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RESEARCH ARTICLE

Phytochemical screening and *in-vitro* antibacterial and DPPH free radical scavenging activities of methanol extract of root of *Combretum album* Pers.

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ABSTRACT

The objective of the study was to investigate *in-vitro* antibacterial and antioxidant activities of methanolic extract of *Combretum album* Pers. root. In brief antibacterial efficacies of methanol extract and its petroleum ether fraction (MePET), chloroform fraction (MeCH), ethyl acetate fraction (MeEA) and water fraction (MeAQ) were determined by agar well diffusion assay along with Minimum Inhibitory Concentrations (MICs). *In-vitro* antioxidant efficacies were evaluated by DPPH radical scavenging method. Preliminary phytochemical assay, Fourier Transform Infrared Spectroscopy and Gas Chromatography-Mass Spectroscopy analyses were employed to detect the plausible active ingredients. Methanol extract showed broad spectrum antibacterial activity having highest inhibition zone against *Staphylococcus aureus* MTCC 2940 (21.67±0.58 mm) and MICs ranged from100 µg/ml to 250 µg/ml. The scavenging activity of methanol extract of root of *C. album* was concentration dependant and IC_{50} value was 136.08 µg/ml. The lowest MIC (5 µg/ml) was noted with MeCH against *B. subtilis* (MTCC 441). MeCH showed highest antioxidant activity with an IC_{50} value of 12.98 µg/mL and MePET, MeEA, MeAQ and Ascorbic acid presented antioxidant potential with IC_{50} values of 16.10, 15.07, 17.44 and 13.40 µg/mL respectively. Preliminary phytochemical tests and FT-IR analysis revealed presence of various phytochemicals and functional groups like hydroxyl, carboxylic acids, amidines, amines, aromatics and esters. Three compounds were elucidated from bioactive TLC fraction of MeCH in GC-MS analysis supported by presence of various functional groups in MeCH as detected in FT-IR analysis. It is concluded that methanolic extract of *C. album* root is a potential antibacterial and antioxidant agent where presence of N-[5-[4-pyridinyl)-1H-1,2,4-triazol-3-yl]-benzamide (compound 1), pyruvic acid (compound 2) and methyloacetone (compound 3) are responsible as possible active ingredients.

Introduction

Amongst the world's leading cause of infection-related morbidity and mortality are various fatal diseases of human caused either by microbial infections or by free radical generation in the body (1). Antibacterial resistance along with unavailability and high cost of new generation drugs has accelerated the premature deaths particularly in patients infected with resistant bacteria (2). On the other hand, normal oxidation-reduction reactions in human body leads to the generation of free radicals like superoxide anion, singlet oxygen, hydrogen peroxide, hydroxyl radicals etc. (3, 4). These highly unstable molecules cause oxidative damages resulting into many chronic and debilitating diseases like coronary heart disease, cancer, diabetes mellitus, atherosclerosis, inflammatory and other neurodegenerative disorders (5-9). Presently available synthetic antioxidants like butylated hydroxyl anisole (BHA), butylated hydroxy toluene (BHT), tertiary butylated hydroquinone (TBH), Trolox, Ascorbic acid (AA) and gallic acid (GA) esters are known to delay or prevent the onset of major free radical induced diseases (10-12). Unfortunately, synthetic antioxidants have been reported as carcinogenic and toxic (13). As a result, the public health is compromised over the emergence of infectious drug resistant bacteria and oxidative stress (14, 15). Hence, attempts are being made to find out the alternatives of synthetic antibiotics and antioxidants. This is evident by the increasing promotion of scientific research in the therapeutic usage of botanicals as antibacterial and antioxidant agents. Plants have been used either as a source of pure compounds or as standardised extracts against

various infectious diseases from antiquity (16). India harbours a rich source of unexploited medicinal plants (17) and in order to get the benefit of these plants, scientific investigations are needed to find their antibacterial and antioxidant activities. Novel compounds from plants could play a central role in the drug development programmes with the ultimate aim to develop plant based drugs for the management of pathogenic infectious diseases and free radical borne diseases (18). The family Combretaceae comprises of about 20 genera and 600 species with tropical distribution around the globe and mainly in Africa and Asia (19). Phytochemical analysis performed on multiple species of the genus *Combretum* has detected the presence of many classes of phytochemicals including triterpenes, flavonoids, lignins and non-protein amino acids (20). Antibacterial activities were reported to be present in methanolic leaf and bark extract and ethanolic leaf extract of *Combretum album*. (21, 22). Although antioxidant and cytotoxic effects of leaf extract of *C. album* (Synonym *C. roxburghii*) were enlisted in literature (23), antibacterial and antioxidant efficacies of root are not yet documented in literature. The aim of the present work was to evaluate antioxidant and antibacterial effects of methanol extract of *C. album* root and its fractions. Presence of probable bioactive compounds in the methanolic root extract was also assessed through preliminary phytochemical tests, FT-IR and GC-MS analyses.

Materials and Methods

Plant material

Roots of *Combretum album* Pers. (Family-Combretaceae) were collected from Joypur forest area (23.0540° N, 87.4345° E), Bankura, West Bengal, India during the month of February, 2018. The plant was identified and authenticated by plant taxonomist Dr. Ambarish Mukherjee, Former Professor, Department of Botany, The University of Burdwan. A voucher specimen (Voucher number- GCSB05) was also kept in Mosquito, Microbiology and Nanotechnology Research Units, Parasitology Laboratory, Department of Zoology, The University of Burdwan. A herbarium specimen of *Combretum album* Pers. has been deposited to Herbarium, Botany Department, The University of Burdwan, West Bengal (Herbarium code: BURD) and obtained voucher specimen Number GCSB06.

Preparation of methanol extract

Roots were rinsed thoroughly until the soil particles were cleaned from the roots and then cut into small pieces for shade drying for 15 days. Two hundred grams of shade dried roots were subjected to Soxhlet extraction with 2000 ml of petroleum ether for 72 hrs extraction with maximum extraction period of 8 hrs per day to obtain a defatted residue and then the residue was subjected to extraction by Soxhlet apparatus with 2000 ml methanol for 72 hrs to get methanol extract following the same method mentioned above. The extract was filtered with Whatman No.1 filter paper. The filtrate was allowed to dry at room temperature.

Preparation of fractions

For preparation of fractions of the methanolic extract liquid-liquid fractionation technique as described (24) was followed with some improvisation. The root methanolic extract obtained from Soxhlet extraction was subjected to fractionations with petroleum ether, chloroform and ethyl acetate in accordance with their polarity index. At first 100 gm of the water soluble dried methanolic extract was dissolved in 500 ml water in a separating funnel (1000 ml) as the primary separating solvent and shaken vigorously till the extract was properly dissolved. To that solution 500 ml petroleum ether was added and shaken vigorously and left stand still for obvious separation of compounds in these 2 solvents. Petroleum ether extract was collected and now referred to as MePET. After separation of petroleum ether fraction same method was followed for preparation of chloroform and ethyl acetate fractions. Chloroform was added to the remaining water dissolved fraction and after getting the chloroform fraction (MeCH), ethyl acetate was added to the remaining water dissolved fraction. After separation of the compounds in the previous solvent i.e., ethyl acetate fraction (MeEA) the remaining water dissolved fraction was denoted as MeAQ. All the 4 fractions were evaporated and experiments were carried out with these fractions.

Microorganisms

The antibacterial activity of methanol extract was tested against four human pathogenic bacterial strains namely *Staphylococcus aureus* (MTCC 2940), *Escherichia coli* (MTCC 739), *Bacillus subtilis* (MTCC 441) and *Pseudomonas aeruginosa* (MTCC 2453) and four fish pathogenic bacterial strains including *B. licheniformis* (MTCC 530), *B. mycoides* (MTCC 7343), *P. putida* (MTCC 1654) and *P. fluorescens* (MTCC 103). The microorganisms were procured from MTCC and were maintained at our laboratory, Mosquito, Microbiology and Nanotechnology Research Units, Parasitology Laboratory, The Department of Zoology, The University of Burdwan. All the strains were separately cultured on sterilized Nutrient Agar (Hi-Media M012) (NA) at 37 °C for 24 hr by streak plate method. Then well isolated 24 hr cultured colonies of each strain were selected from the culture media and transferred to sterilized Nutrient Broth (Hi-Media M002) (NB) and incubated at 37 °C. Overnight grown bacteria inoculated NA culture plates and NB culture tubes were maintained at 4 °C for future use. This method was repeatedly done for maintenance of the strains.

Antibacterial activity assay

The antibacterial activity of methanol extract was examined by agar well diffusion and broth dilution method (25, 26). Nutrient Agar (NA) and Muller Hinton broth (MHB) were used for the bioassay. Overnight cultures were grown at 37 °C in MHB. Bacterial suspensions of 1.0×10^8 colony-forming units (CFU) per ml were obtained (OD_{600} = 0.08 nm). Petri plates containing 20 ml of NA were used for the well diffusion assay and wells were punched using sterile well puncher of 5 mm diameter. Dried methanol extract was dissolved in 1% (v/v) Dimethyl sulfoxide (DMSO) to obtain a concentration of 1000 µg/ml solution for antibacterial bioassay and 1% (v/v)

DMSO was used as control. The plates were incubated overnight at 37 °C. The antibacterial activity against each test organism was quantified by estimating average diameter of the zone of inhibition around the wells in millimetres. The tests were performed thrice and average diameters of zones were determined.

Preparation of inocula

Inocula were prepared by putting the bacterial cells into Müller-Hinton Broth and were grown in the incubator for 24 hr. The concentration of the broth was adjusted to 1×10^8 CFU/ml with the help of UV-VIS spectrophotometer.

Determination of minimum inhibitory concentration (MIC)

Broth dilution method (27-29) was used to determine MIC of methanol extract against all eight bacterial strains with slight modification. Stock solution of 1000 µg/ml of methanol extract was subjected to dilutions and twelve different concentrations (750, 700, 650, 600, 550, 500, 450, 400, 350, 300, 250 and 200 µg/ml) of methanol extract were prepared. Again, 10 µl of 1×10^8 CFU/ml bacterial suspensions were added to each test tube containing each of the ten concentrations of methanol extract. The tubes were allowed to incubate at 37 °C for 24 hr. The highest dilution of the extract that inhibited the growth of the bacteria in each test tube was taken as MIC for those bacteria.

The same standard method was followed to determine the MICs of MeAQ, MePET, MeCH and MeEA fractions. Concentrations ranged from 10-50 µg/ml for each fraction.

Antibiotic Susceptibility Test

Antibiogram was prepared by disc diffusion method using standard antibacterial sensitivity test (28). Antibiotics were purchased from HiMedia Laboratories Limited, Bombay. Antibiotics used for this purpose were Tetracycline 10 µg/disc, Ciprofloxacin 30 µg/disc, Penicillin 10 µg/disc, Kanamycin 5 µg/disc and Novobiocin 30 µg/disc. Nutrient agar was prepared and poured in the Petri plates for solidification and test organisms were swabbed by sterile swab spreader. Five standard antibiotic discs were then placed on the inoculated agar plates and incubated for 24 hr at 37° C. Following that the diameter of inhibition zones were recorded.

Antioxidant assay

2, 2 diphenyl-1-picryl hydrazyl radical scavenging activity assay

The antioxidant activity in terms of electron donation ability of methanol extract was measured by DPPH method using the stable radical, DPPH (30, 31) with slight modifications. The basis of radical scavenging ability was the bleaching of the purple-coloured solution of DPPH to yellow coloured solution of reduced DPPH-H. A methanol stock solution of 10 ml (1 mg/ ml) was prepared and from those stock solution concentrations of 50, 100, 150, 200 and 250 µg/ml were obtained through serial dilution method. Ascorbic acid was used as standard antioxidant. For determination of DPPH radical scavenging activity 1 ml of methanol extract (0.2 to 1 mg/ml) was added to 3 ml of a methanol solution of 0.1 mM DPPH. The mixture was shaken vigorously and incubated at room temperature in dark condition for 30 min and absorbance measurements were commenced immediately. Similar method was applied for ascorbic acid standard. The decrease in absorbance at 517 nm was determined by spectrophotometer. All experiments were carried out in triplicate. The absorbance (Abs_{sample}) of the resulting solution was calculated at 517 nm in spectrophotometer and converted into percentage of antioxidant activity (AA) and the percentage inhibition of the DPPH radical by the samples was calculated according to the following formula (32):

$AA\% = 100–[\{(Abs_{sample}–Abs_{blank}) \times 100\}/Abs_{control}]$. A methanol (3.0 ml) and plant extract (1.0 ml) solution was used as the blank (Abs_{blank}). A 0.1mM DPPH (3.0 ml) and methanol (1.0 ml) solution was used as the control ($Abs_{control}$).

[Abs_{sample} = absorbance of the reaction in presence of sample (sample dilution+DPPH solution), Abs_{blank} = absorbance of the blank for each sample dilution (sample dilution+ methanol), $Abs_{control}$ = absorbance of control reaction (methanol +DPPH solution)].

The antiradical activity was presented as IC_{50} (µg/ml), the concentration required to cause a 50% DPPH inhibition.

Same method was followed to determine radical scavenging property of the fractions (MeAQ, MePET, MeCH and MeEA) as described above.

Determination of Minimum Inhibitory Concentration of MeAQ, MePET, MeCH and MeEA

Standard method (27-29) with slight modification was followed to determine the MICs of MeAQ, MePET, MeCH and MeEA fractions. Concentrations ranged from 10-50 µg/ml for each fraction.

Identification of active ingredients

Preliminary phytochemical tests

Methanol extract was subjected to preliminary phytochemical screening according to the standard protocol (33-36) for the presence of different plant secondary metabolites.

Separation with preparative Thin Layer Chromatographic plates

As MeCH showed highest antibacterial property as well as lower MICs for all the tested strains it was subjected to separation with preparative Thin Layer Chromatographic plates for isolation of active ingredients (37). For separation Chlorofom : Methanol was used as mobile phase in different ratios in which one distinct band (spot) was detected in 9:1 ratio. The R_f value was 0.157. The plates were also examined for their fluorescent property by the non-destructive UV light visualization method in the short and long wavelengths of UV light at 254 and 365 nm respectively (38).

Fourier Transform Infrared Spectroscopy (FT-IR Spectroscopy) analysis

The presence of different functional groups was studied using Fourier Transform Infrared Spectroscopy (FT-IR) based on the peak value in the region of infrared radiation. The hydraulic pellet press method (39) was followed where dried powder

of methanolic extract was taken in 1:100 ratios with potassium bromide and mixed uniformly in a porcelain mortar with pestle. Sample incorporated pellets were loaded after performing Potassium bromide pelleting onto FT-IR spectroscope (JASCO FT/IR Model-4700) in a scan range of 400-4500 cm-1.

For FT-IR analysis of active TLC fraction same method was followed as described above.

Antibacterial Bio-autography test

For antibacterial bio-autography test (40) 1×10^5CFU/ml of bacterial inocula, each of the tested bacteria was sprayed onto the bands visible in TLC plates. After incubation at 25 ^{0}C temperature for 48 hrs duration in a dark chamber in humid condition aqueous solution of 2,3,5-triphenyltetra-zolium chloride (TTC) was sprayed over the plates. The plates were then incubated at a temperature of 25 ^{0}C for one day. The appearance of the purple-red colour of aqueous solution of 2,3,5-triphenyltetra-zolium chloride (TTC) changed to creamy white around the detected bands, which indicated the inhibition of bacterial growth by the compounds present in the TLC spots.

TLC bio-autography assay with DPPH reagent

In order to evaluate the antioxidant property of the detected spots/band in TLC, a TLC bio-autography method (41) was performed. DPPH reagent was sprayed on the TLC plates and yellowish colour of the spots detected in TLC against the purple colour of the DPPH reagent confirmed presence of antioxidant property of the compounds present in the band/spots.

GC-MS analysis of active compound

GC-MS analysis was carried out at Bose Institute, Kolkata by using Thermofisher Scientific India Pvt. Ltd., TRWAX column of POLARIS Q (30 m×0.25 mm×0.25 µm) using 1 µl sample injection. The carrier gas used was Helium with a flow rate of 1 ml/min. The oven temperature was programmed initially at 40 °C for 2 min, then an increase to 130 °C and then programmed to increase to 270 °C and held for 15 min. Total run time was 59 min and 21 sec. The MS transfer line was maintained at a temperature of 270 °C. The injector temperature was 240 ^{0}C , the sample was diluted in methanol, and split ratio was 1:20. Identification of the bioactive compound on the Mass spectrum was done by using the data on NIST (National Institute of Standard Technologies) library. Structures of the identified compounds were drawn by Chem Draw software.

Statistical Analysis

The data were expressed as mean ± S.E. (n=3) and analysed in MS Excel 2007 and STATPLUS PRO 5.9.8. A difference was considered statistically significant if $p\leq0.05$.

Results and Discussion

This is so far the first study centred on the determination of antibacterial and antioxidant activities of methanol extract of *C. album* root as no research has been carried out on the biological activities of the root of *C. album*.

Yield of extract:

The yield of the methanol extract obtained was 31.50% (w/w).

Antibacterial activity assay

The results obtained for the antibacterial activity of methanol extract of *C. album* root by agar well diffusion method are presented in Table 1. Zone of inhibition against *S. aureus* (21.67±0.58 mm) was highest followed by *P. aeruginosa* (21.33±0.58 mm), *E. coli* (21.00±0.00 mm), *B. mycoides* (20.33±0.58 mm), *B. licheniformis* (18.67±0.58 mm), *P. fluorescens* (18.67±0.58 mm), *B. subtilis* (18.33±0.58 mm) and finally lowest against *P. pudita* (18.00±0.00 mm). Thus, from the results we have found that methanol extract of *C. album* root has broad spectrum antibacterial activity against both Gram negative and Gram positive bacteria under study. Antibiogram of five different antibiotics (Table 1) revealed that Penicillin was less effective against *B. subtilis* (8.67±0.58 mm) and *B. mycoides* (9.00±0.00 mm). Ciprofloxacin showed highest antibacterial activity in terms of zone of inhibition of 32.00±0.00 mm against *P. aeruginosa.* Minimum inhibitory concentration (MIC) of methanol extract ranged from 100 µg/ ml to 250 µg/ ml (Fig. 1). MICs of the solvent fractions are depicted in Table 3. MeCH being the most potent

Table 1. Antibacterial activity of methanol extract of root of *Combretum album* against pathogenic bacterial strains in terms of Zone of inhibition in agar well diffusion assay

Compounds	Mean diameter of zone of inhibition in mm							
	Sa	**Ec**	**Pa**	**Bs**	**Bm**	**Bl**	**Pp**	**Pf**
Methanolic Extracts of *Combretum album* root 1000µg/ml	21.67±0.58	21.00±0.00	21.33±0.58	18.33±0.58	20.33±0.58	18.67±0.58	18.00±0.00	18.67±1.15
Tetracycline 10 µg/disc	22.67±0.58	17.00±0.00	14.67±0.58	11.67±0.58	13.83±0.28	16.33±0.58	9.33±0.58	12.00±0.00
Ciprofloxacin 30µg/disc	NA	26.67±0.58	32.00±0.00	NA	NA	NA	29.50±0.50	26.67±0.58
Penicillin 10 µg/disc	0.00±0.00	NA	NA	8.67±0.58	9.00±0.00	0.00±0.00	NA	NA
Kanamycin 5 µg/disc	NA	13.33±0.58	14.00±0.58	NA	NA	NA	14.83±0.28	8.16±0.28
Novobiocin 30 µg/disc	NA	16.33±0.58	22.00±0.00	NA	NA	NA	19.16±0.28	19.00±0.00
Distilled water	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Dimethylsulfoxide 1% (v/v)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Key: Well diameter = 6.0 mm, 6.0 ± 2.0 = No activity or non-significant activity, N/A = Not applicable.

Sa = *Staphylococcus aureus* MTCC 2940, **Ec** = *Escherichia coli* MTCC 739, **Pa** = *Pseudomonas aeruginosa* MTCC 2453, **Bs** = *Bacillus subtilis* MTCC 441, **Bm**= *Bacillus mycoides* MTCC 7343, **Bl** = *Bacillus licheniformis* MTCC 530 **Pp**= *Pseudomonas putida* MTCC 1654, **Pf**= *Pseudomonas fluorescens* MTCC 103.

antibacterial showed lowest MIC (5 µg/ml) against the Gram positive human pathogenic bacteria, *B. subtilis.*

Antioxidant assay

DPPH radical scavenging assay showed that all the concentrations of methanolic root extract of *C. album* exhibited scavenging activity (Table 2) and the highest concentration showed 82.35 ± 0.216 % inhibition of DPPH free radical. IC_{50} value for methanolic extract was 136.08 µg/ml (Table 2). Fig. 2 contains the IC_{50} values of different fractions of methanol extract. Results of DPPH scavenging activity also revealed that scavenging activity was dependant on the concentration of the extract. Among the fractions, MeCH was the most effective antioxidant with IC_{50} value of 12.98 µg/ml. This result indicates that efficacy of *C. album* root extract is much higher than the previous reports on different species of the same genus namely *C. acutum* Laws (leaves) and *C. sericeum* G. Don (aerial part) (42).

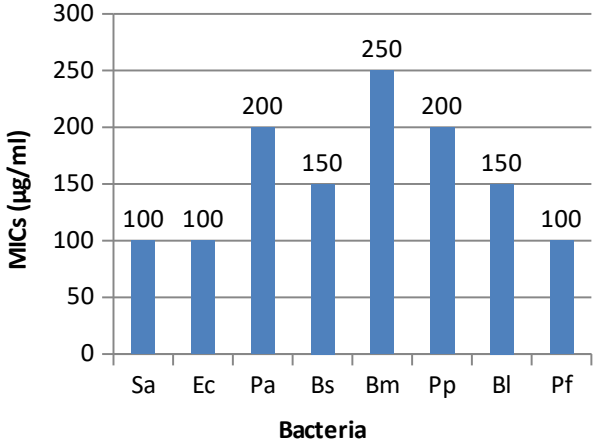

Fig. 1. Minimum inhibitory concentration (MIC) of methanol extract of *Combretum album* root.

Sa = *Staphylococcus aureus* MTCC 2940, ***Ec*** = *Escherichia coli* MTCC 739, ***Pa*** = *Pseudomonas aeruginosa* MTCC 2453, ***Bs*** = *Bacillus subtilis* MTCC 441, ***Bm***= *Bacillus mycoides* MTCC 7343, ***Bl*** = *Bacillus licheniformis* MTCC 530 ***Pp***= *Pseudomonas putida* MTCC 1654 , ***Pf***= *Pseudomonas fluorescens* MTCC 103.

Identification of active ingredients

FT-IR spectrum (Fig. 4) of methanol extract showed a number of absorption bands hinting at the presence of active functional groups in methanolic extract of *C. album* root (Table 4). The peak at 2944.77 cm^{-1} corresponds to O-H stretching vibrations of carboxylic acids. The peak at 1613.16 cm^{-1} and 1047.16 cm^{-1} indicate C-O stretching of hydroxyls (Fig. 4). Other functional groups and their respective peaks are also enlisted in Table 4.

From TLC analysis of MeCH a distinct band (spot) was detected in visible light with R_f value 0.157 and their fluorescence property was depicted in Fig. 3. Preliminary phytochemical assay of MeCH revealed the presence of flavonoids, tannins, terpenoids, alkaloids and saponins. FT-IR chromatogram of MeCH is depicted in Fig. 5 and

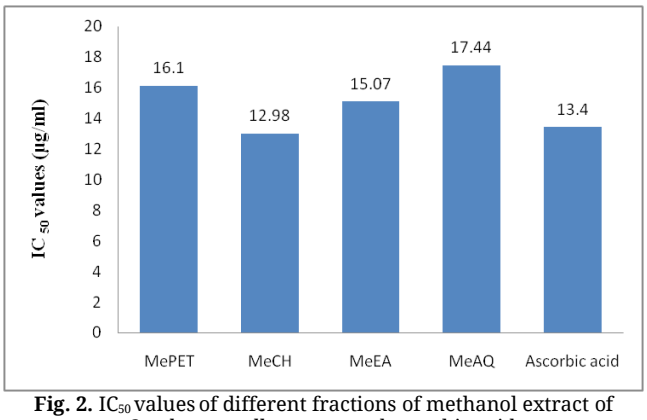

Fig. 2. IC_{50} values of different fractions of methanol extract of *Combretum album* root and ascorbic acid.

details of probable functional groups in the MeCH are mentioned in Table 5. The active spot in the TLC plate when analysed through GC-MS, distinct peaks of 3 different compounds that match with the available data on the system were identified in the chromatogram (Fig. 6) by comparing the peaks with existing NIST library of the system. Structures of the detected compounds e.g., N-[5-[4-pyridinyl)-1H-1,2,4-triazol-3-yl]-benzamide (Compound 1), pyruvic acid (Compound 2) and methyloacetone (Compound 3) are depicted in Fig. 6a, 6b and 6c respectively.

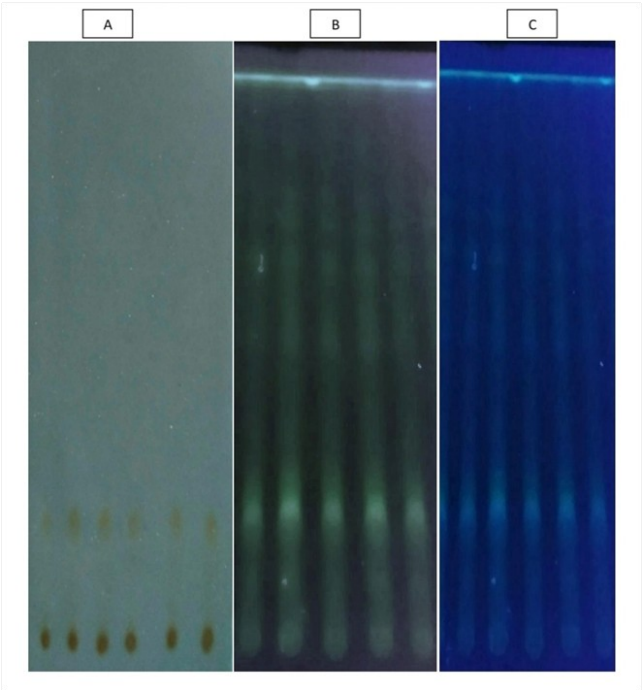

Fig. 3. Visibility of effective spots on TLC plates (R_f value = 0.157) in naked eye (A), in the short wavelengths of UV light at 254 (B) and long wavelengths of UV light at 365 nm.

A perusal of the literature reveals that plants produce many chemicals that have antimicrobial and antioxidant potencies (43–45). However, it is difficult to compare our results with the documented data in literature due to several factors like choice of solvent, seasonal effect of phytochemicals and different testing methods (46). In the present work, presence of antioxidant and antibacterial compounds in MeCH is clearly evidenced by the results found in preliminary phytochemical analysis as well as functional groups

Table 2. Antioxidant activity obtained in DPPH assay of methanol extract of *Combretum album* root

Compounds	Concentration	Free radical-scavenging activity (% inhibition)	IC_{50} µg/ml
Methanolic Extracts of *Combretum album* root	50 µg/ml	19.35± 0.187	136.08
	100 µg/ml	32.82 ± 0.234	
	150 µg/ml	67.17± 0.234	
	200 µg/ml	71.55 ± 0.045	
	250 µg/ml	82.35 ± 0.216	

Table 3. Minimum Inhibitory Concentrations (MICs) of different solvent fractions of methanol extract of *Combretum album* root [(-) = No visible growth in test tubes; (+) = visible growth in test tubes]

Name of Bacteria		Concentration (in µg/ml)									
		50	**40**	**30**	**20**	**10**	**5**	**2.5**	**1.25**	**0.625**	**0.3125**
Staphylococcus aureus (MTCC 2940)	MePET	-	-	-	+	+	+	+	+	+	+
	MeCH	-	-	-	-	-	+	+	+	+	+
	MeEA	-	-	-	-	+	+	+	+	+	+
	MeAQ	-	-	-	+	+	+	+	+	+	+
	Tetracycline	-	-	-	-	-	-	+	+	+	+
Escherichia coli (MTCC 739)	MePET	-	-	-	+	+	+	+	+	+	+
	MeCH	-	-	-	-	-	+	+	+	+	+
	MeEA	-	-	-	-	+	+	+	+	+	+
	MeAQ	-	-	-	+	+	+	+	+	+	+
	Tetracycline	-	-	-	-	-	-	+	+	+	+
*Bacillus subtilis (*MTCC 441)	MePET	-	-	-	+	+	+	+	+	+	+
	MeCH	-	-	-	-	-	-	+	+	+	+
	MeEA	-	-	-	+	+	+	+	+	+	+
	MeAQ	-	-	-	+	+	+	+	+	+	+
	Tetracycline	-	-	-	-	-	-	+	+	+	+
Pseudomonas aeruginosa (MTCC 2453)	MePET	-	-	-	+	+	+	+	+	+	+
	MeCH	-	-	-	-	+	+	+	+	+	+
	MeEA	-	-	-	-	+	+	+	+	+	+
	MeAQ	-	-	-	+	+	+	+	+	+	+
	Tetracycline	-	-	-	-	-	-	+	+	+	+
Bacillus licheniformis (MTCC 530)	MePET	-	-	-	-	-	+	+	+	+	+
	MeCH	-	-	-	+	+	+	+	+	+	+
	MeEA	-	-	-	-	-	+	+	+	+	+
	MeAQ	-	-	-	+	+	+	+	+	+	+
	Tetracycline	-	-	-	-	-	-	+	+	+	+
Bacillus mycoides (MTCC 7343)	MePET	-	-	-	-	-	-	+	+	+	+
	MeCH	-	-	-	+	+	+	+	+	+	+
	MeEA	-	-	-	-	+	+	+	+	+	+
	MeAQ	-	-	-	+	+	+	+	+	+	+
	Tetracycline	-	-	-	-	-	-	+	+	+	+
Pseudomonas putida (MTCC 1654)	MePET	-	-	-	+	+	+	+	+	+	+
	MeCH	-	-	-	-	+	+	+	+	+	+
	MeEA	-	-	-	+	+	+	+	+	+	+
	MeAQ	-	-	-	+	+	+	+	+	+	+
	Tetracycline	-	-	-	-	-	-	+	+	+	+
Pseudomonas fluorescens (MTCC 103)	MePET	-	-	-	+	+	+	+	+	+	+
	MeCH	-	-	-	-	+	+	+	+	+	+
	MeEA	-	-	-	+	+	+	+	+	+	+
	MeAQ	-	-	-	+	+	+	+	+	+	+
	Tetracycline	-	-	-	-	-	-	+	+	+	+

detected in FT-IR analysis and three bioactive compounds were tentatively identified in GC-MS analysis viz. N-[5-[4-pyridinyl)-1H-1,2,4-triazol-3-yl]-benzamide (Compound 1), pyruvic acid (Compound 2) and methyloacetone (Compound 3).

There are previous reports (47, 48) describing the antibacterial efficacy of the benzamide derivatives whereas one report described the benzamide derivatives as promising antioxidant agents (49). According to one report (46), benzamide

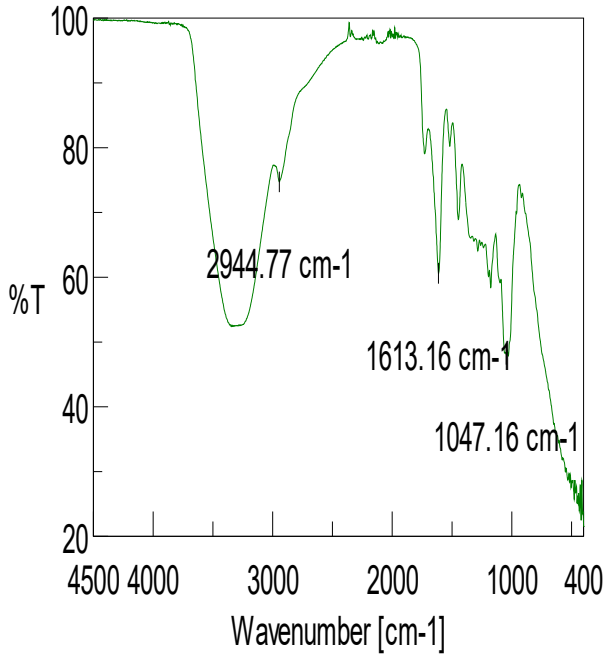

Fig. 4. FT-IR spectrum analysis of methanolic root extract of *Combretum album.*

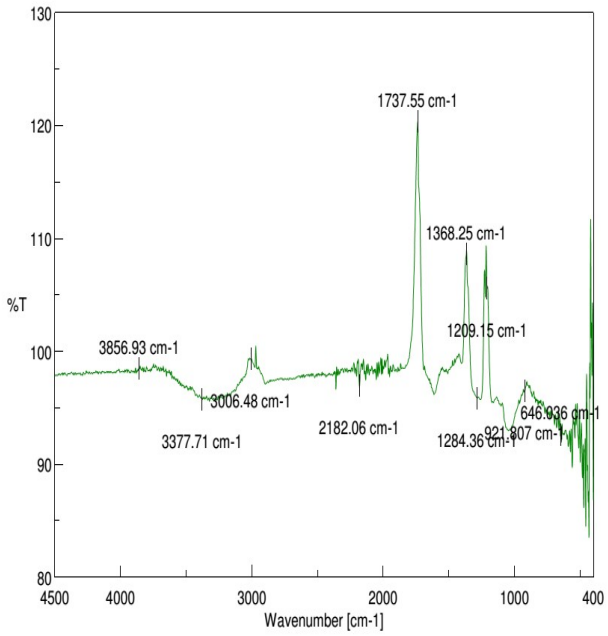

Fig. 5. FT-IR spectrum analysis of active principle in MeCH detected in TLC plates.

Table 4. Functional group analysis of methanolic root extract of *Combretum album* by using FT-IR spectroscopy

Sl. No.	Sample Peak values (cm^{-1})	Reference Peak values (cm^{-1}) (50-56)	Bond	Classification of functional groups	Functional group detected
1.	1740-1720	1740-1725	C=O stretching	Aldehydes	R-CHO
2.	3100-2900	2500-3300	O-H stretching	Carbo-Acids	C-CX-COOH
3.	1075-1000	1080	C-O stretching	Alcohols	$R-CH_2-OH$
4.	1680-1600	1590-1570	C=N stretching	Amidines	N=CH-N
5.	1650-1590	1650-1590	NH_2 deformation may cause overtone around 3200 cm-1	Amines	$(R)_3\,C-NH_2$
6.	1625-1590	1615-1580	Ring stretching	Aromatics	1,3,5-trisubstituted
7.	1300-1000	1120-1100	C-O-C stretching	Esters	COO
8.	1180-1160	1225-950	CH bending	Aromatics	1,3,5-trisubstituted
9.	1210-1140	1425-1250	CN stretching amide III	Imides	5-membered ring Imide

Table 5. Functional group analysis of active principle in MeCH detected in TLC plates by using FT-IR spectroscopy

Sl. No.	Sample Peak values (cm^{-1})	Reference Peak values (cm^{-1}) (57-64)	Bond	Classification of functional groups	Functional group detected
1.	3400-3200	3354.08	Stretching, hydrogen bonded broad peak	Alcohols	$R-CH_2-OH$
2.	1650-1590	1580-1490	NH deformation	Amines	$CH_2-NH-CH_2$
3.	1715-1690	1700	C=O, stretching	Carbo-Acids	COOH
4.	1285-1270	1376	Symmetric stretching	Nitrates	$R-O-NO_2$
5.	1700-1600	1607.30-1636	C=N, stretching	Oximes	C=N-OH
6.	1745-1725	1750-1700	C=O, stretching	Ketones	C-(C=O)-C=C-OH;C=O
7.	920-880	920-820	-	Alkanes	Cyclohexyl
8.	1750-1650	1700-1500	C=O stretching	Amides	R-CO-NH-CO-NHR
9.	3079-3010	3130-3070	OH, stretching	Aromatics	1,2,3-trisubstituted

derivatives showed antibacterial potency at concentration of 50 mg/ml which was higher than the concentration used in our study. So, probably the other two compounds, Pyruvic acid and Methyloacetone present in the active band in the TLC plates are enhancing the efficacy of N-[5-[4-pyridinyl)-1H-1,2,4-triazol-3-yl]-benzamide as antibacterial agent.

Thus, we can conclude that the presence of N-[5-[4-pyridinyl)-1H-1,2,4-triazol-3-yl]-Benzamide, as detected in GC-MS analysis of active spot in TLC plates (Rf value = 0.157), may be the reason for the antibacterial and antioxidant efficacies of the root methanol extract of *C. album*. This study has showed how isolated bioactive compounds from a single plant can show potent antimicrobial activity against eight different strains of pathogenic bacteria

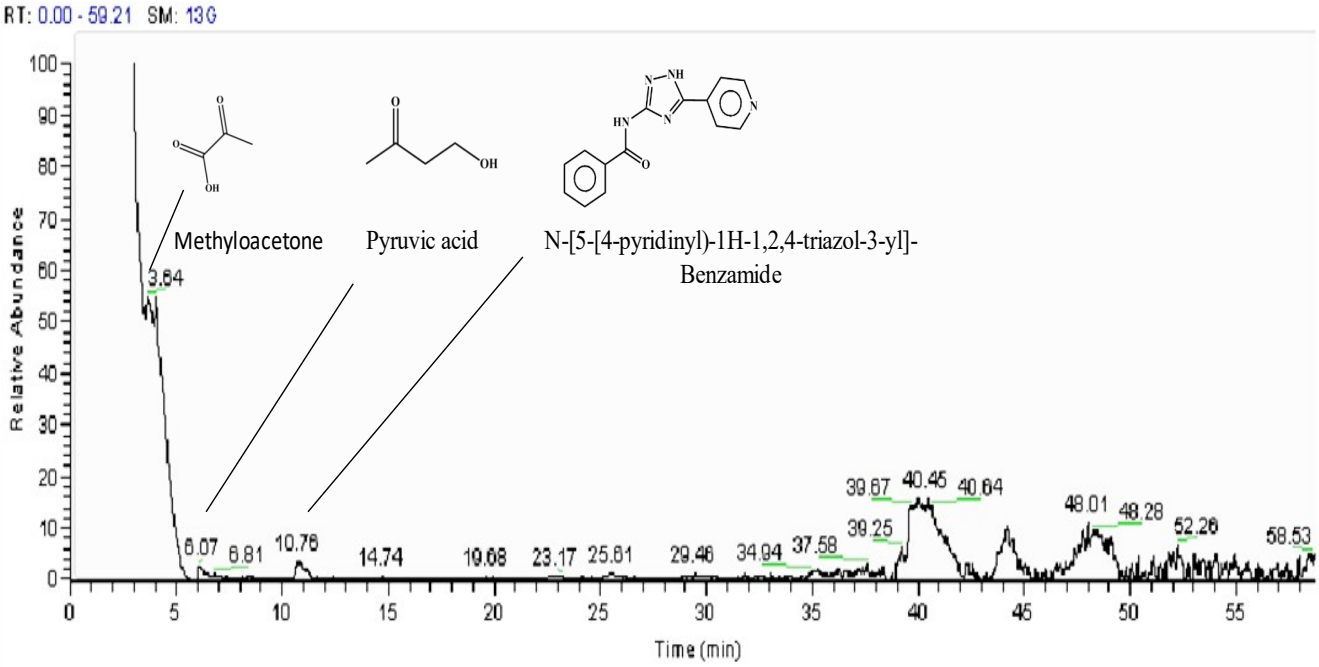

Fig. 6. Total chromatogram in GC-MS analysis of active principle in MeCH detected in TLC plates.

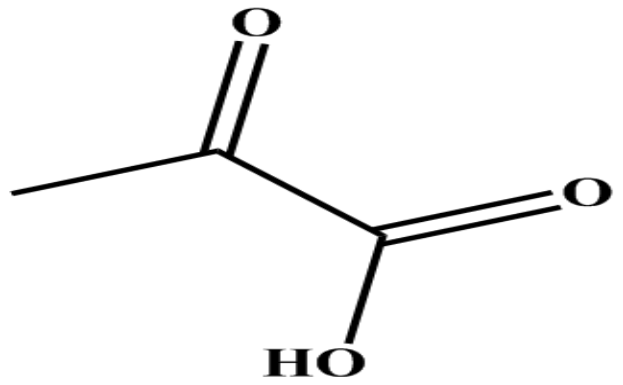

Fig. 6a. Pyruvic acid.

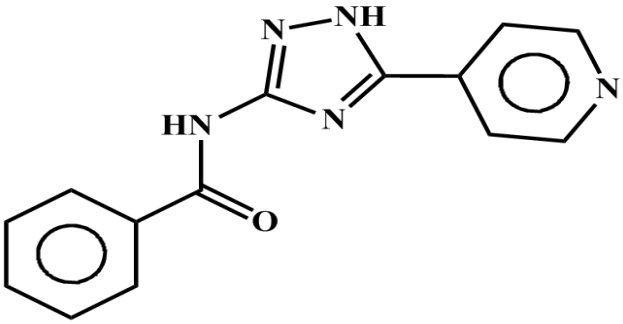

Fig. 6b. N-[5-[4-pyridinyl)-1H-1,2,4-triazol-3-yl]-Benzamide.

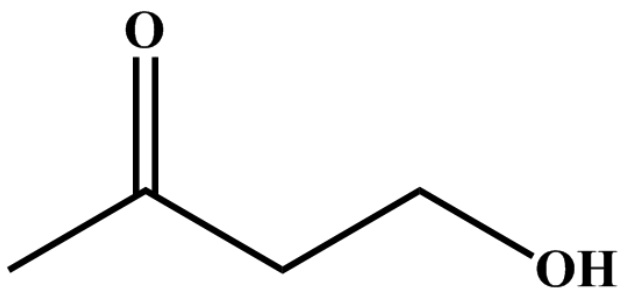

Fig. 6c. Methyloacetone.

including both Gram negative and Gram positive strains besides having promising antioxidant efficacy. This study can be a guideline for further investigations on the biological activities of *C. album.*

Conclusion

To the best of our knowledge, this is the first ever report citing the antibacterial and antioxidant activities of methanolic extract of *C. album* Pers. root. In conclusion, the obtained results from the present investigation could form a reliable foundation for the selection of this particular plant for further investigation on its new bioactive principles.

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Authors' contributions

SB conducted the experiments, analysed the data statistically and prepared the first draft of the manuscript. GC designed and supervised the work and revised the manuscript. Both authors approved the manuscript for publication.

Conflict of interests

Authors declare no competing interests.

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