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Chemical composition, antimicrobial and antioxidant activities of the essential oil of *Conyza canadensis* growing wild in Kashmir valley

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Abstract
Chemical composition of the essential oil of *Conyza canadensis* has been carried out using Gas chromatography-mass spectrometry (GC-MS). A total of 26 components, constituting 93.94% of the total volatile constituents present in the aerial part, have been identified. The hydrodistilled extract of the aerial part of *C. canadensis* was dominated mainly by monoterpene hydrocarbons (53.08%) followed by sesquiterpene hydrocarbons (37.25%). The major components identified in the essential oil were limonene (23.78%), (*Z*)-lachnophyllum ester (21.25%), (*E*)-*β*-ocimene (16.02%), *β*-pinene (11.83%) and (*E*)-*β*-farnesene (7.84%). The essential oil and the individual constituents exhibited significant antibacterial activity against the tested Gram-positive and Gram-negative food-borne bacteria with MIC values in the range of 12.00-40.00 µg/mL for the essential oil and 32.00-600 µg/mL for the individual constituents, respectively. The essential oil showed maximum inhibitory activity against *Candida albicans* and *Candida parapsilosis* with MICs of 2.50 and 1.80 µg/mL, respectively. The essential oil showed promising antioxidant activity using DPPH (2, 2-diphenyl-1-picrylhydrazyl) and hydroxyl radical scavenging assays.

Keywords: Monoterpene hydrocarbons, Limonene, (*Z*)-lachnophyllum ester, Free radical-scavenging activity

1. Introduction
The contamination and deterioration of food articles by oxidation and microbial damage not only lead to reduction of shelf life but also to the onset of diseases and therefore the economic losses. The food industry is facing an enormous pressure because of the food deterioration caused by afore mentioned factors [1]. It has been reported that in 2005, almost two million people died globally because of diarrhoea alone. A large proportion of these deaths are attributed to microbial contamination in food and drinking water. From the storage of raw materials to processing using various treatments, oxidation is another factor responsible for the degradation of lipids and proteins which leads to deterioration in flavour and nutritional qualities of foods [2]. Reactive oxygen species (superoxide radicals, hydroxyl radicals, peroxy radicals and other free radicals) trigger many diseases such as carcinogenesis, coronary heart disease, aging and many other health related issues [3]. If on one hand the use of synthetic preservatives in food items increase the shelf life but on the other hand it leads to undesirable consequences and ill effects which need to be addressed. The synthetic antioxidants like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) which are currently used as food preservatives have been found to be carcinogenic [4]. In recent years, the resurgence for natural preservatives for prevention of oxidation and microbial growth has gained considerable attention due to their health benefits [5]. Since the multidrug resistance (MDR) of microbes constitutes one of the main reasons for the failure of antimicrobial drugs, the search for nontoxic substitutes that reduce MDR is a task of great importance. In this connection, the diversity of natural products and the formulations prepared thereof may be of great use. The structural diversity and efficiency of natural products can be exploited to eradicate the dread created by MDRs. Keeping in view the above facts, it is demanding to develop natural products with antioxidant and antimicrobial activities. They have been shown to be an excellent source for the development of food additives in food industry, and new drugs in medical fields [5]. Among the natural products, essential oils have shown remarkable promise in the fields of perfumery, make-up products and sanitary products, in food industry as food additives and food preservatives, in dentistry as natural remedies and in agriculture as

green pesticides [1]. As evident from literature, combinations of agents have been used clinically for the therapeutic advantages they provide over single agents especially in the treatment of malignant and infectious diseases [6]. This is because in synergistic interactions, the cooperation of several agents may be needed for a full effect [6]. Drug combinations have been advantageous in the treatment of cardiac failure [7], myocardial infarction [8], hypertension [9], asthma and in the prevention of graft rejection [6]. Thus interactions between bioactive agents are of great importance in the field of pharmacology for the development of effective therapeutic agents.

Conyza canadensis (L) Cronquist also known as horseweed, belongs to the family Asteraceae (Compositae). It is an annual herb, widely distributed in Western Himalayas, Punjab, Kashmir and Pakistan [10]. It is used as astringent, stimulant, haemostatic and diuretic. It also finds use in treatment of diarrhoea, dysentery and uterine haemorrhages [11]**.** In Chinese folk medicine, horseweed has also been used for the treatment of wounds, swellings and pain caused by arthritis [12]. In North America its decoction has traditionally been used to treat cancerous diseases [13]. In Poland, the alcoholic extract from fresh herb called Hemorigen is used as haemostatic agent [14]. Extracts of the aerial parts of *C. canadensis* from Tunisia have shown antibacterial, antioxidant and cytotoxic activities [15]. Besides antiviral activity against human cytomegalovirus (HCMV) AD-169 and Cox-B3 viruses [16].

Previous phytochemical studies of horseweed from various parts of the world show the presence of acetylenes [17], flavonoids [18], alkaloids, sterols, triterpenes, sphingolipids [19], phenylpropanoyl esters [20].

Sphingolipids are biologically important class of compounds [21], some of which have been reported to exhibit antihepatotoxic [22], antitumor and immunostimulatory activities [23]. Bioassay-directed isolation and identification of acetylenes from *Conyza canadensis* unveiled their phyto toxic and fungi toxic activities. [24]. Yan *et al* [25] reported the antiproliferative constituents from the methanol extract of *C. canadensis* which were found to exert considerable cell growth-inhibitory activity against human cervix adenocarcinoma (HeLa), skin carcinoma (A431), and breast adenocarcinoma (MCF-7) cells. Erigeronol, a triterpene derivative isolated from *Conyza canadensis* has shown potent cytotoxic activity with IC_{50} value of 7.77 ± 0.47 µg/mL against melanoma B16 determined by the 3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method[26]. *Conyza canadensis* has been reported to be allelopathic in nature. Its allelopathic properties may be a significant factor in its success as a weed [27]. The essential oil of *C. canadensis* (syn. *Erigeron canadensis* L.) was referred in the *United States Pharmacopoeia* as diuretic, tonic, astringent and for arresting internal haemorrhage [28]. The extracts of *C. canadensis* have been found to possess anti-inflammatory activity [29]. As part of the Institute's programme to explore the aromatic flora of Kashmir Valley for new essential oils, aroma chemicals and other bioactive phytochemicals, the essential oil analysis of *C. canadensis* was carried out and was screened for antimicrobial and antioxidant properties. Some of the constituents found in the essential oil of *C. canadensis*, available in our institute were subjected to the antibacterial screening to identify the bioactive constituents of the essential oil. To the best of our knowledge and literature survey, there is no information available documenting the chemical composition, antimicrobial and antioxidant activities of *C. canadensis* essential oil of Kashmir origin. The essential oil of *C. canadensis* has shown interesting antimicrobial and antioxidant properties which could make it a successful candidate in food and pharmaceutical industries. The oil could be used as an alternative against the synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) which have recently been documented to initiate tumorigenesis [4].

The oil could also find the use against food-borne pathogens to avoid food adulteration, but further study in this direction needs to be done to check the toxicological parameters of the oil which will be the future plan of our work.

2. Material and Methods

2.1 Chemicals

DPPH (2,2-dipheny-1-picryl hydrazyl), L-ascorbic acid, dry ferric chloride, methanol, dimethyl sulfoxide and all the pure essential oil components were purchased from Sigma Chemical Co. (St. Louis, MO). All the chemicals/solvents were of analytical grade.

2.2 Plant Material and Essential oil extraction

The plant material of *C. canadensis* was collected from Srinagar (Jammu and Kashmir), where it grows as a weed. After proper identification, a voucher specimen (No. 2101/11) was deposited in the Herbarium of the Indian Institute of Integrative Medicine, Srinagar. Fresh above ground part (aerial part) was subjected to hydro distillation using a modified Clevenger-type apparatus for 3 hours as per the procedure described in the European Pharmacopoeia (Council of Europe, 2007). Anhydrous sodium sulphate was used to remove water after extraction. The essential oil was stored in air tight glass container in a refrigerator at 4.0 ˚C. The essential oil yield of the aerial part was found to be 0.90% (V/W) as calculated on fresh weight basis.

2.3 GC-FID analysis

GC/FID was carried out on Perkin Elmer auto system XL Gas Chromatograph 8500 series with flame ionization detector (FID) and head space analyzer using a fused silica capillary RTX-5 column (30m x 0.32 mm, film thickness 0.25µm) coated with dimethyl polysiloxane. Oven temperature was programmed from 60 to 280 ˚C at 3 ˚C/min, with injector temperature 230 ˚C and detector temperature 250 ˚C. Injection volume 0.5µL, nitrogen was used as a carrier gas (1.3 mL/min).

2.4 Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS analysis was carried on a Varian Gas Chromatograph series 3800 fitted with a VF-5 ms fused silica capillary column (60m x 0.25mm, film thickness 0.25µm) coupled with a 4000 series mass detector under the following conditions: injection volume 0.5 µL with split ratio 1:60, helium as carrier gas at 1.0 mL/min constant flow mode, injector temperature 230 ˚C, oven temperature was programmed from 60 to 280 ˚C at 3 ˚C /min. Mass spectra: electron impact (EI+) mode,70 eV and ion source temperature 250 ˚C. Mass spectra were recorded over 50-500 a.m.u range.

2.5 Identification of components

Identification of the essential oil constituents was done on the basis of Retention Index [RI, determined with respect to homologous series of *n*-alkanes (C_5-C_{28}, Polyscience Corp., Niles IL) under the same experimental conditions], co-injection with standards (Sigma Aldrich and standard

isolates), MS Library search (NIST 05 and Wiley) and by comparing with the MS literature data [29-30].

2.6 Determination of antimicrobial activity

2.6.1 Microbial strains and culture media

The antibacterial activity of essential oil of *C. canadensis* and its individual constituents available in the laboratory was tested against a panel of 6 bacterial and 6 fungal strains. The bacterial strains used were Gram positive *Staphylococcus aureus* ATCC-25923 and *Bacillus subtilis* ATCC-25955, Gram negative bacteria such as *Escherichia coli* ATCC-25922, *Pseudomonas aeruginosa* ATCC-27853, *Klebseilla pneumoniae* ATCC-25924, *Shigella flexneri* ATCC-12022 obtained from American Type Cultures Collection (Manassas, VA, USA). The medium used was Muller Hinton Agar and Muller Hinton Broth (Becton-Dickinson, Cockeysville, MD, USA; DIFCO laboratories). The cultures were maintained on Tryptone soya agar and stored at -70 °C containing 50% glycerol (Himedia, Mumbia, India). The five fungal strains i.e. *Candida albicans*, *Candida krusei*, *Candida parapsilosis*, *Candida tropicalis* and *Aspergillus niger* obtained from Mycology section, Department of Microbiology, SKIMS, J&K, India and Institute of Basic Medical Sciences (IBMS) were grown on Sabouraud dextrose agar (SDA) plates at 25 °C and maintained on SDA slants. Cell suspension of microorganisms in 0.9% NaCl was adjusted at 0.5 McFarland to obtain approximately 10^6 CFU/mL.

2.6.2 Antibacterial activity assay

MIC was determined as per the guidelines of Clinical and Laboratory Standards Institute (Formerly the National Committee for Clinical Laboratory Standards) [31]. Bacterial suspensions were prepared by suspending 18 h grown bacterial culture in sterile normal saline. The turbidity of the bacterial suspension was adjusted to 0.5 McFarland standards (equivalent to 1.5×10^8 CFU/mL) at wavelength 625nm. The 2-fold serial of the essential oil and individual constituents (stock solution prepared in dimethylsulphoxide) were prepared in Mueller Hinton Broth (MHB; DIFCO laboratories) in 100 µL volume in 96-well U bottom microtitre plates (Tarson, Mumbai, India). The above mentioned bacterial suspension was further diluted in the MHB and 100 µL volume of this diluted inoculum was added to each well of the plate resulting in the final inoculum of 5×10^5 CFU/mL in the well and the final concentrations of samples ranged from 2000 to 3.90 µg/mL till 10th column. Column 11 and column 12, containing 100 µL and 200 µL of medium without drug, served as growth and medium control respectively. Gentamicin, streptomycin and ciprofloxacin (Sigma-Aldrich) were used as positive control. The plates were incubated at 37 °C for 18 h. The plates were visually read and the minimum concentration of the compound showing no turbidity was recorded as MIC.

2.6.3 Antifungal activity assay

A macro dilution broth method was used to determine the Minimal Inhibitory Concentrations (MIC) according to NCCLS references [32-33]. The serial doubling dilution of each oil was prepared in dimethyl sulfoxide (DMSO), with concentrations ranging from 0.05 to 130 µg/mL. Final concentration of DMSO never exceeded 2%. Recent cultures of each strain were used to prepare the cell suspension adjusted 1-2 × 10^4 cells/mL. The concentration of cells was confirmed by viable count on Sabouraud agar. The test tubes were incubated aerobically at 35 °C for 48 h/72 h and MICs were determined. In addition, two reference antifungal compounds, amphotericin B (Fluka) and fluconazole (Pfizer) were used to control the sensitivity of tested microorganisms. All tests were performed in RPMI medium. For each strain tested, the grow conditions and the sterility of the medium were checked in two control tubes. The innocuity of the DMSO was also checked at the highest tested concentration. All experiments were performed in triplicate and repeated if the results differed.

2.7 Antioxidant activity

The essential oil of *C. canadensis* was subjected to screening for the possible antioxidant activity by two methods i.e. DPPH free radical scavenging and hydroxyl radical scavenging assays.

2.7.1 DPPH Free radical-scavenging activity

DPPH Free radical scavenging activity was evaluated by measuring the scavenging activity of the essential oil on stable 2, 2-diphenyl-1-picryl hydrazyl radical (DPPH). A 0.5 mM solution of DPPH in methanol was prepared and a stock solution of oil sample (1 mg/mL) in methanol was prepared. Various concentrations (10-100 µg /mL) were added to 1.0 mL (0.5 mM DPPH) and final volume was made to 3.0 mL with methanol. The mixture was shaken thoroughly and kept standing at room temperature for 10 min. Then the absorbance of the mixture was measured at 517 nm on spectrophotometer. The decrease in the absorbance indicates an increase in DPPH-radical scavenging activity. The percentage inhibition was calculated by the following equation.

DPPH radical scavenging (%) = $\{(A_c-A_s)/A_c)\}$ X 100

Where, A_c is the absorbance of control, A_s is absorbance of sample.

L-Ascorbic acid and butylated hydroxyl toluene (BHT) (Sigma-Aldrich) served as positive control. The experiment was done in triplicate and mean values were recorded. IC_{50} value was calculated as the concentration of sample, required to scavenge 50% of DPPH free radicals.

2.7.2 Hydroxyl radical scavenging activity

Hydroxyl radical scavenging was carried out by measuring the competition between deoxyribose and the essential oil for hydroxyl radicals generated in Fenton reaction. Hydroxyl radicals degrade deoxyribose leading to the formation of thiobarbituric acid reactive substances (TBARS) that could be measured spectrophotometrically at 532 nm. The reaction mixture containing 25 mM deoxyribose, 10 mM ferric chloride, 100 mM ascorbic acid, 2.8 mM H_2O_2 in 10 mM KH_2PO_4 (pH 7.4) and various concentrations of essential oil of *C. canadensis* was incubated at 37 °C for 1h. Then 1.0 mL of 1.6% thiobarbituric acid and 1.0 mL of 3% trichloroacetic acid were added and heated at 100 °C for 20 min. The TBARS was measured spectrophotometrically at 532 nm. The results were expressed as percentage inhibition of deoxyribose oxidation, as determined by the following formula.

Percent inhibition = $\{(A-B)/A\} \times 100$

Where A was the malonaldehyde produced by Fenton reaction treated alone, and B was the malonaldehyde produced in the presence of essential oil of *C. canadensis* and known antioxidant (L-Ascorbic acid and butylated hydroxyl toluene).

2.8 Statistical analysis

Each experiment was done in triplicate and mean values were calculated. The data were recorded as means ± standard deviations. Analysis of variance for individual parameters was

performed on the basis of mean values to find out the significance at $p < 0.05$.

3. Results and Discussion

3.1 Chemical composition of the essential oil

The various chemical constituents identified in the essential oil of *C. canadensis* are shown in Table 1 in order of their elution from an RTX-5 column. GC-MS analysis of the essential oil led to the identification of 26 constituents accounting for 93.94% of the total oil composition. The hydrodistilled extract of the aerial part of *C. canadensis* is dominated by the presence of monoterpene hydrocarbons (53.08%) followed by sesquiterpene hydrocarbons (37.25%). The principal components of the essential oil are limonene (23.78%), (*Z*)-lachnophyllum ester (21.25%), (*E*)-*β*-ocimene (16.02%), *β*-pinene (11.83%), (*E*)-*β*-farnesene (7.84%), ar-curcumene (2.99%) and (*E*)-*α*-bergamotene (2.07%). Other minor constituents identified comprise (*Z*)-sesquisabinene hydrate (1.83%), myrcene (0.82%), *β*-isocomene (0.80%), daucene (0.64%) and khusinol (0.49%). The chemical composition of essential oil of *C. canadensis* from Kashmir valley using hydrodistillation technique shows both qualitative as well as quantitative differences with that reported from other parts of the world [14].

Table 1: Chemical composition of the essential oil of *Conzya canadensis* growing wild in Kashmir (India).

Components [a]	Peak area (%)	RI	Methods of identification [b]
β-Pinene	11.83	974	MS,RI,Std
Myrcene	0.82	982	MS,RI
α-Terpinene	0.15	1014	MS,RI,Std
Limonene	23.78	1024	MS,RI,Std
(Z)-β-Ocimene	0.22	1031	MS,RI,std
(E)-β-Ocimene	16.02	1039	MS,RI
n-Nonanal	0.14	1107	MS,RI
p-Mentha-1,3,8-triene	0.26	1115	MS,RI
Daucene	0.64	1380	MS,RI
β-Isocomene	0.80	1404	MS,RI
β- Caryophyllene	0.14	1417	MS,RI,std
(E)-α-Bergamotene	2.07	1435	MS,RI
(Z)-β-Farnesence	0.28	1447	MS,RI
α-Himachalene	0.34	1449	MS,RI
(E)-β-Farnesence	7.84	1456	MS,RI
ar-Curcumene	2.99	1474	MS,RI
Germacrene-D	0.31	1483	MS,RI,Std
Bicyclogermacrene	0.40	1500	MS,RI
β-Bisabolene	0.19	1508	MS,RI,Std
(Z)-Lachnophyllim ester	21.25	1530	MS,RI
(Z)-Sesquisabinene hydrate	1.83	1540	MS,RI
(E)-Nerolidol	0.31	1566	MS,RI
Spathulenol	0.18	1573	MS,RI
Caryophyllene oxide	0.23	1582	MS,RI,Std
α-Cadinol	0.43	1658	MS,RI
Khusinol	0.49	1673	MS,RI
Total identified (%)	93.94		
Monoterpene hydrocarbons	53.08		
Sesquiterpene hydrocarbons	37.25		
Oxygenated sesquiterpenes	3.47		
Alcohols	0.14		

RI = retention indices were experimentally measured using homologous series of n-alkanes (C5–C28) on the RTX-5 column.

[a] Compounds are listed in order of their elution from RTX-5 column.

[b] Identification methods: MS, by comparison of the mass spectra with those from computer mass libraries, NIST 05 library, Wiley and Adams; RI, As per literatutre reports.

Essential oil of *C. canadensis* from Poland was found to be a rich source of limonene (80-81%).Limonene is generally predominant in the oils of European origin [14]. There are striking differences in the overall essential oil composition of Kashmiri *C.* canadensis and that of Poland. Unlike Kashmiri *C. canadensis* the Polish *C. canadensis* oil contains polyacetylenic (*Z, Z*)-matricaria ester while as the Kashmir *C. canadensis* was found to contain polyacetylenic (*Z*)-lachnophyllum ester in good concentration (21.25%). In addition to this, certain constituents like *α*-pinene, *trans*-limonene oxide, *cis*-piperitol, *cis* and *trans*-carveol, carvone and bisabolol are present in the essential oil of *C. canadensis* of Polish origin only [14]. On the other hand certain volatile constituents like *n*-nonanal, daucene, *β*-isocomene, *α*-cadinol and khusinol were present in the essential oil of *C. canadensis* of Kashmir origin only. The essential oil of *C. canadensis* from France also shows differences with that of Kashmir origin both qualitatively as well quantitatively. The major volatile compound in French oil is limonene (76%). In addition, the other major contstituents in French *C. canadensis*, absent in Kashmir *C. canadensis*, are *β*-myrcene, *δ*-3-carene, thujone, camphor and α-santalene. Essential oil of American *C. canadensis* comprises of much higher concentration of limonene (67.25%) along with some polyacetylenes like matricaria methyl ester, matricaria ethyl ester, lacnophylum methyl ester, matricaria lactone and lacnophylum lactone [34]. These polyacetylenes were not found in the essential oil of Kashmir *C. canadensis.*

3.2 Antibacterial activity

The in vitro antibacterial activity of the essential oil of *C. canadensis* and its major chemical constituents against the tested Gram-positive and Gram-negative bacteria was qualitatively and quantitatively assessed by the inhibition zones. The results (MICs) are given in Table 2 and indicate that the essential oil and its chemical constituents exhibited a broad spectrum and potent inhibitory effect against all tested bacterial strains (*S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa*, *K. pneumonia*, and *S. flexneri*). The essential oil showed a better antimicrobial effect against the two Gram-positive bacterial strains *S. aureus* and *B. subtilis* with the MIC values of 12.00 µg/mL and 16.00 µg/mL respectively. Gram-negative bacterial strains were a little resistant than their Gram-positive counterparts with the MIC values in the range of 28.00 µg/mL to 600 µg/mL. In order to identify the active principles of the essential oil, some of the individual components of the oil available in the laboratory were also screened and it was observed that almost all the major components of the oil showed moderate to high degree of antibacterial activity against the tested bacterial strains with *β*-pinene, limonene, *β*-caryophyllene and germacrene D being the most potent antibacterial agents. The individual essential oil constituents were not as active as the essential oil itself (Table-2). Taking cue from the findings of (6), the antibacterial activity results shown in table-2 reflect the synergistic effect of constituents in the essential oil. Therefore lower dose of essential oil was required as compared to the individual constituents to inhibit the growth of bacteria. Terpenes in combined state (essential oils) have been found to exert their enhanced activity by polyvalence effect by attracting multiple targets (multi-target principle) [6]. Because of their large lipophilicity the terpenoids have great affinities for cell membranes and, therefore a high potential to permeate through cell walls of the body or bacteria which can strongly enhance overall efficacy, if they possess a sufficiently high

bioavailability [6]. Also, the observed variation in the antibacterial activity of the samples against each culture may be attributed to the structural differences between the microorganisms under study [35]. In general, the two Gram-positive bacterial strains were the most sensitive both against the essential oil as well as the individual chemical constituents while as the Gram-negative strains showed some resistance against the samples under study. It is maintained that the hydrophobic components usually diffuse easily across the lipid bilayer membranes compared to hydrophilic components which are transported passively via channels present in the membranes. Gram-negative bacteria are surrounded by lipopolysaccharide outer membrane thereby making them less susceptible to various hydrophobic antimicrobials. Thus higher doses of antimicrobial agents are required compared to Gram-positive bacteria (36-37). The antibacterial activity of the essential oil and individual chemical components was compared with that of standard antibiotics: gentamicin, streptomycin and ciprofloxacin.

Table 2: Antibacterial activity (Minimum Inhibitory Concentration) of the essential oil from *C.canadensis*.

Sample	Minimum Inhibitory Concentration (µg/ml)					
	S. aureus	***E. coli***	***P. aureoginosa***	***S. flexneri***	***K. pneumoniae***	***B. subtilis***
Essential oil	12.00	40.00	32.00	28.00	32.00	16.00
β-Pinene	40.00	132.0	76.00	64.00	128.00	44.00
Myrcene	NT	NT	NT	400	400	320.20
Limonene	32.00	NT	52.00	56.00	NT	32.00
(*Z*)-*β*-Ocimene	NT	600	NT	600	600	132.00
(*E*)-*β*-Ocimene	NT	NT	NT	NT	560	132.00
β-Caryophyllene	32.00	96.00	60.00	60.00	96.00	32.00
Germacrene-D	32.00	NT	NT	NT	92.00	32.00
Gentamicin	3.90	7.80	7.80	7.60	7.80	5.90
Streptomycin	11.20	11.60	11.50	11.20	11.64	7.70
Ciprofloxacin	6.44	5.90	6.40	6.50	6.65	8.80

NT=not tested

Gentamicin, Streptomycin, Ciprofloxacin were used as standard antimicrobial agents.

3.3 Antifungal activity

The essential oil from *C. canadensis* was tested against a panel of five different fungal strains and showed broad antifungal activity at against all the tested fungal strains (Table 3). Among the fungal strains tested, *C. albicans* and *C. parapsilosis* were the most susceptible with MICs of 2.50 and 1.80 µg/mL, respectively while as *C. kreusei*, *C. tropicalis* and *A. niger* were a little resistant towards the essential oil. Amphotericin B and fluconazole were used as positive controls respectively. The overall results revealed that the inhibitory activity of microorganisms is concentration dependent. Polyacetylenes have been identified as strong broad spectrum antifungal compounds found in many Apiaceae plant species inhibiting [38]. Spore germination of different fungi Several studies have demonstrated that limonene,(*Z*)-lachnophyllum ester, myrcene, *β*-pinene, (*E*)-*β*-ocimene and related terpenes possess broad spectrum antifungal and antibacterial activities [39]. These volatile chemical constituents exert their antimicrobial effect through the disruption of membrane integrity [40]. Since these compounds constitute the major proportion of the essential oil composition, they along with other chemical constituents could be responsible for the antifungal properties of the essential oil from *C. canadensis*.

Table 3: Antifungal activity (Minimum Inhibitory Concentration) of the essential oil from *C. canadensis*.

Strains	Minimum Inhibitory Concentration (Mic)[A]		
	Essential Oil	Fluconazole	Amphotericin B
Candida albicans	2.50 ± 0.09	1.10 ± 0.07	NT[b]
Candida parapsilosis	1.80 ± 0.08	0.88 ± 0.05	NT
Candida krusei	120.00 ± 1.20	65.5 ± 1.50	NT
Aspergillus niger	4.50 ± 0.10	NT	1.22 ± 0.10
Candida tropicalis	7.20 ± 0.05	4.12 ± 0.25	NT

MICs were determined by a macrodilution method and expressed in µg/mL (W/V).

3.4 Antioxidant activity

In order to ascertain the antioxidant potential of the essential oil of *C. canadensis*, two spectrophotometric methods (DPPH and hydroxyl radical assays) were employed.

3.4.1 DPPH free radical scavenging activity

DPPH radical scavenging assay, a standard assay in antioxidant activity studies, offers a rapid technique for screening the radical scavenging ability of specific compounds, essential oils or extracts. DPPH possesses a characteristic absorption at 517 nm, which decreases significantly on exposure to radical-scavengers (antioxidants) by providing hydrogen atom or electron donation. A lower absorbance at 517 nm indicates a higher radical-scavenging activity of the sample under study. DPPH radical scavenging activity of the essential oil from *C.canadensis* at various concentrations is shown in Fig. 1. At the concentrations of 10-100 µg/mL, scavenging ability of the essential oil on DPPH radical was in the range of 35.17-85.1%. Ascorbic acid and BHT were used as the reference standard. The essential oil exhibited a concentration dependant scavenging of DPPH radicals with an IC_{50} value of 34.5 µg/mL which was comparable to the reference standards at the same doses.

3.4.2 Hydroxyl radical scavenging activity

Hydroxyl radical scavenging effect of the essential oil from *C. canadensis* at various concentrations is shown in Fig. 2. Hydroxyl radical scavenging ability was estimated by

generating hydroxyl radicals using ascorbic acid-iron-H_2O_2 (Fenton reaction). Antioxidant activity of the essential oil was determined as the ability to scavenge the hydroxyl radicals generated. At the concentrations of 10-100 µg/mL, scavenging ability of the essential oil on hydroxyl radicals was in the range of 19.7-65.22%. Ascorbic acid and BHT were used as the reference standard. The essential oil exhibited a concentration dependant scavenging of hydroxyl radicals with an IC_{50} value of 53.4 µg/mL which was comparable to the reference standards at the same doses.

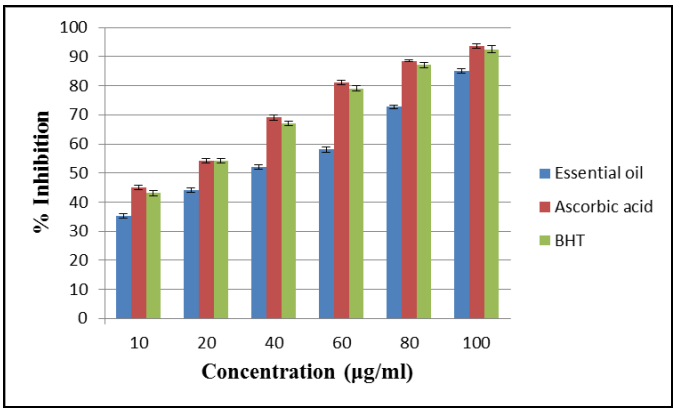

Fig 1: Antioxidant activity of the essential oil from *Conyza canadensis* using DPPH radical scavenging assay.

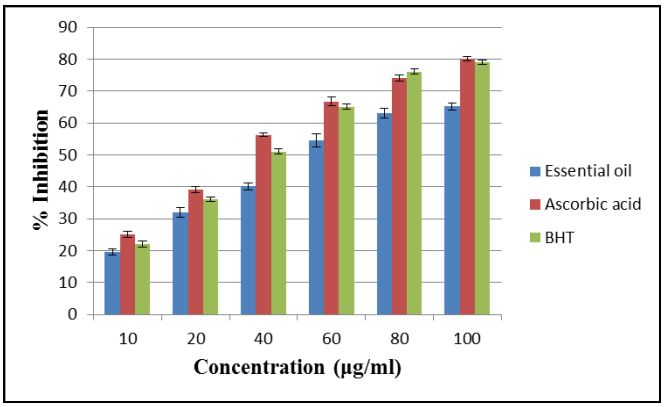

Fig 2: Antioxidant activity of the essential oil from *Conyza canadensis* using hydroxyl radical scavenging assay.

4. Conclusion

Essential oil analysis of *C. canadensis* lead to the identification of 26 constituents constituting 93.94% of the total oil. The essential oil was dominated by limonene (23.78%),(*Z*)-lachnophyllum ester (21.25%), (*E*)-*β*-ocimene (16.02%), *β*-pinene (11.83%), (*E*)-*β*-farnesene (7.84%), *ar-curcumene* (2.99%) and (*E*)-*α-bergamotene* (2.07%). The essential oil exhibited broad spectrum antibacterial and antifungal activities against the tested bacterial and fungal pathogens. The essential oil showed a better activity against the Gram-positive bacterial strains: *S. aureus* and *B. subtilis* with the MIC values of 14.62 µg/mL and 16.00 µg/mL respectively. Among the fungal strains *Candida albicans* and *Candida parapsilosis* were the most susceptible strains. The essential oil showed promising antioxidant activity using DPPH (2,2-diphenyl-1-picrylhydrazyl) and hydroxyl radical scavenging assays. The results suggested that the essential oil from *C. canadensis* may find a potential use as natural antimicrobial and antioxidant agents applied in food systems. Further studies will be required to check the toxicological parameters of the essential oil and the components responsible for its bioactivity.

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