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Research Article

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

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ABSTRACT
In this study, phytochemical analysis of the leaves and stem barks of *Tridax procumbens* Linn. indicated the presence of alkaloids, flavonoids, coumarins, diterpenes, steroids, saponins, and terpenoids. In addition, the qualitative and quantitative profiles of the hexane and dichloromethane (DCM) extracts were determined by GC-MS. Twenty-six (26) compounds were identified (18 and 11 compounds from the leaf and stem bark extracts, respectively; 3 compounds having been simultaneously identified from these two extracts). The most abundant compounds in the leaf extracts were caryophyllene (62.97%), cedrene (11.85%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (6.43%), caryophyllene oxide (5.67%), trans-α-bergamotene (4.32%), and 3,5,11,15-tetramethyl-1-hexadecen-3-ol (3.10%) from hexane and phytol (31.708%) and 3,7,11,15-tetramethyl-2-hexadecen-1-ol (25.763%) from the DCM extract. Likewise, the most abundant compounds in the stem barks were 6,10,14-trimethyl-2-pentadecanone (34.76%), caryophyllene oxide (18.40%), 4,8,12,16-tetramethylheptadecan-4-olide (4.29%), tetracosane (3.28%), and eicosane (1.38%) from hexane, and 4-methyldecane (42.69%) and tridecane (35.85%) 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 extracts were the most active (exhibiting moderate activity, MIC ≈ 1000 µg/mL) against *S. aureus* while the remaining extracts were inactive (MIC > 1000 µg/mL) against both test bacteria.

Keywords: *Tridax procumbens*, Antibacterial, Chromatography, GC-MS.

INTRODUCTION

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).

Tridax procumbens (Linn.) (Asteraceae) is a semi-prostrate annual or short-lived perennial herb, native to the tropical Americas but that has been introduced to tropical, subtropical, and mild temperate regions of the world (Kale and Deshmukh, 2014). It is denoted by different names, including Fododo or Issongo (Central African Republic; Amégninou et al., 2013), Agnamalaobe (Madagascar; Rivière et al., 2005), Coat Buttons or Tridax Daisy (English), Herbe Caille (French), Ghamra (Hindi), Cadillp Chisaca (Spanish), etc. (Ankita and Jain, 2012). The leaves are membranous, scaberulous above, glabrate beneath, auricled at base, irregularly toothed. Flower heads have long stalk, yellow hard, rounded, 2.4-3.9 cm across, often 2-5 clustered together in the axils of leaves or terminal. Petals are about 2 cm long, tubular, yellow in colour. Achenes are curved, compressed *ca*. 8 mm long, tip narrowed, with one rib on each face (Sankaranarayanan et al., 2013). Known as Wilikumbe in DR Congo (Konda et al., 2012), *T. procumbens* has been in use in various traditional systems of medicine as anticoagulant (Sankaranarayanan et al., 2013), anticancer (Manjamalai et al., 2012a; Priya et al., 2012; Sankaranarayanan et al., 2013), antidiabetic (Sonawane et al., 2014), antiinflammatory (Manjamalai et al., 2012b), antioxidant (Sindhuja et al., 2014), antibacterial, antifungal, and insect repellent (Alka and Padma, 2013; Bharathi et al., 2012; Manjamalai et al., 2012a; Kale and Dhake, 2013; Sankaranarayanan et al., 2013; Mohale et al., 2014; Sindhuja et al., 2014). This weed has also been reported for the treatment of bronchial catarrh, dysentery, malaria, stomachache, diarrhea, high blood pressure, and to check haemorrhage from cuts, bruises, and wounds; and to prevent hair fall (Sankaranarayanan et al., 2013). Several previous studies have shown that this plant contains alkaloids (Kumar et al., 2012; Sankaranarayanan et al., 2013; Sawant and Godghate, 2013; Agme and Agme, 2014), amino acids (Sawant and Godghate, 2013; Agme and Agme, 2014), carotenoids (Kumar et al., 2012), coumarins (Sawant and Godghate, 2013), essential oils (Agme and Agme, 2014), flavonoids (Kumar et al., 2012; Sankaranarayanan et al., 2013; Sawant and Godghate, 2013), glycosides (Sankaranarayanan et al., 2013; Sawant and Godghate, 2013), saponins (Kumar et al., 2012; Sankaranarayanan et al., 2013; Sawant and Godghate, 2013), terpenoids (Sankaranarayanan et al., 2013; Sawant and Godghate, 2013), steroids (Sawant and Godghate, 2013; Agme and Agme, 2014), and tannins (Kumar et al., 2012; Sankaranarayanan et al., 2013; Agme and Agme, 2014). Some specific compounds that have been reported from this plant include β-sitosterol (Saxena and Albert, 2005; Bhalerao and Kelkar, 2012), luteolin, glucoluteolin, quercetin, isoquercetin, oleanolic acid, dexamethasone, bis-bithiophene, taraxasteryl acetate, β-amyrenone, lupeol, 8,3'-dihydroxy-3,7,4'-trimethoxy-6-*O*--D-glucopyranosylflavone, 6,8,3'-trihydroxy-3,7,4'-trimethoxyflavone, puerarin, esculetin, and

betulinic acid (Mundada and Shivhare, 2010; Bhalerao and Kelkar, 2012). Moreover, earlier workers had reported the use of this plant in agricultural crop protection of mango plants against the attack of *Xanthomonas campestris* (Pawart, 2014), and as bioadsorbent for phytoremediation of industrial wastewater for metals such as chromium (VI) (Mundada and Shivhare, 2010; Kale and Deshmukh, 2014).

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 *T. procumbens* 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 *T. procumbens* 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 *T. procumbens* 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 3017 of July 1, 1976) 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 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-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

From this plant, a total of 18 compounds were identified from the leaf extracts (10 components from the hexane and 9 from the DCM extract, 1 being common to the two extracts), and the most abundant being caryophyllene (62.97%), cedrene (11.85%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (6.43%), caryophyllene oxide (5.67%), trans-α-bergamotene (4.32%), and 3,5,11,15-tetramethyl-1-hexadecen-3-ol (3.10%) from hexane, and phytol (31.708%) and 3,7,11,15-tetramethyl-2-hexadecen-1-ol (25.763%) from the DCM extract.

In addition, eleven (11) compounds were identified from the stem bark extracts (9 components from hexane and 2 from the DCM extract). The most abundant compounds were 6,10,14-trimethyl-2-pentadecanone (34.76%), caryophyllene oxide (18.40%), 4,8,12,16-

tetramethylheptadecan-4-olide (4.29%), tetracosane (3.28%), and eicosane (1.38%) from hexane, and 4-methyldecane (42.69%) and tridecane (35.85%) from the DCM extract.

Table 1. Chemical composition of *T. procumbens* leaf extracts

Entry	Name	Retention Time (min)	Peak Area	Relative % Composition	Match Factor (%)
	I. Hexane extract				
1	trans-α-Bergamotene	9.6153	2989752	4.32	96
2	Caryophyllene	9.7716	205108	62.97	89
3	Camphene	10.3953	1535	0.03	82
4	Cedrene	11.0071	562827	11.85	82
5	Caryophyllene oxide	11.7871	269414	5.67	80
6	1-Hexadecene	13.4573	17594	0.37	82
7	1-Dodecanol	13.9959	3000	0.06	85
8	Tridecane	14.2223	1997	0.04	84
9	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	15.2313	305170	6.43	82
10	1-Hexadecen-3-ol, 3,5,11,15-tetramethyl-	15.661	146961	3.10	90
	II. Dichloromethane extract				
11	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	14.8346	1092980	25.763	92
12	Hexadecanoic acid, methyl ester	15.4495	7786	0.184	89
13	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.0859	5225	0.123	83
14	Phytol	17.2823	1345215	31.708	85
15	1-Undecanol	20.4375	2417	0.057	83
16	Pyrazine, 2,6-dimethyl-	21.0055	1082	0.026	86
17	Pentadecane, 2,6,10,14-tetramethyl-	23.3656	17264	0.407	83
18	Hexadecane	23.3656	14118	0.333	91
19	Eicosane	24.7255	24039	0.567	91

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 DCM extracts exhibited moderate activity (MIC ≈ 1000 µg/mL) against *S. aureus* while 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).

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Table 2. Chemical composition of *T. procumbens* stem bark extracts

Entry	Name	Retention Time (min)	Peak Area	Relative % Composition	Match Factor (%)
	I. Hexane extract				
1	Caryophyllene oxide	11.8372	723772	18.40	88
2	Decane, 2-methyl-	13.0808	1108	0.03	82
3	2-Pentadecanone, 6,10,14-trimethyl-	14.6497	1367276	34.76	93
4	Hexadecanoic acid, ethyl ester	16.0984	12003	0.31	93
5	1-Hexadecanol	16.9597	21922	0.56	96
6	1-Undecanol	18.7569	1341	0.03	87
7	**A**	19.363	168627	4.29	91
8	Eicosane	23.347	54365	1.38	85
9	Tetracosane	24.7057	129190	3.28	85
	II. Dichloromethane				
10	Decane, 4-methyl-	10.1858	1679	42.69	87
11	Tridecane	12.489	1410	35.85	88

A: 4,8,12,16-Tetramethylheptadecan-4-olide

Interestingly, several similar studies (Suksamrarn et al., 2004; Pisutthanan et al., 2005; Owolabi et al., 2010), on the evaluation of the antimicrobial activity of *T. procumbens* L. from elsewhere have reported the activities as well as the occurrence of some of the compounds we found in *T. procumbens* 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, caryophyllene and caryophyllene oxide (Owolabi et al., 2010; Rajeswari et al., 2011; Mazimba et al., 2012; Elisabeth and Arumugam, 2014), copaene (Mazimba et al., 2012), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (Musa et al., 2015) 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 3,7,11,15-tetramethyl-2-hexadecen-1-ol, phytol, 4-methyldecane, and tridecane towards *T. procumbens* activity may be particularly important since they were found to be the major compounds of the most active DCM extracts. The remaining compounds have previously been reported from different plants species and have shown similar activities (Mazimba et al., 2012; Saravanan et al., 2013; Elisabeth and Arumugam, 2014), etc. 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 and could be identified and/or isolated from *T. procumbens* 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 stem barks of *T. procumbens* L. collected in Kinshasa / DR Congo. The present investigation has led to the identification of a total of 26 compounds (18 and 11 compounds from the leaf and stem bark extracts, respectively; 3 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 ≈ 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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Conflict of interest :Nil

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Mihigo received his Licence (Bachelor of Science) (2000) in Chemistry at the University of Kinshasa/DR Congo (Central Africa) and a Master of Science (2005) and PhD (2011) in Natural Products Chemistry (Organic synthesis and Green Chemistry) from the University of Botswana, under Professor Berhanu M. Abegaz. He is currently serving as an Associate Professor and lectures Organic chemistry and Green Chemistry at the University of Kinshasa.

Dr S.O. Mihigo's main research interests include the phytochemical investigations of Congolese plants, the development of antimicrobial compounds for human health and Agricultural crop protection, the development of anti-venomous natural products against scorpions and snake bites, the use of Green Chemistry-based approaches for the synthesis of heterocyclic compounds, including chromenes, flavonoids and biflavonoids, and the promotion of safety and good laboratory practices, and good scientific writing.