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# Chemical Composition and Antibacterial Activity of Essential Oil from Kenyan *Conyza bonariensis* (L.) Cronquist

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## Abstract

The utilization of plants to treat sicknesses and to prevent diseases, including epidemics, has been in practice for thousands of years. The understanding of their therapeutic properties has been passed over the centuries within and among individual communities. Secondary metabolism produces active compounds which are usually responsible for the biological and pharmacological properties of some plant species used for the management of communicable diseases. Presently, the antimicrobial activity of several plants, up to now considered empirical has been scientifically established. Several studies have aimed to explain the chemical composition and the mechanisms involved in microbial growth inhibition, either separately or together with conventional antimicrobial compounds. In this study, the essential oil from aerial parts of *Conyza bonariensis* (L.) Cronquist collected from Kabianga division, Kericho County, Kenya was obtained by hydro-distillation and analyzed by GC-MS. The major components were monoterpene hydrocarbons; limonene (8.26%), *trans*-ocimene (3.74%) and sesquiterpene hydrocarbon *trans*-β-farnesene (3.17%), and among other compounds isolated an aldehyde, 1H-Indene-3-carboxaldehyde, 2,6,7,7a-tetrahydro-1,5-dimethyl yielded 49.14%. The antibacterial activity indicated that the oil was active against the bacterial strains tested. The minimum inhibitory concentration for the oil against *Escherichia coli* was 12.5%, while that of *Salmonella typhi* was 6.25%. The results suggested that the essential oil of *C. bonariensis* can be developed as a safe, natural and alternative method for controlling bacterial infections.

## Introduction

Plants and their essential oils are potentially useful sources of antimicrobial compounds. There are numerous studies and published data on the antimicrobial activities of plant compounds against many different types of microbes. The main constituents of essential oils mono- and sesquiterpenes including carbohydrates, phenols, alcohols, ethers, aldehydes and ketones are responsible for the biological activity of aromatic and medicinal plants as well as for their fragrance. Due to these properties, spices and herbs have been added to food since ancient time, not only as flavoring agents but also as preservatives [1]. The genus *Conyza* belongs to the family *Asteraceae* and comprises about seventeen species of annual, biennial and perennial herbs, particularly inhabiting highland and mountain regions [2]. Seven species have been reported: *Conyza canadensis* (L.) Cronquist, *C. laevigata* (Rick) Pruski, *C. primulifolia* (Lam.) Cuatrec. & Lourteig, *C. sumatrensis* (Retz) E. Walker, *C. uliginosa* var. *columbiana* (Hieron) Cuatrec, *C. uliginosa* var. *uliginosa* (Benth) Cuatrec and *C. bonariensis* (L) Cronquist [3]. *C. bonariensis* belongs to the flowering plants; it grows widely in Kenya, and is known as 'taplile' by the Kalenjin community of Kenya. An infusion from *C. bonariensis* aerial parts is used in traditional medicines as antiseptic, hemostatic and antispasmodic [4]. The leaves are also used as a decoction in the treatment of malaria, fever, jaundice, skin rashes, sickle cell anemia, inflammations of the fallopian tubes and backache [5]. In women, the leaves are used to induce uterine contraction, abortion and stimulate lactation [6]. Diseases such as rheumatism, cystitis, gout, nephritis, toothache, and headache have been managed by the use of *C. bonariensis* [5]. Some of the biological activities of the *Conyza* genus are: *C. sumatrensis*, antimalarial activity [4]; *C. Canadensis*, antibacterial, antioxidant, cytotoxic and anti-proliferative activity [7]; *C. aegyptiaca*, anti-inflammatory, analgesic and antiviral activity [8-10]; *C. dioscorides*, antischistosomal activity [11]; *C. filaginoides*, anti-protozoa and anti-diarrheal activity [12, 13] and *C. bonariensis*, mulluscicidal, anti-inflammatory, antipyretic and antimicrobial properties [14]. Previous investigations of *C. bonariensis* have revealed glycosides, polyphenolic compounds, flavonoids sesquiterpenic lactones and essential oils [15, 16]. The current research was done to look at the chemical constituents and antibacterial activity of the essential oil from the Kenyan *C. bonariensis*; this is the first report on the essential oil of the Kenyan *Conyza* species.

## Materials and Methods

### Plant material

*C. bonariensis* (L.) Cronquist leaves were collected in Kabianga location, Kericho, Kenya, in February 2016, and identified by a botanist from the Biological Sciences Department. A voucher specimen (Cb 01) was deposited in the University herbarium, School of Agriculture and Biotechnology, Kabianga.

### Essential oil isolation

Fresh leaves (about 1000 g) were subjected to hydro-distillation for 4 h using a Clevenger-type apparatus (Garg process glass, India). This apparatus consists of three parts: a round bottomed flask in which the material containing essential oil and given the quantity of water are placed, a separator in which the oil is automatically separated from the distillate and a condenser. The oil (0.4 ml) was dried over anhydrous sodium sulfate in order to remove any traces of water and then stored in sealed vials at 2 °C in the dark until analyzed and tested. The yield (0.04%) was calculated based on the dry weight of the plant material.

### Gas chromatography-mass spectrometry

About 1 mg of *C. bonariensis* essential oil was diluted in 1 ml volume of dichloromethane and analyzed by gas chromatography–mass spectroscopy (GC-MS). The peak area was used for relative quantification. The essential oil was analyzed in full scan mode of GC-MS. A GC column HP-5 MS ultra-inert column (30 m × 0.25 mm; 0.25 μm) (J & W, Folsom, CA, USA) with the flow rate of 1.2 ml/min was used. The injection mode was splitless and the oven temperature of 35 °C was maintained

for 5 min and then programmed to 280 °C at 10°C/min for 10.5 min. The total run time was 50 min and the injection volume was 1 µl. The mass spectrometer (MS) was operated in the positive electron impact (EI) mode at 70 eV, in m/z range of 42-350. The identification of the compounds was done by comparing their retention times and mass spectra with those found in NIST 11 chemical mass spectral database. The relative proportions of the essential oil constituents were expressed as a percentage obtained by the peak area normalization.

**Antibacterial assay**

The antibacterial potential of the essential oil was tested according to the national committee of clinical laboratory (NCCL) standards at the Biotechnology Laboratory, Egerton University. The Gram-negative bacterial strains *Escherichia coli* ATCC 25922 and *Salmonella typhi* ATCC 13311 were used for the analysis. The antibacterial activity was determined using disc diffusion method [17]. All the apparatus for the culturing process were sterilized at 120$^{o}$C for 20 minutes. The Mueller Hinton agar was used as the plating media to culture both bacterial strains [18]. The bacterial strains of *E. coli* and *S. typhi* were primarily incubated in the nutrient broth at a temperature of 37◦C to 0.5 on the McFarland scale. The cultures were then diluted to the concentration of $1.0 \times 10^5$ CFU/ml with sterilized water, transferred to Muller Hilton media and added into the Petri plates [19]. The stock solution weighed I ml of the pure oil was serially diluted to 75%, 50%, 25%, 12.5%, and 6.25 % using dimethyl sulfoxide as a diluting agent. Later, 4 mm filter paper discs were placed on the medium and added with different dilutions of *C. bonariensis* oil in triplicate. The pure dimethyl sulfoxide was used as a control. Later, the plates were incubated at 37◦C and the inhibition zones were measured in mm after two days [20].

## Results and discussion

The hydrodistilled essential oil from the leaves of *C. bonariensis* was analyzed by GC–MS and 37 compounds were identified (Fig. 1). The retention times and area percentages of the compounds identified by GC–MS are given in Table 1. The

**Table 1** The chemical composition of *C. bonariensis* essential oils as determined by gas chromatography–mass spectroscopy.

| RT (min) | Compound name | Percentage |
|---|---|---|
| | **Monoterpene hydrocarbons** | |
| 9.48 | α-Phellandrene | 0.90 |
| 9.58 | α- Pinene | 2.25 |
| 10.21 | Benzaldehyde | 2.20 |
| 10.52 | β-Pinene | 5.37 |
| 10.84 | Myrcene | 1.30 |
| 11.48 | *o*-Cymene | 0.82 |
| 11.56 | Limonene | 8.26 |
| 11.73 | Sylvestrene | 0.90 |
| 11.92 | (E)-β-Ocimene | 3.74 |
| 12.11 | γ- Terpinene | 0.93 |
| 12.63 | Terpinolene | 0.70 |
| 13.29 | neo-allo-Ocimene | 0.70 |
| 13.33 | Verbenene | 0.70 |
| 14.11 | Terpinen-4-ol | 0.85 |
| 16.46 | Camphene | 1.10 |
| | **Oxygenated monoterpenes** | |
| 16.73 | Eugenol | 0.68 |
| | **Sesquiterpene hydrocarbons** | |
| 17.00 | α-Copaene | 0.68 |
| 17.06 | Daucene | 0.89 |
| 17.19 | β-Elemene | 0.86 |
| 17.61 | (E)-Caryophyllene | 1.59 |
| 17.73 | β-Cubebene | 1.14 |
| 17.96 | (E)-β-farnesene | 3.17 |
| 18.05 | α-Humulene | 1.01 |
| 18.16 | *trans*-Muurola-4(14),5-diene | 0.79 |
| 18.27 | γ- Cadinene | 1.47 |
| 18.39 | Germacrene D | 1.55 |
| 18.58 | Bicyclogermacrene | 1.02 |
| 20.08 | γ- Muurolene | 0.81 |
| | **Oxygenated sesquiterpenes** | |
| 19.39 | Nerolidol | 1.47 |
| 19.66 | Spathulenol | 1.32 |
| 19.82 | 1,10-Cubeno | 1.65 |
| | **Others** | |
| 18.78 | 1H-Indene-3-carboxaldehyde, 2,6,7,7a-tetrahydro-1,5-dimethyl- | 49.14 |

RT = retention time

results indicated that, the dominant constituent in the oil was the monoterpene hydrocarbons, which accounted for 30.72%, of which limonene (8.26%), β- pinene (5.37 %) and β-ocimene (3.74%) were the major compounds. The previous investigation of the leaf oil of *C. bonariensis* collected from Merida, Venuzuela also showed that these monoterpenes were the main compounds; however, a higher concentration of trans-β-ocimene (20.7%) was also reported [20]. The sesquiterpene hydrocarbons accounted for 14.98% of the total oil, with β-farnesene (3.17%), E-caryophyllene (1.59%) and γ-cadinene (1.47%) being the major compounds. These results were in accordance with those of Lambert et al. [17] who found that trans-β-farnesene

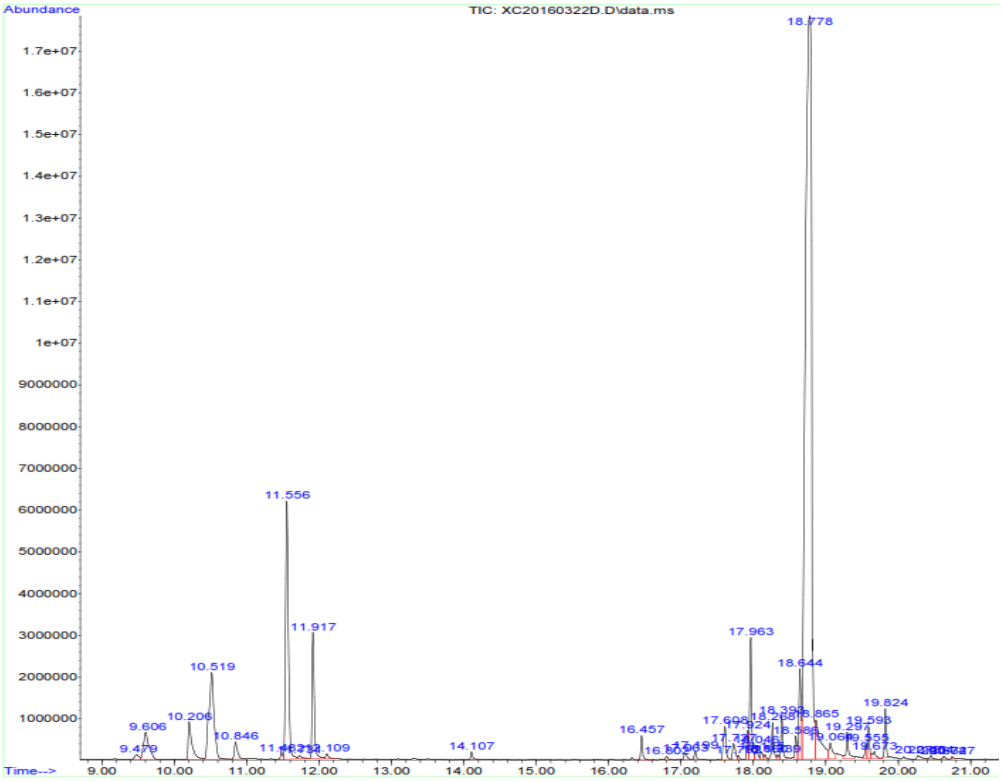


**Fig. 1** Representative total ion chromatogram of *Conyza bonariensis* essential oils with retention time of each compound detected by gas chromatography–mass spectroscopy.

(37.8%), trans-ocimene (20.7%) and β-sesquiphellandrene (9.8%) were present as the major components of the *C. bonariensis* collected from Venezuela. The findings of this study were also in agreement with those of Mabrouk et al. [16] and Maia et al. [21], who also reported *trans*-β-farnesene as one of the main compounds of the essential oil of this species. On the other hand, Šoškić et al. [22] reported limonene and *trans*-ocimene as the main proportions in the essential oil of *C. bonariensis* found in Rio de Janeiro, Brazil. β-Sesquiphellandrene was only observed in low amounts in the essential oil studied by Souza et al. [23]. Limonene was detected in 5.1% in the same species collected in Rio de Janeiro, Brazil [24]. The oxygenated derivatives of both monoterpenes and sesquiterpene contributed to 0.68% and 4.44%, respectively, it was found that nerolidol, spathulenol and 1,10-cubeno were the major components in the present investigation. *C. bonariensis* found in Kenya and reported herein had the same components as those found in other regions but in low concentration. This variability in the chemical composition could be attributed to numbers of factors such as genetics (species or variety) of the plant, environmental conditions (geographical, climatic or seasonal) and processing of plant material. Variability in the qualitative and quantitative composition most probably depended on the genotype of the plant and the influence of different environmental factors [22].

The essential oil was tested for antibacterial activity against the pathogenic Gram-negative bacterial strains (*E. coli* and *S. typhi*). The oil exhibited activity against the two bacterial strains tested as seen by their zones of inhibition (Table 2). However, the activity of the oil is known to differ with its concentration and the kind of bacteria. The activity response to *S. typhi* was greater than in *E. coli* at different concentrations, except 50% oil concentration. The minimum inhibitory concentration for the oil against both bacterial strains was 12.5% for *E. coli* and 6.25% for *S. typhi*. The essential oil tested in this work has a range of compounds that could account for antibacterial activity as a result of their synergistic effect.

**Table 2** The antibacterial activity of different concentration of essential oil of *Conyza bonariensis* against *Escherichia coli* and *Salmonella typhi*.

| Microorganisms | Zone of Inhibition (mm) | | | | | |
|---|---|---|---|---|---|---|
| | 100% | 75% | 50% | 25% | 12.5% | 6.25% |
| *Escherichia coli* ATCC 25922 | 14.7 ± 1.3 | 11 ± 0.8 | 8.7 ± 0.5 | 7.7 ± 0.4 | 6.3 ± 0.5 | 0.0 ± 0.0 |
| *Salmonella typhi* ATCC 13311 | 16 ± 0.0 | 12 ± 0.8 | 8.7 ± 0.5 | 7.3 ± 0.5 | 7.3 ± 1.3 | 6.0 ± 0.2 |

The essential oil was diluted with dimethyl sulfoxide and antibacterial activity was determined using disc diffusion assay.

Although, essential oils usually occur as complex mixtures of compounds and its antimicrobial activity can generally be accounted for their major monoterpenoid components. This is because that the monoterpenes can diffuse into and harm cell membrane structures [24]. Previous research indicated that, limonene, β-pinene, β-ocimene, β-caryophyllene and β-farnesene possess moderate antibacterial activity [25], it has also been reported that oxygenated components of essential oil have a higher potential, mainly phenol-type compounds, for example, thymol and carvacrol while hydrocarbon monoterpenes demonstrate the lowest antibacterial activity. Other reports indicated that oxygenated monoterpenes, display strong antimicrobial activity, mostly on whole cells, while hydrocarbon compounds show lower activity, because of their limited diffusion through the medium insolubility in water [26]. Generally, hydrocarbons are relatively inactive regardless of their structural type, and this inactivity is closely related to their limited hydrogen bound capacity and water solubility [27]. Ketones, aldehydes and alcohols are active, but with differing specificity and levels of activity, which is related to the present functional group, but also associated with hydrogen-bounding parameters in all cases. Previous results showed that greater antimicrobial potential could be ascribed to the oxygenated terpenes, especially phenolic compounds [28].

## Conclusions

In this study, the essential oil from the plant *C. bonariensis* was extracted and analyzed. The results showed that oil obtained could be developed into a natural antibiotic because its compounds demonstrated potential activity against bacterial strains. These components displayed potential for synergistic coupling with antimicrobial agents to advance therapeutic effectiveness, in the face of increasing bacterial resistance; however, this needs further investigation.

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## Conflict of interest

There is no conflict of interest.

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