Fitoterapia 129 (2018) 191–197

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

Chemical composition and biological activities of the essential oil from *Cleome rutidosperma* DC

Megil J. McNeil[a], Roy B.R. Porter[a,*], Lois Rainford[b], Oliver Dunbar[c], Sheena Francis[c], Nicola Laurieri[d], Rupika Delgoda[c]

[a] Department of Chemistry, University of The West Indies, Mona, Kingston 7, Jamaica
[b] Department of Microbiology, University of the West Indies, Kingston 7, Jamaica
[c] Natural Products Institute, University of the West Indies, Mona, Kingston 7, Jamaica
[d] Department of Pharmacology, University of Oxford, Mansfield Road, Oxford OX1 3JA, UK

ARTICLE INFO

Keywords:
Cleomaceae
(*Z*)-phytol
Bacillus cereus
Antimicrobial
Arylamine N-acetyltransferase

ABSTRACT

Cleome rutidosperma DC, commonly known in Jamaica as 'consumption-weed' is a plant traditionally used in folklore for treating tuberculosis and other infectious and chronic ailments. We evaluate for the first time the chemical composition and biological activities of the essential oil components of the complete aerial parts of this plant. The essential oil obtained by steam distillation (0.02%) was analyzed by a combination of gas chromatography-flame ionization detector (GC-FID), gas chromatography-mass spectroscopy (GC–MS) and retention index (RI). The volatile oil of *C. rutidosperma* was dominated by oxygenated diterpenes (67.6%); with (*Z*)-phytol (65.1%) being the single most abundant constituent. *C. rutidosperma* aerial essential oil exhibited moderate inhibition against the activity of recombinant arylamine N-acetyltransferase (NAT) from *Mycobacterium marinum* (IC_{50} 22.20 ± 1.80 μg/μL), while, racemic phytol had an inhibition with an IC_{50} of 22.33 μg/μL ± 0.50 μg/μL, thus accounting for the NAT inhibition imparted by the crude oil. Inhibition of NAT, a key enzyme in mycobacterial growth may be the pathway in which phytol, shown in this study to interact with the active site using *in-silico* methods, renders its previously demonstrated anti-tubercular properties. The phytol rich essential oil also demonstrated antimicrobial activity against all nine human pathogenic bacteria and the fungus strain assayed, with the most significant inhibitory activity against *Bacillus cereus* and justifies the need for further evaluation and development of the essential oils from this plant.

1. Introduction

Cleome (Cleomaceae) species are often grown as garden ornamentals, and universally known under the synonym 'spider flower.' The common name refers to the long stamens, which protrude from their flowers and resemble a spider. According to Hall et al. [1], as many as 150 *Cleome* species, are widely distributed in the tropical countries of both the Old and New Worlds [2,3]. In the flora of Jamaica, ten introduced *Cleome* species have been recorded [4] and in this study, we examined *Cleome rutidosperma*.

C. rutidosperma DC (syn. - *C. ciliata*) is a native plant to tropical West Africa, and has become naturalized in various parts of Tropical America and South-East Asia [5]. The plant, colloquially known as 'consumption weed' in Jamaican folk medicine, tend to occur commonly on disturbed sites and sandy waste lands as low growing aggressive herb with erect or trailing branches reaching up to 1 m long. It produces violet-blue flowers, which turn pink as they age. In the rural communities of Jamaica, the entire plant is often boiled to make a tea for the treatment of cold, and as a traditional remedy for stomach, chest and joint complaints [4], in addition to its use as anti-tubercular agent. Different parts of *C. rutidosperma* are well documented in the literature to have extensive use in various traditional systems of medicine with a variety of therapeutic effects attributed to it. For example, in Nigerian folk therapy, a decoction of its aerial parts is used to manage skin rashes, aching legs and to cure ear infections [6]. In East and West Africa, the young shoots and leaves are boiled and consumed as a green vegetable by the indigenous population [7]. On the other hand, the popular uses of the roots, refer mainly to its analgesic, anti-inflammatory, wound healing [8] and anthelmintic properties [5]. In the literature, previous studies on *C. rutidosperma* have focused mainly on the phytochemical and biological investigations from different parts of the plant. For example, phytochemical examinations of the seeds and aqueous extracts

* Corresponding author.
E-mail address: roy.porter@uwimona.edu.jm (R.B.R. Porter).

https://doi.org/10.1016/j.fitote.2018.07.006
Received 30 May 2018; Received in revised form 4 July 2018; Accepted 4 July 2018
Available online 05 July 2018

(leaves and stems) from the plant have revealed the presence of tannins, saponins, flavonoids and other phytoconstituents [6,9]. In addition, several authors have confirmed significant *in vitro* and *in vivo* antioxidant [10], insecticidal [11], antimicrobial [12], hepatoprotective [13] and antidiabetic properties [14,15], as well as anticancer and anti-HIV activities of compounds isolated from different members of *Cleome* [14,16–18]. Despite the considerable phytochemical studies reported in the literature on *Cleome* species, no previous reports can be found on the volatile constituents of *C. rutidosperma*. To fill this gap in information, it is pertinent to characterize the volatiles of *C. rutidosperma* and to gain an understanding of its biological activities.

Although some anti-tubercular drugs have been developed from natural products [19], none appear to have originated from the *Cleome* species, despite its common affiliation with the disease as a remedy for 'consumption'. An alamar-blue-assay based investigation with an extract containing phytol as its major constituent, had displayed moderate inhibition of the growth of *Mycobacterium tuberculosis* in comparison to standard drugs [20], and both *Z* and *E* phytol isomers were shown to have anti-tuberculosis activity [21]. We therefore evaluated the potential inhibitory impact of essential oil and its most abundant component, Z phytol, on the activity of mycobacterial Arylamine N-acetyltransferase (NAT) enzyme, which has been long established as a potential novel target against tuberculosis [22]. NAT is a phase II drug metabolizing enzyme found expressed in both eukaryotes and prokaryotes, and catalyses the transfer of an acetyl moiety from acetyl CoA to arylamines and arylhydrazines [23]. The drug Isoniazid (INH), which is the leading anti-tubercular drug, is acetylated in humans by the NAT enzyme and the discovery of an active NAT homolog in *Mycobacterium tuberculosis*, the causative agent of tuberculosis, led to research on the role of NAT in the pathogen. The *nat* gene appears to be essential for survival of the pathogen in the macrophages infected, since it encodes for a protein that is involved in the synthesis of cell wall complex lipids including mycolates [22]. Although initially thought to play a significant role in drug resistance, the small increases in the sensitivity to INH for strains whose *nat* gene has been knocked out, led to a change in the focus of the research on the pivotal role of NAT in mycobacterial growth, such as *M. Bovis*, in macrophages [22]. Therefore, NAT enzyme has become a useful target for designing novel anti-tubercular therapeutics, and a screening procedure has been developed to identify selective NAT inhibitors, including natural products [18], as novel anti-tuberculars [24–27]. Due to the relative insolubility of recombinant NAT protein from *M. tuberculosis*, other pure prokaryotic NAT proteins with similar primary sequence and substrate specificities have been used to carry out the biochemical characterization of small molecule inhibitors [28], such as NATs from *Pseudomonas aeruginosa* and *Mycobaterium marinum*, termed PANAT and MMNAT respectively, have been employed in enzymatic assays of this study. Small inhibitors of MMNAT [28] have been useful in identifying the endogenous role that NAT plays in bacterial arylamine N-acetyltransferases.

We report herein for the first time, the chemical composition of the volatile extract from the complete aerial parts of *C. rutidosperma* grown in Jamaica and its impact on prokaryotic NAT proteins, in particular MMNAT and PANAT. We anticipate that an understanding of the impact of essential oil extract and its major constituents on the activity of prokaryotic NAT *in-vitro* and *in-silico* will provide an initial insight into their potential as effective anti-tubercular chemicals. The oils were further evaluated for its *in-vitro* impact on a panel of microbes.

2. Materials and methods

2.1. Plant material

C. rutidosperma growing wild was collected from the campus of the University of West Indies, Mona, Jamaica, a tropical country, with minimal seasonal changes. It was identified as *C. rutidosperma* and voucher specimen was deposited in the Herbarium, Department of Life Sciences, University of the West Indies, Mona (Accession # 32,490).

2.2. Essential oil collection

Fresh aerial parts of *C. rutidosperma* (1.00 kg) were chopped, weighed and hydrodistilled for 4 h using a Clevenger-type apparatus. The resulting distillate was dried over anhydrous sodium sulfate to yield clear yellow oils of 0.02% (w/w). All extractions were done in triplicates. The extracts were stored below 5 °C in the refrigerator for further analysis.

2.3. Antioxidant assay

The DPPH radical scavenging capacity of extracts of *C. rutidosperma* were evaluated by the method described elsewhere [29]. Each test extract, as well as the ascorbic acid control, was made up in methanol to a starting concentration of 50 μg/mL and serially diluted in ninety-six well microtitre plates and added to a 0.02% (w/v) solution of the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol. After a thirty-minute incubation period, in the dark, at room temperature, the absorbance was read at 517 nm. All tests were carried out in duplicate for at least two independent experiments.

2.4. GC-FID analysis

Analytical GC was carried out on a Hewlett-Packard 5800 Series II gas chromatograph fitted with a HP-5 ms capillary column (30 m × 0.25 mm; 0.25 μm film thickness). Helium was used as the carrier gas with a flow rate of 1 mL/min. The temperature program employed was from 70 °C (3 min.) to 220 °C (12 min.) at a rate of 17 °C/min. Injector and detector temperatures were maintained at 250 °C and 300 °C, respectively. Injection volume for all samples was 1 μL. Quantitative data were obtained by electronic integration of FID area counts without the use of correction factors.

2.5. GC–MS analysis

The volatile oils were analyzed by GC–MS using Hewlett-Packard 6890 gas chromatograph equipped with a SLB-5 ms fused silica capillary column (30 m × 0.25 mm; 0.25 μm film thickness) and a HP-5973 mass-selective detector. Injector and detector temperatures for both oven temperatures were maintained at 250 °C and 300 °C, respectively. The oven temperature program employed was from 120 °C (1 min.) to 280 °C (5 min.) at a rate of 5 °C/min. Helium was employed as the carrier gas with a flow rate of 1 mL/min. Injection volume for all samples was 1 μL. The mass spectral data were obtained with ionization energy of 70 eV and a mass range of 30–450 amu.

2.6. Identification of components

The oils were spiked with a standard mixture of homologous nC_5 to nC_{26} paraffin series and analyzed by GC under the above mentioned conditions. Retention indices were directly obtained by the application of Kovàts procedure as described by Sun et al. [30]. The components of the essential oil were identified by comparison of mass spectral data with those of Wiley and NIST 98 library or with authentic compounds when thought necessary and were confirmed by comparison of their Kovàts retention indices with published data in the literature [31–36].

2.7. NAT assay

All chemicals and reagents for the NAT - 5,5′-dithiobis-2-nitrobenzate (DTNB) colorimetric assay were purchased from Sigma-Aldrich (St. Louis, MO). Heterologously expressed prokaryotic NAT from *Pseudomonas aeruginosa* and *Mycobacterium marinum* were purified as described elsewhere [28,37] and were donated as a gift by Professor