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(RESEARCH ARTICLE)

Chemo-profiling and assessment of antioxidant activity and antibacterial potentials of selected plants of family Combretaceae

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Abstract

Traditional medicine is used all over the world, especially in developing countries. Though synthetic drugs are very effective but are harmful to human beings because of their side effects. Genus of *Terminalia* and *Combretum* species belongs to the family Combretaceae, which contains 20 genera and 600 species as herbs, shrubs, and trees. These plants are used in different traditional medicine for their antioxidant property and also for the treatment of hepatitis and malaria due to the presence of antibacterial properties. The present study is focused on chemo- profiling, screening of the antioxidant compound, antioxidant activities and antimicrobial properties of different *Terminalia* and *Combretum* species, namely *Terminalia arjuna*, *T. alata*, *T. bellerica, T. T. cataappa, T. chebulla, T. bucera* and *T. mauritiana*,, *Combretum albidum*, *C. roxburghii* and *C. indicum*. Four different concentrations (20, 40, 60 and 80 mg/ml) of methanolic extracts of leave and bark of these plant species were tested against six bacterial pathogens i.e *Streptococcus epidermis*, *Salmonella enterica*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtillis* and *Pseudomonas aeruginosa* using disc diffusion method. It was observed that the methanolic leaf and bark extract *T. arjuna, T. chebulla*, and *C. albidum* proved remarkable antibacterial properties against the different bacterial strains. This study will help to know the properties of medicinal plants for making different formulation of different types of green medicine.

Keywords: Antibacterial assay; Antioxidant; Chemo-profiling; *Combretum* species. HPTLC; Terminalia spp.

1. Introduction

The family Combretaceae comprises 20 genera with 600 species of which the genus *Combretum* and *Terminalia* contain 370 and 200 species, respectively. The genus *Combretum* is used in folk medicine for the treatment of various diseases such as stomach pain and diarrhoea [1]. Medicinal plants have been used since ancient times in virtually all cultures as a source of medicines [2, 3, 4]. The family Combretaceae is widely distributed in tropical Africa, South America and Asia [5]. *Combretum* (Loefl.) has been used in the treatment of syphilis, abdominal pains, conjunctivitis, diarrhoea, toothache, and several other diseases. Several plant species of family Combretaceae have antimicrobial activities [6, 7, 8]. Compounds like flavonoids, phenanthrenes, stilbenes, cyclobutanes, and triterpenoids are present in the plants. Now-a-days, oxidative stress or excessive production of ROS is being implicated in many diseases such as cancer, aging, and diabetes. The potential targets for the ROS in cells are membrane lipids, DNA and proteins. External supplementation through antioxidants is recommended to protect cells from the deleterious effects of such oxidative stress conditions. Earlier studies reported the presence of secondary metabolites (combretastatins) in the bark of the tree *C. caffrum* [9] and which is active against colon, lung and leukemia cancers. Some species of the family Combretaceae are used in African traditional medicines for the treatment of a variety of diseases, including hepatitis and malaria. However, no reports are available so far on any of the Indian species of family Combretaceae used as anticancer drug. Cambretastins A-4 compound is an anticancer molecule, which has a potent target in cancer chemotherapy in cell division [10]. In

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Ethiopia, *Combretum paniculatum* is used for the treatment of ringworm and wounds [11]. The combretastatin is a family of stableness which act as anti-angiogenic agents, causing a vascular shutdown in tumor and resulting in tumour necrosis. A water-soluble analog, combretastatin A-4 phosphate (CA4), has shown promise in early clinical trials. This chemical has served as a model for the synthesis of a host of analogs containing the essential trimethoxy aryl moiety (Flavopiridol, Roscovitine) linked to substituted aromatic moieties through a variety of two or three atom bridges including heterocyclic rings and sulfonamides. This is an impressive display of the power of a relatively simple natural product structure to spawn a prolific output of medicinal and combinatorial chemistry. Keeping this in view, we have focused on the present investigation to identify the biologically active principle from the plants under family Combretaceae.

2. Material and methods

2.1. Collection of plant materials

The leaves and bark samples of *Terminalia arjuna, T. alata, T. bellerica, T. catappa, T. chebulla, T. bucera, T. mauritiana, Combretum albidum, C. roxburghii*, and *C. indicum* were collected from the different locations of Odisha. The herbarium of ten species under family combretaceae was presented in Fig.1. The leaves and barks were washed with deionized water, dried under shade for 48 hours. The dried leaves and barks were milled into a fine powder with the mechanical grinder and used for biochemical analysis.

Figure 1 Ten different plant species of genus Terminalia and Combretum under family Combretaceae

2.2. Extraction of plant samples

For solvent extraction, 10gm of dried leaf and bark powder of each plant sample were taken separately and placed in the Soxhlet extraction apparatus. Acetone (150 ml) was used as a solvent for extraction. Extraction was carried for 72 hours at the boiling point (65^0C) of the solvent. Then, the solvent was removed from crude extract at a reduced pressure with the help of a rotary vacuum evaporator to yield a viscous dark green residue and the extract was stored in 4ºC until further use. The fixed quantity of residue was dissolved in methanol and used for TLC & HPTLC experiment.

2.3. Chromatography

A standard of Combretastatin (A-4) (Sigma, USA) was used for the experiment. The standard solution of Combretastatin (A-4) (1mg/ml) was prepared in methanol. The Silica gel 60 F254 aluminium plates (Merck, India) was used as a stationary phase. Ethyl acetate: methanol: water in the ratio of 40:5.4:4 was prepared and used as a mobile phase. All extracts were dissolved in methanol to have a final concentration of 1mg/ml each. Chromatography was performed on aluminium plate pre-coated with silica gel (60 F254 20x10 cm) [12,13].. Samples and standards were loaded on the plate as 6 mm wide bands with a CamagLinomat-V automatic TLC applicator positioned 15 mm from the lower edge of the plate and 20 mm from the side of the plate. The application parameters were identical for all the analyses performed. The plates were developed with the help of a mobile phase containing Ethyl acetate: methanol: water in the ratio (40:5.4:4 v/v/v) under saturated condition (25–30º C and 40–50 % relative humidity). After completion of the separation of compounds, the plate was air-dried and then vanillin spray solution (0.1 g vanillin, 28 ml methanol and 1 ml sulphuric acid) was uniformly sprayed over the chromatogram. Then the plate was heated at 105ºC for the development of bands. Spots corresponding to the standard were scanned at 366 nm and Rf values were recorded with Camag Scanner–III in conjunction with win CATSV1.2.3 software. The quantity of Combretastatin (A4) present in each extract was calculated by comparing the peak area of the reference standard and respective samples.

2.4. Qualitative analysis of antioxidant activity

To detect antioxidant activity, a qualitative 2, 2 diphenyl-1-picrylhydrazyl (DPPH) assays was carried out. Qualitative screening of the constituents in each of the samples for antioxidant activity was done by TLC analysis. About 6µl of each sample was loaded on the TLC sheet and the chromatogram was developed in a solvent system, Ethyl acetate: methanol: water in the ratio (40:5.4:4 v/v/v). After that, the plates were first air-dried and then the chromatograms were sprayed with 0.2% 2, 2 diphenyl-1-picrylhydrazyl (DPPH) in methanol as an indicator [14]. The presence of antioxidant compounds was detected by yellow spots against a purple background on the TLC plates sprayed with 0.2 % DPPH in methanol.

2.5. Estimation of antioxidant enzymes

Nature has endowed each cell with adequate protective mechanisms against any harmful effects of free radicals i.e. superoxide dismutase (SOD), Catalase (CAT), Guaiacol peroxidase (GP). The protein content of plants understudy was estimated by the Lowry method [15]. Antioxidants are substances that neutralize free radicals or their actions.

2.6. Estimation of superoxide dismutase (SOD)

Protein samples were taken out from liquid nitrogen for thawing and kept them on ice. Then, pre-warmed of all reagents except protein samples necessary for the SOD mixture. After that prepared blank test sample mixture without protein and buffer-A. Also prepared the first test samples, the mixture contains all components except H2O2. Reading was taken at 560 nm. The calculation of SOD activity as = SOD % Inhibition = Control OD – Treatment OD / Control X 100 [16].

2.7. Estimation of catalase (CAT)

Protein samples were removed from liquid nitrogen for thawing and kept them on ice. Then pre-warmed all reagents except protein samples necessary for the catalase reaction then prepared a blank test sample without protein sample buffer A only. After that prepared mixture for the first test sample, the mixture contains all components except the protein sample. The sample was taken in a spectrophotometer cuvette and added the necessary amount of the protein sample and quickly mixed and measured absorbance against the blank test sample at 240 nm in 0 min and 3 min. Calculation of CAT activity (mM of H_2O_2 /min mg of protein). Catalase activity = (A sample 3 – A sample 0) / (ε. d. t. c)ε = 39.4 M-1cm-1 (H_2O_2 extinction coefficient) [17].

2.8. Estimation of guaiacol peroxidase (GP)

One gram of fresh leaf of each plant sample was taken in 3 ml of 0.1M phosphate buffer for grinding in pre-chilled sterile mortar and pestle. After that ground leaf samples were centrifuged at 18,000 rpm at 5ºC for 15 minutes. Then the supernatant was collected as an enzyme source. After collection of supernatant in the eppendorf tube stored in the ice till the assay was carried out. Then 3 ml of buffer solution, 0.5ml guaiacol solution, 0.1 ml enzyme extract and 0.03 ml H_2O_2 was added in a cuvette and properly mixed for each sample separately. After that cuvette was placed in spectrophotometer and reading was taken at 436 nm and noted time required in minutes to increase the absorbance by 0.1. Activity of Peroxidase = µ mols/min/mg.protein = Δ OD / ΔT X 1/C X mg protein/10.

2.9. Antibacterial activity of plant crude extract

Six bacterial strains viz. *Streptococcus epidermis*, *Pseudomonas aeruginosa*, *Escherichia coli, Salmonella enterica*, *Staphylococcus aureus*, and *Bacillus subtillis* were used in antibacterial potentiality test. These organisms were procured from the microbial type culture collection and gene bank, Institute of Microbial Technology, Chandigarh. From pure culture of each bacterial strain were transferred to Luria Britani liquid medium. Forty-eight hours of culture was taken for the test of the antibacterial assay using the disk diffusion technique. The discs (6 mm in diameter) were impregnated with 20 mg/ml, 40 mg/ml, 60 mg/ml, 80 mg/ml leaf and bark extracts of ten species and the discs were placed on seeded agar of each organism. The test plates were kept at 37ºC in an incubator for 24 hours. The test plates were inspected visually to determine the growth of organisms and the diameter of zone of inhibition was measured in mm. The experiment was performed in triplicate and the average zone of inhibition.

3. Results and discussion

3.1. Analysis of compound through TLC and HPTLC

Combretastatin was identified from all of the leaf and bark extracts of tested plants through chromatograms after performing TLC (Figs. 2 & 3). The HPTLC profile was developed by using the solvent system and individual peak scanned at a short wavelength of 366 nm to measure the retention value (Rf) and area unit (AU). The Rf value indicates the ratio of the distance moved by the solute (i.e. active constituents under test) and the distance moved by the solvent along with the stationary phase (i.e. HPTLC plate). The HPTLC images (after derivatization) have shown in Figs 4 & 5 indicate that the reference standard (combretastatin) and sample constituents were clearly separated on silica gel 60 F254 TLC plates. The constituent of the sample extracts was identified by comparison of bands in the sample with reference substances on the same plate. The identification of combretastatin in the leaf and bark extract was confirmed by UV–visible spectra of the extract with that of the standard within the same Rf value. The Rf values of the bands for a reference standard (combretastatin) were 7.0 and of the sample containing Combretastatins Rf value varies from 7.0-7.6. The phytochemical constituents of the twenty sample extracts were recognized by comparison of bands in the sample with reference to standard on the same HPTLC plate (Figs.3 & 5). Combretastatin was found to be the major compound in all leaf and bark extracts of all the species of the family Combretaceae under study. The leaves of ten species of Combretaceae family exhibited a higher quantity of combretastatin than the bark samples of the same species under the family Combretaceae. The maximum content of combretastatin was recorded in the leaf extract of *C.albidum* (18565.6 AU). The maximum content of combretastatin content in the bark extract of *Terminaliaarjuna* (18266.4 AU).

3.2. Enzymatic activity and antibacterial assay

The presence of antioxidant enzyme activities was detected in the plant extracts using a qualitative 2, 2 diphenyl-1-picrylhydrazyl (DPPH) assay which gives positive results in each of the experimental plants extract (Fig.6). Antioxidant activity of methanol and ethanol extract of all the parts *T.arjuna* and *T. bellerica* plants. The phenolic content was also high in methanolic extracts of *T. bellerica* leaves. DPPH free radical scavenging activity was studied by computation percentage inhibition of methanolic and ethanolic extracts in *Terminalia* species, the maximum percentage inhibition value was observed in methanolic extract of T.arjuna stem as well as bark [8]. SOD activity was reported good response in *T. arjuna*, *T. bellerica*, *T. catappa*, *T. chebulla*, *T. bucera*, *Combretum albidum, C. roxburhii* and *C. indicum* and less response in *T. alata* and *T. mauritiana*. *Combretum albidum* (61.013% Inhibition of H_2O_2) was found with the best SOD activity (Fig.7). Catalase activity of ten plant species from family Combretaceae during the experiment was reported good response in *T. catappa* (1.8693mM of H_2O_2/min mg of protein) and *T. chebulla* (1.638 mM of H_2O_2/min mg of protein) more remarkable than rest of the plant under study (Fig.8). Guaiacol peroxidase activity of ten plant species from family Combretaceae showed variability. *C. albidum* was found to be the maximum activity (i.e. 0.1498 µmols/min/mg of protein) during the enzyme assay as compared to other plant species (Figure.9). *Terminalia* species have been reported to have good antioxidant, anticancer, antidiabetic, antiseptic, cardiotonic and anti-inflammatory effects have been established [18].

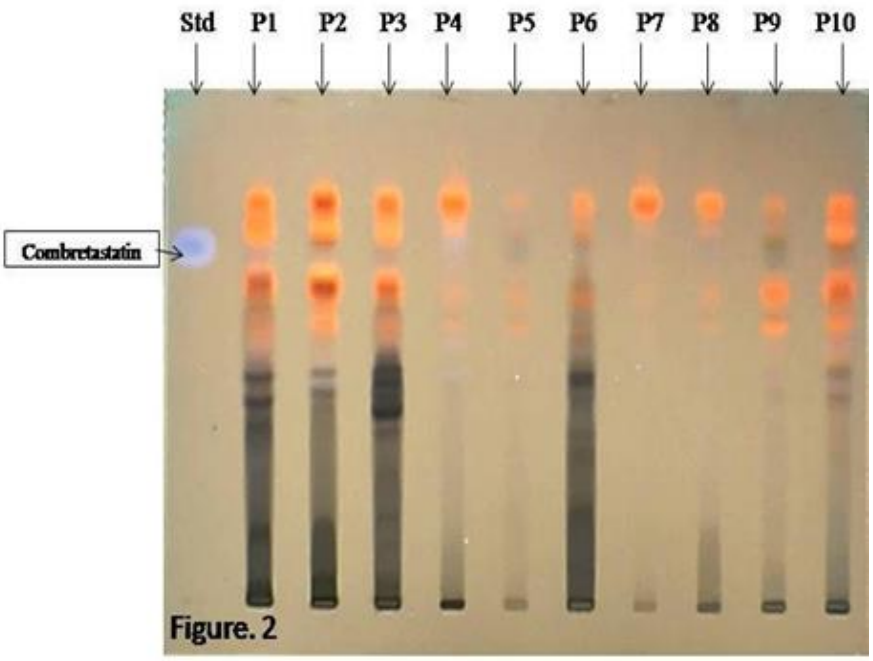

Figure 2 TLC profile of leaf extracts of 10 plant species under family Combretaceae . Standard ¬- Combretastatin, P1-*T. arjuna*, P2-*T. alata*, P3-*T. bellerica* ,*T. T. cataappa*, P5-*T. chebulla*, P6 - *T. bucera*, P7-*Terminalia mauritiana*, P8-*C. albidum*, P9-*C. roxburghii* and P10- *C. indicum*

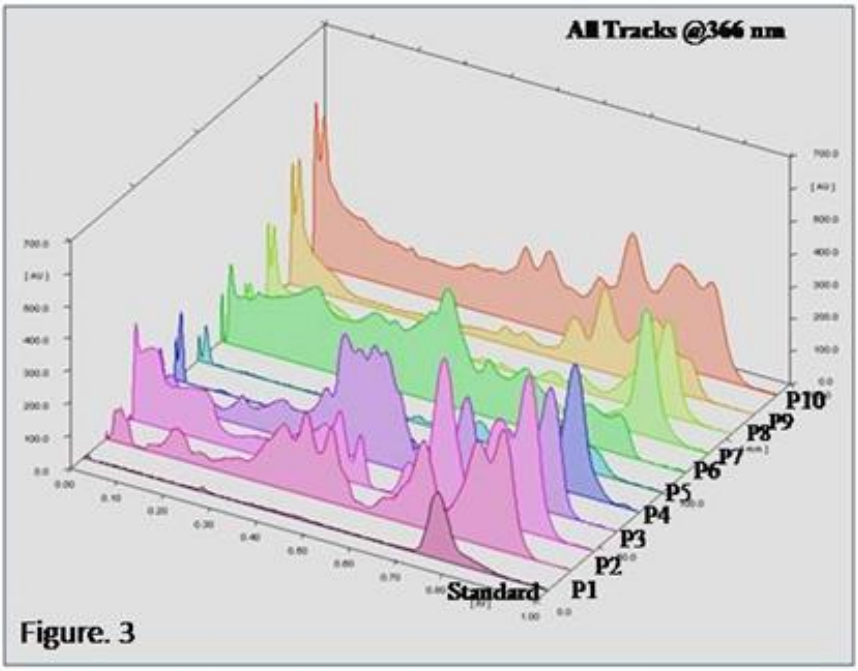

Figure 3 HPTLC profile of leaf extracts; of 10 plant species under family Combretaceae. Standard ¬- Combretastatin, P1-*T. arjuna*, P2-*T. alata*, P3-*T. bellerica* ,*T. cataappa*, P5-*T. chebulla*, P6 - *T. bucera*, P7-*Terminalia mauritiana*, P8-*C. albidum*, P9-*C. roxburghii* and P10- *C. indicum*

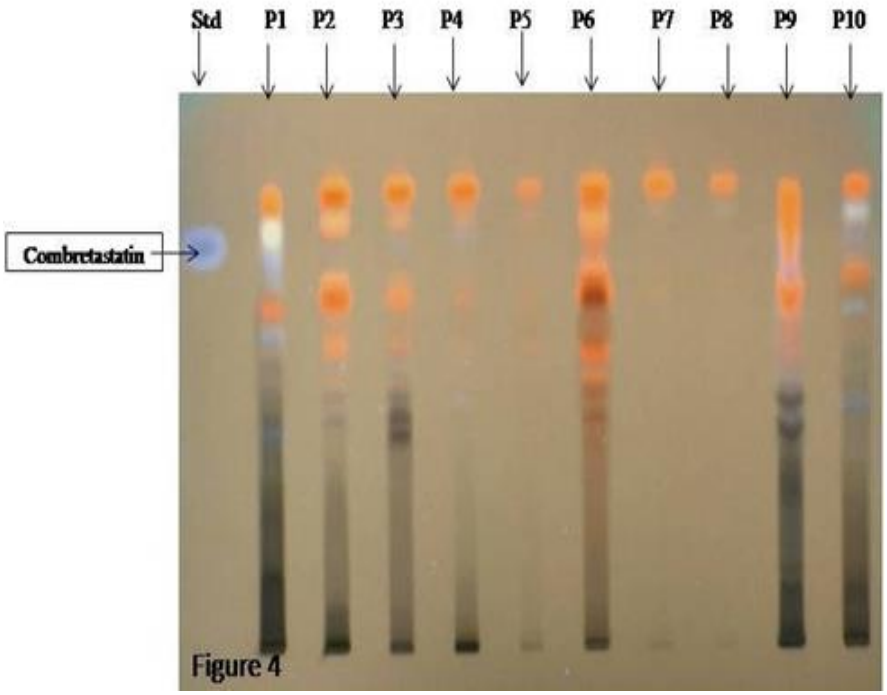

Figure 4 TLC profile of bark extracts of 10 plant species under family Combretaceae. Standard ¬- Combretastatin, P1-*T. arjuna*, P2-*T. alata*, P3-*T. bellerica* ,*T. cataappa*, P5-*T. chebulla*, P6 - *T. bucera*, P7-*Terminalia mauritiana*, P8-*C. albidum*, P9-*C. roxburghii* and P10- *C. indicum*

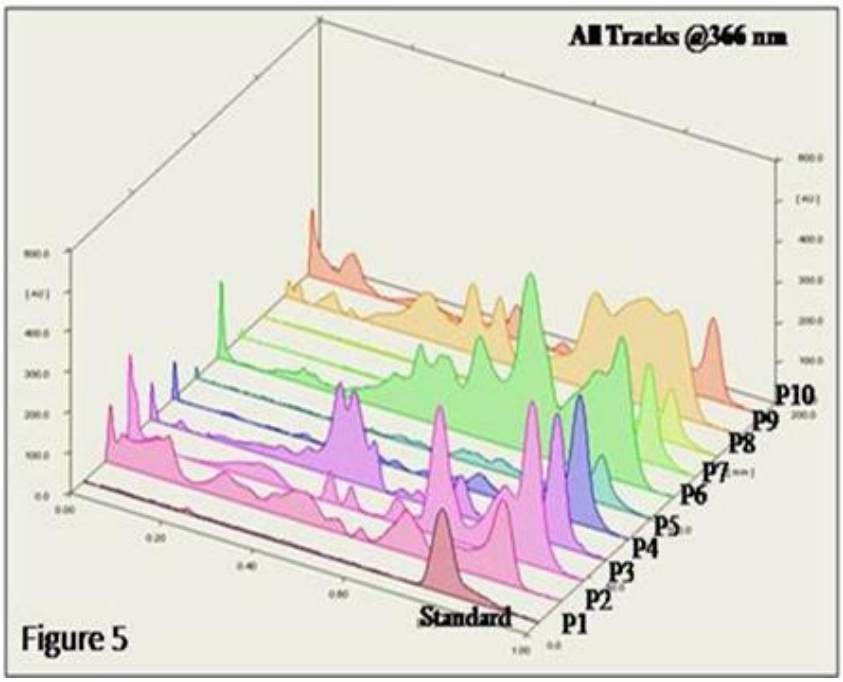

Figure 5 HPTLC profile of bark extracts of 10 plant species under family Combretaceae. Standard ¬- Combretastatin, P1-*T. arjuna*, P2-*T. alata*, P3-*T. bellerica* ,*T. cataappa*, P5-*T. chebulla*, P6 - *T. bucera*, P7-*Terminalia mauritiana*, P8-*C. albidum*, P9-*C. roxburghii* and P10- *C. indicum*

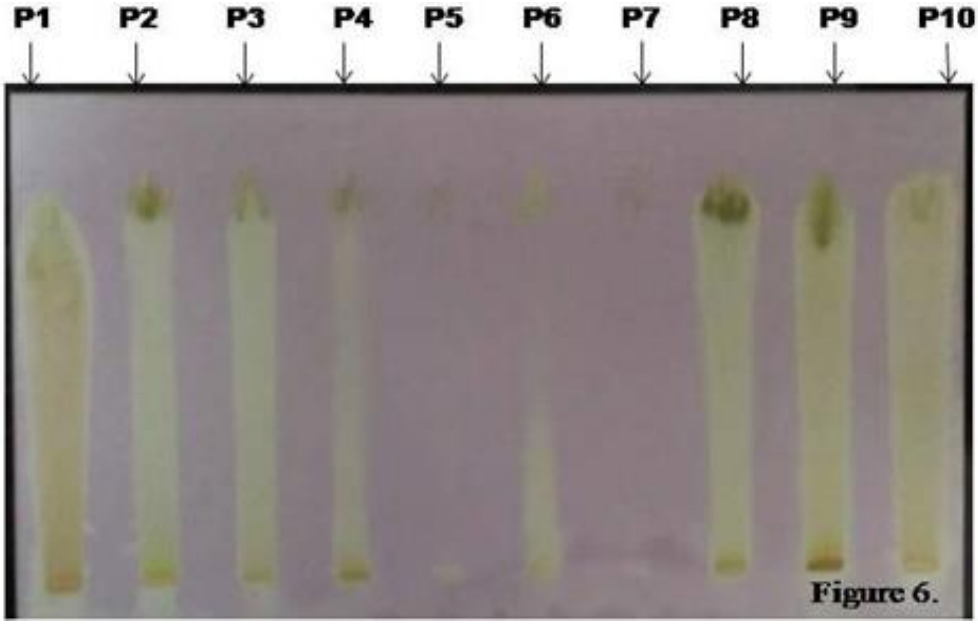

Figure 6 Qualitative analysis of antioxidant activity of leaf extracts of 10 species under family Combretaceae using DPPH method. P1-*Terminalia arjuna*, P2-*T. alata*, P3-*T. T. cataappa*, P5-*T. chebulla,* P6-*T. bucera*, P7-*T. mauritiana*, P8-Combretumalbidum, P9-*C. roxburghii* , P10- *C. indicum*

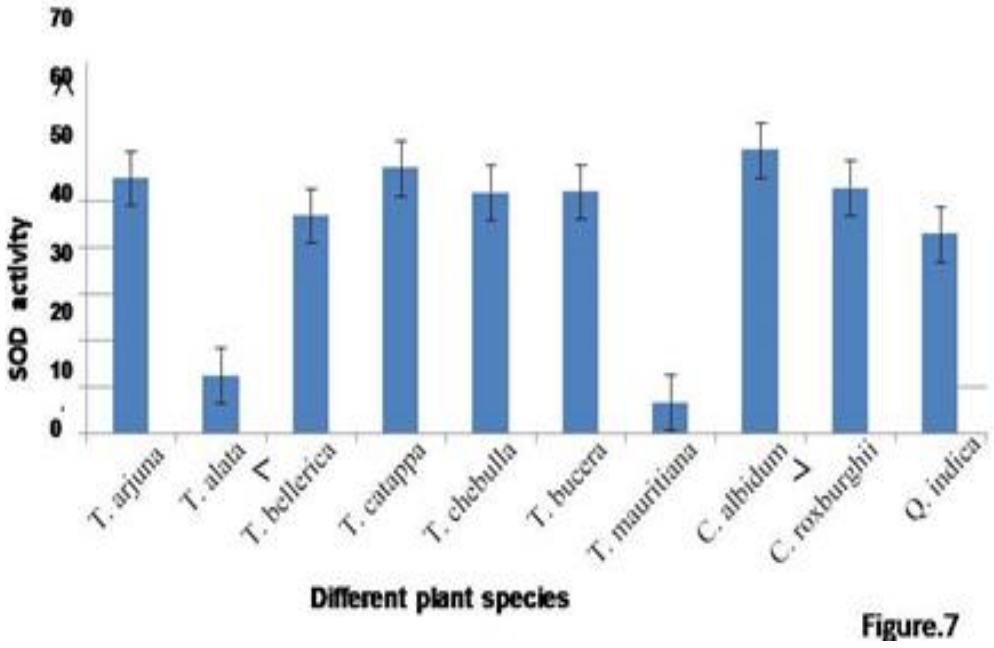

Figure 7 SOD activity of different plant species under family Combretaceae

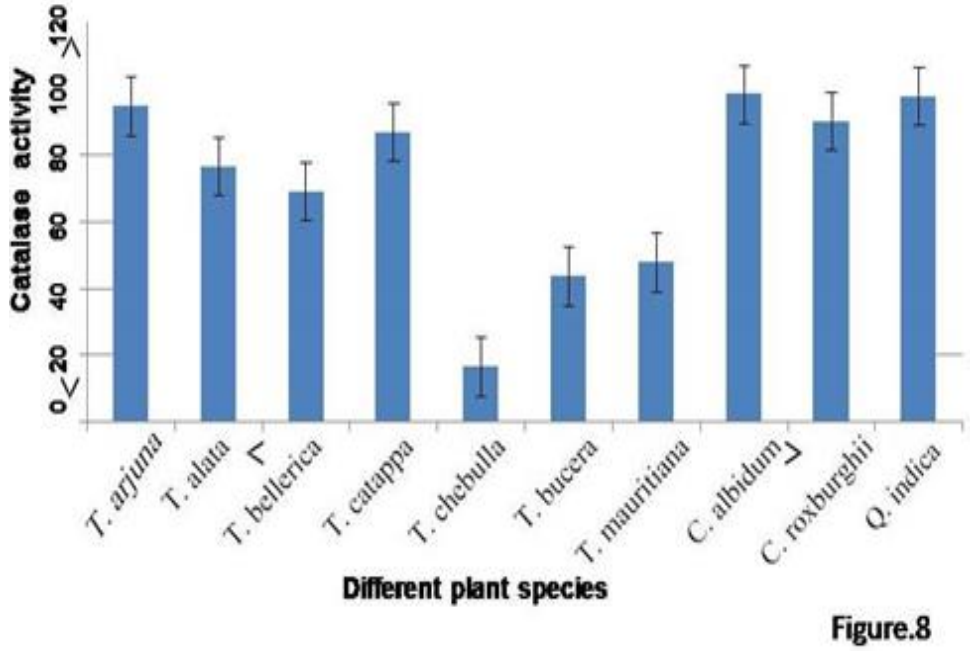

Figure 8 Catalase activity of 10 different plant species under family Combretaceae

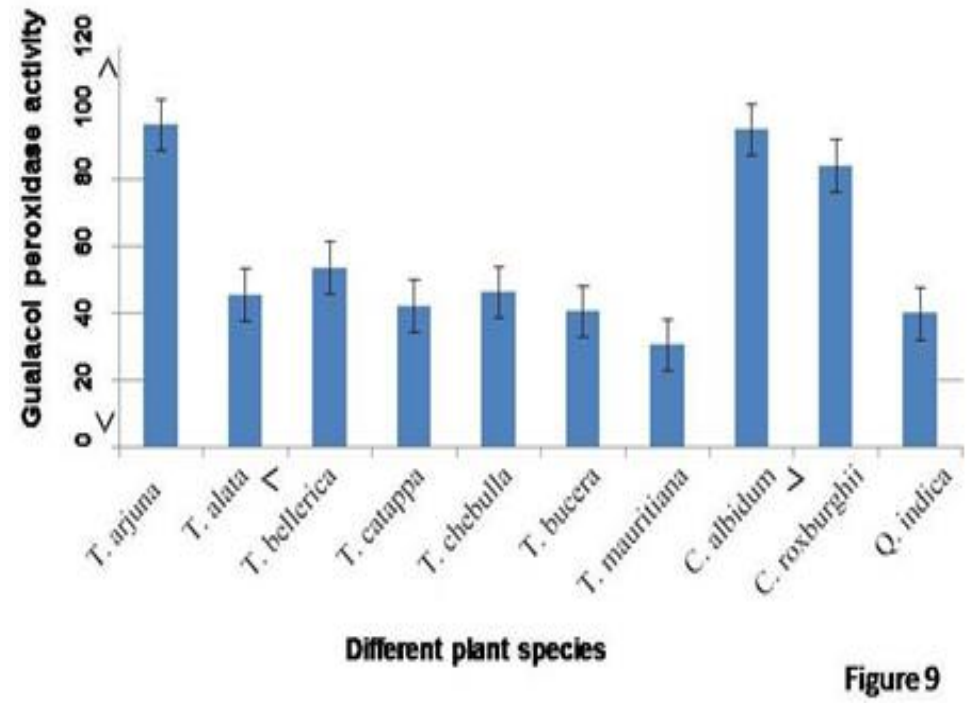

Figure 9 Guaiacol peroxidaseactivity of 10 different plant species under family Combretaceae

Antibacterial properties of ten different species such as *Terminali aarjuna, Terminalia alata, T. bellerica, T.T. cataappa, T. chebulla, T. bucera* and *T. mauritiana, Combretum albidum, C. roxburghii* , and *C.indicum* were tested under control condition. Four different concentrations (20, 40, 60 and 80 mg/ml) of methanolic extracts of leave and bark of these ten plant species were tested against six bacterial pathogens i.e.*Streptococcus epidermis, Salmonella enterica, Staphylococcus aureus, Escherichia coli, Bacillus subtillis* and *Pseudomonas aeruginosa* using disc diffusion method. Methanolic extract of *Terminalia arjuna* leaf and bark given best result against *Streptococcus epidermis* with a remarkable zone of inhibition i.e. 17.6 mm and 15.3 mm in 80 mg/ml concentration respectively and also against of

Escherichia coli . *T.arjuna* leaf and bark exhibited the best result with 21.2 mm and 16.4 mm zone of inhibition respectively. In the case of Pseudomonas aeruginosa, leaf and bark of *T.arjuna* showing remarkable good results with 17.5mm and 14.8mm respectively. Leaf and bark extract of *T. chebulla* showed the best result against Salmonella enterica showing 19.2 mm and 16.7 mm of the zone of inhibition respectively. *T. chebulla* leaf and bark extract showing the best result against *Staphylococcus aureus* having 22.6mm and 20.1mm of the zone of inhibition respectively. Leaf and bark extract of *Combretum* albidum showed the best result against *Bacillus subtillis* showing 22.4mm and 18.6mm respectively. Phenolic compounds from the fruit of *T.chebula* exhibited good antioxidant properties and remarkable recovery of balancing the nervous system as reported earlier [7,19]. *Terminalia*and *Combretum*species have various medicinal properties including antibacterial, anti-inflammatory and therapeutic potential as reported by various researchers [6, 19, 20].

4. Conclusion

In conclusion, the chemo-profiling study of the chemical constituents of the leaves and bark extract of ten different plant species under family Combretaceae namely *T.arjuna*, *T. alata*, *T. bellerica*, *T.T. cataappa*, *T. chebulla*, *T. bucera* and *T. mauritiana*, *Combretum albidum*, *C. roxburghii* and *C.indicum* showed the presence of an anticancer compound (Combretastatin A-4). For the detection of the presence of antioxidant activity in the plant extract, qualitative 2, 2 diphenyl-1-picrylhydrazyl (DPPH) assay showed a positive results. On the basis of the antibacterial study, it is observed that the pronounceable activity of *T.arjuna*, *T. chebulla* and *Combretum albidum* leaf and bark extract against the different bacterial strains.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no conflicts of interest.

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