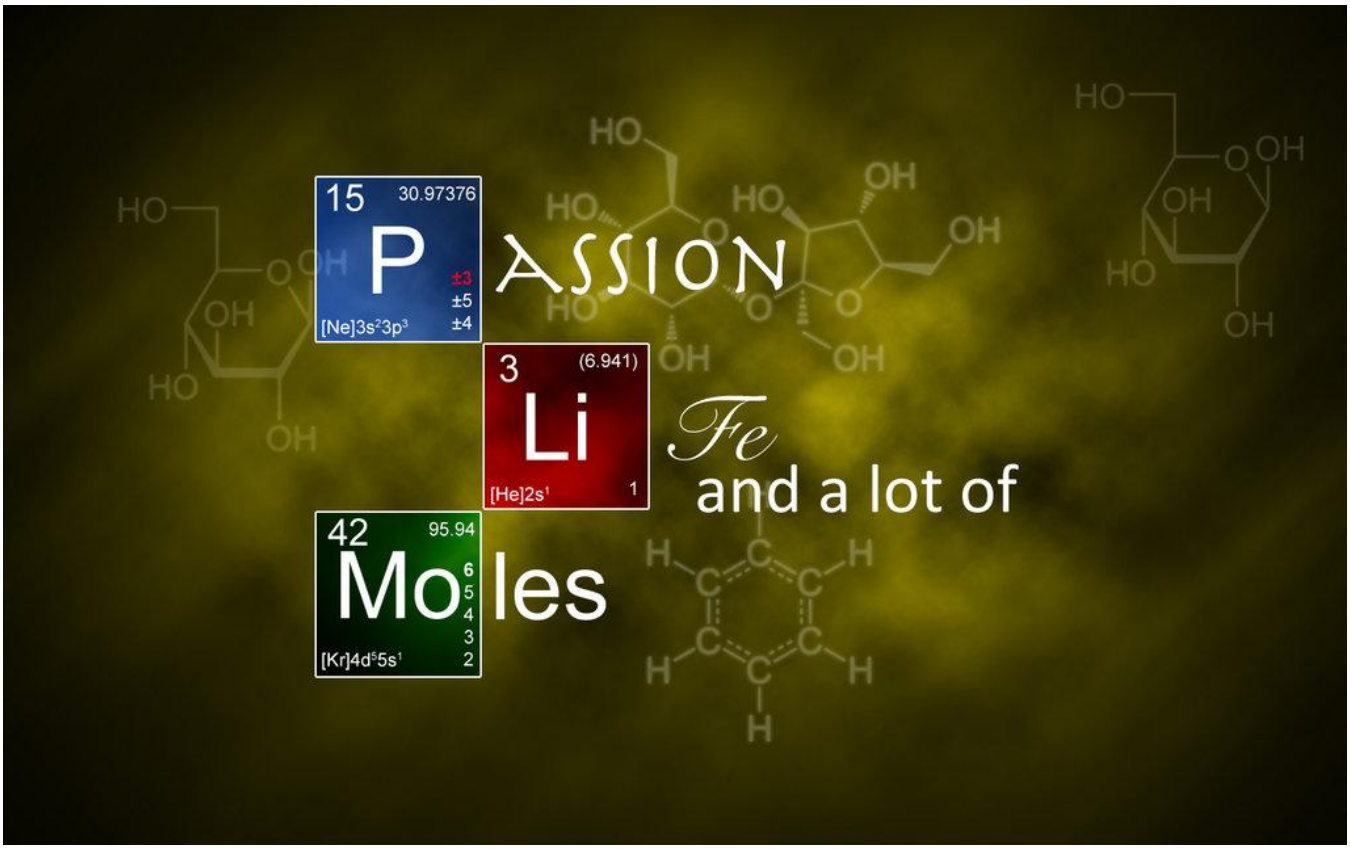

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PRELIMINARY GC-MS PROFILING AND ANTI-BACTERIAL ACTIVITY INVESTIGATION OF *EMILIA COCCINEA* (SIMS) G. DON (ASTERACEAE)

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ABSTRACT

In this study, phytochemical analysis of the leaves and stem barks of *E. coccinea* (Sims) G. Don indicated the presence of alkaloids, flavonoids, coumarins, quinones, saponins, and triterpenes. In addition, the qualitative and quantitative profiles of the hexane and dichloromethane (DCM) extracts were determined by GC-MS. Twenty-four (24) compounds were identified (19 and 7 compounds from the leaf and stem bark extracts, respectively; 2 compounds having been simultaneously identified from these two extracts). The most abundant compounds in the leaf extracts were caryophyllene (22.07%), 1-octadecanol (19.34%), caryophyllene oxide (17.74%), 1-tridecene (7.70%), geranylgeraniol (7.46%), tetracosane (5.50%), and ethylhexadecanoate (2.82%) from hexane and pentadecanal (40.03%), 1,E-11,Z-13-octadecatriene (11.35%), 1-octadecanol (7.31%), 1-tridecene (6.39%) and 4,8,12,16-tetramethylheptadecan-4-olide (2.59%) from the DCM extract. No compound was identified from the root bark hexane extract but seven (7) compounds were identified from its DCM extract; the most abundant being methyl-11,14-eicosadienoate (46.95%), pentadecanal (25.51%), (E)-3-eicosene (8.67%), 1-hexadecanol (3.64%) and menthol (2.19%). The leaf extracts were evaluated for antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* using a 96-well microdilution broth assay. The hexane extract exhibited moderate activity (MIC = 500-1000 µg/mL) but the DCM part was found inactive (MIC > 1000 µg/mL) against both test bacteria. The root extracts were not tested due to limited and insufficient quantities of sample.

Keywords: *Emilia coccinea*, Antibacterial, Chromatography, GC-MS.

INTRODUCTION

Medicinal plants of various types and from different settings/habitats are of great importance to both human and 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 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).

E. coccinea (Sims) G. Don (Asteraceae) is a semi-erect annual herb that grows up to 50 cm and reproduces by seed. This herb is commonly found throughout the plain of the Central Africa and in dry areas up to 2000 m altitude in the eastern Africa. It belongs to the genus *Emilia* represented by *ca.* 100 species, with 50 of them found in Africa. It has a hollow stem and simple leaves (Simplice et al., 2014; Nduche et al., 2015). *E. coccinea* has been in use in various traditional systems of medicine. For instance, the leaves are used in the treatment of convulsions, epilepsy, and spleen enlargement. The plant serves as laxative and is also effective in the treatment of sore throat (Nduche et al., 2015). It is also used to calm down toothache in Cameroon. In Nigeria, the leaves are used for cleaning wounds, as remedy for sore eyes, and are also eaten raw and can be mixed with guinea corn and lime juice to serve as a remedy for sore throat (Agbor, 2015). Additional medicinal benefits associated with *E. coccinea* include the treatment of syphilis, hernia, gonorrhoea, ulcer, craw-craw, abscesses of the breast, ringworm, lice, measles, cough, diarrhea, microbial and fungal infections (Okiei et al., 2009; Faleye et al., 2012; Chaddha et al., 2013; Erhabor et al., 2013; Mensah et al., 2013; Simplice et al., 2014; Agbor, 2015). This plant was also found to have neuroprotective activity (Simplice et al., 2014).
Several previous studies have shown that *E. coccinea* contains alkaloids (Faleye et al., 2012; Mensah et al., 2013; Roger et al., 2015), phenols (Faleye et al., 2012), cardiac glycosides (Mensah et al., 2013; Roger et al., 2015), flavonoids (Faleye et al., 2012; Mensah et al., 2013; Agbor, 2015; Roger et al., 2015), glycosides (Agbor, 2015), saponins (Faleye et al., 2012; Mensah et al., 2013), terpenoids (Agbor, 2015; Roger et al., 2015), steroids (Mensah et al., 2013; Agbor, 2015; Roger et al., 2015), and tannins (Faleye et al., 2012; Mensah et al., 2013; Agbor, 2015; Roger et al., 2015).

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

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 a preliminarily GC-MS analysis of *E. coccinea* and the investigation of its anti-bacterial potential against *Escherichia coli* and *Staphylococcus aureus*.

Materials and Methods

Plant materials

The leaves and stem barks of *E. coccinea* 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 (P. Compère 542 of October 10, 1959) 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 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 □m) 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 □L

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 g/mL to 62.5 g/mL were obtained. The sample was first sterilized, then stirred, and inoculated with 100 L 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 L) 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

In this study, the non-polar extracts of a Congolese weedy plant (*E. coccinea*) was submitted to phytochemical, GC-MS, and antibacterial activity analyses. The phytochemical analysis revealed the presence of alkaloids, flavonoids, coumarins, quinones, saponins, and triterpenes.

From the GC-MS analysis of this plant, a total of 24 compounds were identified from the leaf extracts (19 and 7 compounds from the Hexane and DCM extracts, respectively; 2 being common to the two extracts). The most abundant components were caryophyllene (22.07%), 1-octadecanol (19.34%), caryophyllene oxide (17.74%), 1-tridecene (7.70%), geranylgeraniol (7.46%), tetracosane (5.50%), and ethylhexadecanoate (2.82%) from hexane and pentadecanal (40.03%), 1,E-11,Z-13-octadecatriene (11.35%), 1-octadecanol (7.31%), 1-tridecene (6.39%) and 4,8,12,16-tetramethylheptadecan-4-olide (2.59%) for the DCM extract.

Table 1. Chemical composition of *E. coccinea* Leaf extracts

Entry	Name	Retention Time (min)	Peak Area	Relative % Composition	Match Factor (%)
	I. Hexane extract				
1	Caryophyllene	9.5946	1489395	22.07	97
2	1-Tridecene	10.496	519892	7.70	93
3	Pentadecane	10.5748	9796	0.15	89
4	Caryophyllene oxide	12.7235	1197558	17.74	83
5	1-Hexadecene	13.3803	23468	0.35	81

6	Hexadecanoic acid, ethyl ester	16.1401	190413	2.82	93
7	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.0916	28201	0.42	88
8	Octadecanoic acid, methyl ester	17.3949	6168	0.09	85
9	Hexadecane	19.6794	5246	0.08	87
10	Eicosane	20.4763	73956	1.10	87
11	Tetracosane	21.9989	371076	5.50	86
12	1-Octadecanol	23.4701	1305553	19.34	85
13	Geranylgeraniol	24.1957	503550	7.46	83
	II. Dichloromethane extract				
14	Cyclohexanone	5.0372	57774	1.13	80
15	Benzene, tert-butyl-	5.8203	15447	0.30	90
16	Phenol, m-tert-butyl-	8.0335	4313	0.08	85
17	1-Tridecene	10.4768	325388	6.39	92
18	4,8,12,16-Tetramethylheptadecan-4-olide	19.3812	132017	2.59	88
19	1-Dodecanol	20.4133	5863	0.12	89
20	1-Octadecanol	23.3934	372546	7.31	85
21	Pentadecanal-	24.3485	2039692	40.03	87
22	Eicosane	24.697	24737	0.49	89

However, no compound was identified from the root bark hexane extract while seven (7) compounds were identified from the root bark DCM extract. The most abundant compounds in the DCM extract were methyl-11,14-eicosadienoate (46.95%), pentadecanal (25.51%), (E)-3-eicosene (8.67%), 1-hexadecanol (3.64%) and menthol (2.19%).

Table 2. Chemical composition of *E. coccinea* Root extracts

Entry	Name	Retention Time (min)	Peak Area	Relative % Composition	Match Factor (%)
	I. Hexane extract				
	No compound identified				
	II. Dichloromethane extract				
1	Menthol	14.4293	6391	2.19	80
2	1-Undecanol	16.947	3368	1.15	93
3	3-Eicosene, (E)-	15.9477	25286	8.67	85
4	11,14-Eicosadienoic acid, methyl ester	17.4512	136971	46.95	80
5	Pentadecanal-	22.9327	57252	19.63	89
6	1-Hexadecanol	23.3543	10605	3.64	96
7	Tridecane	24.6798	1544	0.53	88

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*. Only the hexane extracts exhibited moderate activity (MIC = 500-1000 g/mL) against *S. aureus* while the remaining extracts were inactive (MIC > 1000 g/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, several 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 the antimicrobial activity of *E. coccinea* from elsewhere have reported the activities as well as the occurrence of some of the compounds we found in *E. coccinea* 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, caryophyllene and caryophyllene oxide (Owolabi et al., 2010; Mazimba et al., 2012; Elisabeth and Arumugam, 2014). 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 1-octadecanol, geranylgeraniol, 1-tridecene, and tetrcosane towards *E. coccinea* activity may be particularly important since they were found in good quantities in the most active hexane extracts. The remaining compounds have previously been reported from different plants species and have shown similar pharmacological properties (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. 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 or could be identified and/or isolated from *E. coccinea*.

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 stem barks of *E. coccinea* collected in Kinshasa / DR Congo. The present investigation has led to the identification of a total of 24 compounds (19 and 7 compounds from the leaf and stem bark extracts, respectively; 2 compounds having been simultaneously identified from the leaves and stem bark extracts). The extracts were preliminarily evaluated for antibacterial activity against *Staphylococcus aureus* and *Escherichia coli.* The DCM extracts exhibited moderate activity (MIC = 500-1000 µg/mL) against *S. aureus* but all remaining extracts were inactive (MIC > 1000 µg/mL) against the test bacteria.

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.

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