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RESEARCH ARTICLE

PHARMACOLOGICAL EVALUATION OF CERIOPS DECANDRA (GRIFF.) DING HOU STEM EXTRACTS

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ABSTRACT

Healing potential of plants has been known for thousands of years. Medicinal use of plants and their products was passed down from generation to generation in various parts of the world throughout its history and has significantly contributed to the development of different traditional systems of medicine. Because of available antimicrobials failure to treat infectious diseases, many researchers have focused on the investigation of natural products as source of new bioactive molecules from mangrove plants. *Ceriops decandra* is an evergreen tree in the inner mangrove forests in Andhra Pradesh, India. It is used in traditional medicine to cure hepatitis and ulcers. In the present study, the work carried out is mainly focussed on phytochemical screening, antibacterial activity, MIC (Minimum inhibitory concentration) and MBC (Minimum Bactericidal Concentration) studies and finally DPPH (2,2-diphenyl-1-picryl hydrazyl) free radical scavenging activity. The crude extracts were prepared in different solvent extracts viz., hexane, benzene, chloroform, ethyl acetate, acetone and methanol. The results revealed that all most all the phytochemicals tested were positive in all the solvent extracts except soluble starch. Acetone extract showed more zone of inhibition against *Micrococcus luteus* and *Enterobacter aerogenes* than the positive control. *Rhodococcus rhodochrous* was inhibited by all the solvent extracts, but the positive control has not shown any action. The MIC and MBC studies of different solvent extracts exposed the antibacterial activity of *C. decandra* stem material, and chloroform and acetone extracts displayed better free radical scavenging activity at different concentrations. The results are showing the medicinal importance of the plant.

INTRODUCTION

Phytotherapy is the use of plants or plant extracts for medicinal purposes (especially plants that are not part of the normal diet). Many of the modern medicines are produced directly or indirectly from medicinal plants. The medicinal effects of plants are due to metabolites especially secondary compounds produced by plant. Mangrove plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, etc, which have been found in vitro to have antimicrobial properties. The public is becoming increasingly aware of the problems with the over prescription and misuse of traditional antibiotics. There is a need of development of new drugs against pathogenic organisms because of the resistance developed by the pathogens. Mangrove plants are the richest source of potent bioactive compounds as they exist under stressful conditions such as violent environments, high concentration of moisture, high and low tides of water, and abundant living microorganisms and insects. There are modifications or alterations in other physiological processes such as carbohydrate metabolism or polyphenol synthesis and due to these reasons; they may have chemical compounds, which protect them from these destructive elements. *Ceriops decandra* is an evergreen tree in the inner mangrove forests in Andhra Pradesh, India. It is a straight columnar tree, usually small to medium sized and it belongs to the family Rhizophoraceae and its vernacular name is “calhasu”. It is used in traditional medicine to cure hepatitis and ulcers. Considerable scientific evidence suggested that under situations of oxidative stress reactive oxygen species (ROS) such as superoxide, hydroxyl and peroxyl radicals are generated and the balance between antioxidation and oxidation is believed to be a critical concept for maintaining a healthy biological system (Davies, 2000). An antioxidant can be defined as ‘‘any substance that, when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate’’(Halliwell *et al*., 1999 and Prabhune *et al*., 2013). There is a need to develop natural antioxidants of plant origin because of the side effects of chemically synthesized antioxidants usage. The plants used in traditional medicine are still a large source of natural antioxidants that might serve as leads for the development of novel drugs. In the present study, made an attempt to find out the Phytochmical screening, antibacterial activity, Minimum

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inhibitory concentration (MIC) , Minimum bactericidal concentration (MBC) and antioxidant activity by DPPH(2,2-diphenyl-1-picryl hydrazyl) free radical scavenging activity.

MATERIALS AND METHODS

Preparation of the plant extracts

Ceriops decandra (Griff.) Ding Hou plant was collected from Coringa Mangrove forest, near Kakinada, Andhra Pradesh, India. Stem part of this plant was thoroughly washed and dried in shade. The dried plant material was made into a coarse powder by means of electrical grinder. The dried powdered plant material was extracted in different solvents viz., Hexane, Benzene Chloroform, Ethyl acetate, Acetone and Methanol. The resulted extracts were filtered and then concentrated on a roto evaporator for solvents elimination and the crude extracts were preserved in sterile, air tight containers for further analysis.

Phytochemical screening

Reagents used for the different phytochemical tests

The following reagents were prepared and tests were carried out according to standard protocols.

Mayer's reagent

Mercuric iodide of 1.36 gm was dissolved in 60 ml of water and mixed with a solution containing 5 gm of Potassium iodide in 20 ml of water.

Dragendroff's Reagent

Basic bismuth nitrate (1.7 gm) and tartaric acid (20 gm) were dissolved in 80 ml of water. This solution was mixed with a solution containing 16 gm potassium iodide and 40 ml water.

Fehling's solution- A

Copper sulphate of 34.64 gm was dissolved in a mixture of 0.50 ml of sulfuric acid and sufficient water and made up to 500 ml.

Fehling's solution- B

Sodium potassium tartarate of 176 gm and sodium hydroxide of 77 gm were dissolved in sufficient water and made up to 500 ml.

Benedicts Reagent

Cupric sulphate (1.73 gm), sodium citrate (1.73 gm) and anhydrous sodium carbonate (10 gm) were dissolved in water and the volume was made up to 100 ml.

Molisch Reagent

Pure α -naphthol of 2.5 gm was dissolved in 25 ml of ethanol.

Liebermann- Burchard Reagent

Acetic acid (5 ml) was carefully mixed under cooling with 5ml concentrated sulfuric acid. This mixture was added cautiously to 50 ml absolute ethanol with cooling.

The following qualitative tests were done to find out the presence or absence of phytochemical constituents like Carbohydrates, Tannins, Steroids, Saponins, Terpenoids, Soluble starch, Flavonoids and Alkaloids.

Test for flavonoids

Ferric chloride test

Two ml of the test solution was boiled with distilled water and filtered. Then, few drops of 10% ferric chloride solution were added to the 2 ml of filtrate. A greenish-blue or violet coloration indicates the presence of a phenolic hydroxyl group.

Shinoda's test

Five grams of each extract was dissolved in ethanol, warmed and then filtered. Small pieces of magnesium chips were then added to the filtrate followed by few drops of conc. HCl. The pink, orange, or red to purple coloration indicates the presence of flavonoids.

Sodium hydroxide test

Extract of 0.2 gm was dissolved in water and filtered. To this, 2 ml of the 10% aqueous sodium hydroxide was added to produce yellow coloration. A change in color from yellow to colorless on addition of dilute hydrochloric acid was the indication for the presence of flavonoids.

Leadacetate test

Extract o f 0.5 gm was dissolved in water and filtered. To the 5 ml of each filtrate, 3 ml of lead acetate solution was added. Appearance of a buff-colored precipitate indicates the presence of flavonoids.

Test for alkaloids

Five grams of crude powder was stirred with 1% aqueous HCl on water bath and then filtered. To the 1 ml filtrate, few drops of dragendroff's reagent was added. Orange- Red precipitate was taken as positive. To another 1 ml filtrate, few drops of Mayer's reagent was added and appearance of buff- colored precipitate will be taken as presence of alkaloids.

Test for soluble starch

Crude extract of 0.2 gm was boiled in 1 ml of 5% KOH, cooled and acidified with H_2SO_4. Yellow coloration indicates the presence of soluble starch.

Test for Saponins

Crude powder of 0.5 gm was shaken with water in a test tube and it warmed in a water bath. The persistent froth indicates the presence of saponins.

Test for terpenoids

Five grams of crude extract was dissolved in ethanol. To this, 1 ml of acetic acid was added followed by conc. H_2SO_4. A change in color from pink to violet confirms the presence of terpenoids.

Test for steroids

Salkowskii test

In 2 ml of chloroform, 0.2 gm of extract was dissolved and added the conc. H_2SO_4. The development of reddish brown color at inter phase indicates the presence of steroids.

Keller-Killiani test

To 0.5 ml of test solution, 2 ml of 3.5% $FeCl_3$, small amount of glacial acetic acid and 2 ml of conc. H_2SO_4 were added carefully. Appearance of reddish brown ring at inter phase is a positive indication for the presence of steroids.

Liebermann-Burchard test

To 0.2 gm of each extract, 2 ml of acetic acid was added and the solution was cooled well in ice followed by the addition of conc. H_2SO_4 carefully. Color development from violet to blue or bluish-green indicates the presence of a steroidal ring (i.e. aglycone portion of cardiac glycoside).

Test for carbohydrates

Molisch's test

Two ml of *Molisch*'s reagent was added to the extract dissolved in distilled water and 1 ml of conc. H_2SO_4 was dispensed along the walls of the test tube. The mixture was allowed to stand for two minutes and then diluted with 5 ml of distilled water. Formation of a dull violet color at the inter phase of the two layers indicates the positive test for carbohydrates.

Fehling's test (for free reducing sugars)

The crude extracts were treated with 5.0 ml of Fehling's solution (A & B) and kept in boiling water bath. The formation of yellow or red color precipitate indicates the presence of free reducing sugars.

Fehling's test (for Combined Reducing Sugars)

Extract of 0.5 gm was hydrolyzed by boiling with 5 ml of dilute hydrochloric acid and the resulting solution neutralized with sodium hydroxide solution. To this, few drops of Fehling's solution were added and then heated on a water bath for 2 minutes. Appearance of a reddish-brown precipitate of cuprous oxide indicates the presence of combined reducing sugars.

Barfoed's test *(for monosaccharide)*

In distilled water, 0.5 gm of the extract was dissolved and filtered. To 1 ml of the filtrate, 1 ml of Barfoed's reagent was added and then heated on a water bath for 2 minutes. Reddish precipitate of cuprous oxide formation is the positive test for the presence of monosaccharide.

Test for tannins

Crude extract of 0.5 gm was stirred with 10 ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2 ml of the filtrate. Occurrence of a blue-black, green or blue-green precipitate indicates the presence of tannins.

Borntrager's Test

Extract of 0.2 gm was shaken with 10 ml of benzene and then filtered. To the filtrate, 5 ml of 10% ammonia solution was added and then shaken the tube well. Appearance of pink, red or violet color in the ammonical (lower) phase indicates the presence of free anthraquinones.

Phlonatanins test

To 0.2gm of extract, 1% HCl solution was added. Formation of red precipitate indicates the presence of tannins.

Antibacterial activity of the plant extracts
Microorganisms used

The antibacterial activity of the crude extracts was determined by using both Gram positive and Gram negative bacteria. Nine Gram positive bacteria namely *Micrococcus luteus* MTCC 106, *Arthrobacte rprotophormiae* MTCC 2682, *Rhodococcus rhodochrous* MTCC 265, *Bacillus subtilis* MTCC 441, *Staphylococcus aureus* MTCC 737, *Bacillus megaterium* MTCC 428, *Enterococcus faecalis* MTCC 439, *Streptococcus mutans* MTCC 497 and *Lactobacillus acidophilus* MTCC 10307. Six Gram negative bacteria viz., *Alcaligens faecalis* MTCC 126,

Salmonella enterica MTCC 3858, *Proteus vulgaris* MTCC 426, *Proteus mirabilis* MTCC 425, *Pseudomonas aeruginosa* MTCC 1688 and *Enterobacter aerogenes* MTCC 10208.

Antibacterial screening by agar well diffusion method

Antibacterial screening was determined by agar well diffusion method (Umamaheswara Rao and Nagababu, 2014). Bacterial suspensions of different bacteria were prepared by using 24 hours old bacterial cultures and were cultivated (100 µl) on agar medium. After solidification, 6mm diameter wells were punched in agar plate with a sterile cork borer. Streptomycin standard antibiotic was used as positive control in the concentration of 10 µg/ml DMSO. A minute quantity of sterile agar suspension was added to the well and 100 µl of the sample, which was prepared by dissolving 100 mg of sample in 1 ml of DMSO, was added to each well. In a separate well, DMSO was also dispensed to maintain the control. The plates were incubated at 37^o C for 24 hrs. After incubation, diameter of the zone of inhibition was measured. For each sample and bacterial species, triplicates were maintained.

Determination of MIC and MBC

Minimum inhibitory concentration (MIC) was determined by using broth dilution method. minimum Inhibitory Concentration and minimum Bactericidal Concentration (MBC) were determined at different concentrations viz., 12.5 mg/ml, 25 mg/ml, 50 mg/ml, 75 mg/ml and 100 mg/ml on those bacterial strains which showed zones of inhibition against the plant extracts.

In vitro antioxidant assay

2, 2-diphenyl-1-picryl hydrazyl (DPPH) Free radical scavenging activity

The DPPH free radical scavenging activity of the different extracts was measured according to the method of Ai Lan Chew *et al*. (2012). The crude extracts in different concentrations viz., 100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml and 500 µg/ml were prepared in DMSO. One ml of each concentration was mixed with 4 ml of the 0.004% (w/v) solution of DPPH prepared in methanol. The reaction mixture was kept for incubation in dark for 30 minutes. Methanol was used as control and Ascorbic acid was used as positive control. The absorbance was measured at 517 nm. The DPPH scavenging activity (%) was calculated by using the following formula

DPPH scavenging activity (%) = $[(A_0 – As) / A_0] \times 100$,
Where, A_0 -- absorbance of the control, As -- absorbance of the plant sample

RESULTS AND DISCUSSION

Phytochemical screening

Phytochemical analysis results of *Ceriops decandra* stem extracts in six various solvents are mentioned in table-1.

Table1 Phytochemical analysis of *Ceriops decandra* Stem extracts indifferent solvents

S.No.	Phytochemicals	H	B	C	E	A	M
1.	Carbohydrates	--	+	+	--	+	+
2.	Monosaccharides	--	--	--	--	+	+
3.	Free reducing sugars	--	+	+	--	+	--
4.	Combined reducing sugars	--	--	--	--	+	+
5.	Tannins	--	--	--	--	+	+
6.	Free anthraquinones	--	--	--	--	+	+
7.	Steroids	+	+	+	+	--	--
8.	Cardiac glycosides	+	+	+	+	--	--
9.	Terpenoids	+	+	+	+	--	--
10.	Saponins	--	--	--	--	++	++
11.	Flavonoids	--	--	--	+	++	+
12.	Soluble starch	--	--	--	--	--	--
13.	Alkaloids	--	+	--	++	++	--

H - Hexane; B - Benzene; C - Chloroform; E - Ethyl Acetate;
A - Acetone; M – Methanol

Table2 MIC and MBC (mg/ml) values of *Ceriops decandra* stem extracts

Test organisms	Hexane		Benzene		Chloroform		Acetone	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Micrococcus luteus MTCC 106	75	100	50	75	50	75	25	50
Arthrobacter protophormiae MTCC 2682	75	100	--	--	75	100	75	100
Rhodococcus rhodochrous MTCC 265	--	--	--	--	75	100	75	100
Bacillus megaterium MTCC 428	50	75	75	100	75	100	--	--
Bacillus subtilis MTCC 441	--	--	75	100	75	100	--	--
Enterococcus faecalis MTCC 439	50	75			50	75	--	--
Streptococcus mutans MTCC 497	--	--	75	100	75	100	75	100
Staphylococcus aureus MTCC 737	--	--	--	--	75	100	75	100
Lactobacillus acidophilus MTCC 10307	--	--	75	100	50	75	--	--
Alcaligens faecalis MTCC 126	--	--	75	100	75	100	75	100
Proteus mirabilis MTCC 425	--	--	75	100	75	100	75	100
Proteus vulgaris MTCC 426	--	--	75	100	50	75	50	75
Enterobacter aerogenes MTCC 10208	--	--	--	--	75	100	75	100
Salmonella enterica MTCC 3858	50	--	--	--	25	50	--	--

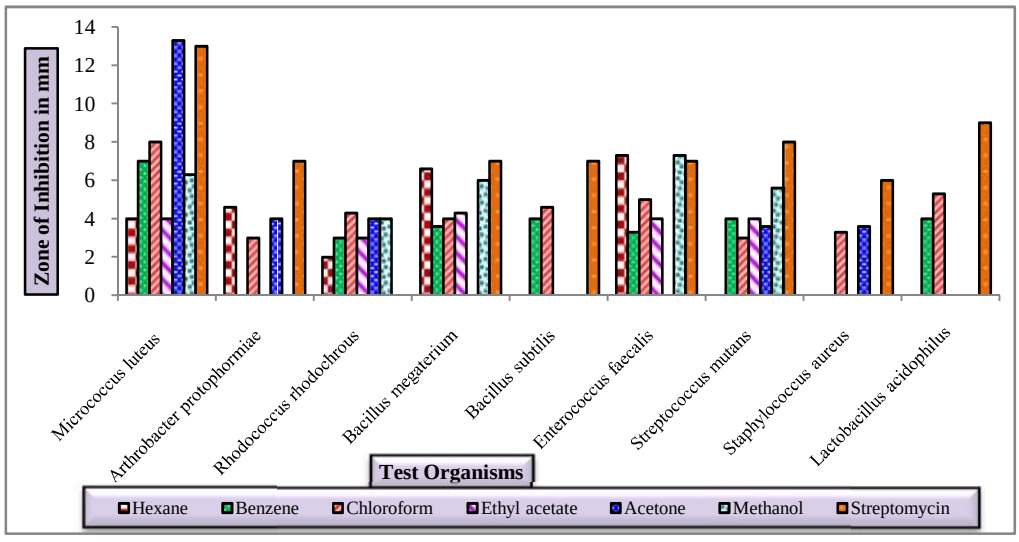


Figure 1 Antibacterial activity of different solvent extracts of *C. decandra* stem

Terpenoids, Steroids like Cardiac glycosides were found in Hexane, Benzene, Chloroform and Ethyl acetate extracts. Alkaloids were present in Benzene, Ethyl acetate and Acetone extracts. Flavonoids were seen in Ethyl acetate, Acetone and Methanol extracts. Apart from that, Carbohydrates were found in Benzene, Chloroform, Acetone and Methanol extracts but, free reducing sugars were observed in Benzene, Chloroform and Acetone extracts. Monosaccharides and Combined reducing sugars were present in Acetone and Methanol extracts. Furthermore, Tannins, Saponins and Free anthraquinones were found in Acetone and Methanol extracts.

Medicinal plants contain some organic compounds which produce definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids (Anjali Soni *et al.*, 2013, Edoga *et al.*, 2005, Mann, 1978). The plant chemicals have been found to possess biocidal activity against

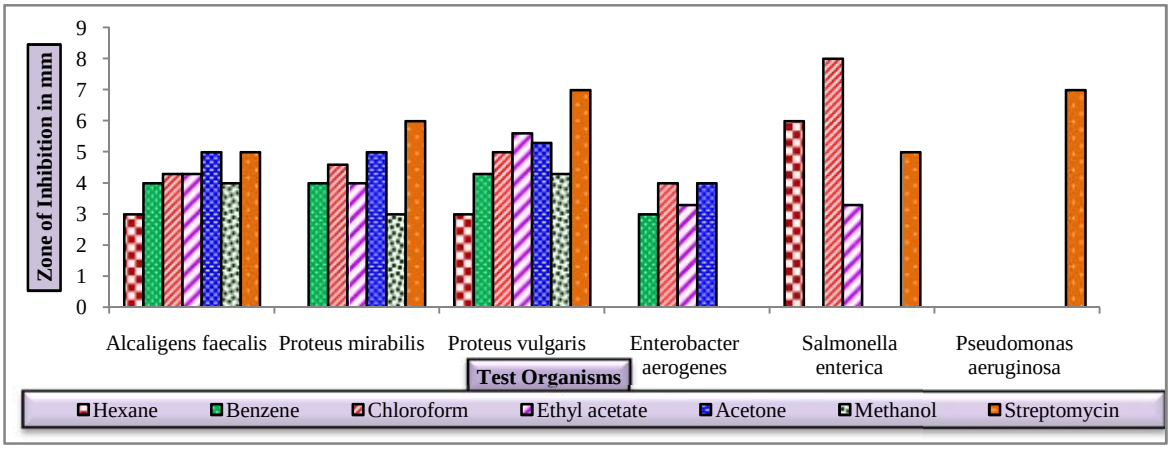


Figure 2 Antibacterial activity of different solvent extracts of *C. decandra* stem

several pests and pathogens (Arora *et al*., 2003, Bharti *et al*., 2012, Charu arora *et al*., 2012).

Antibacterial activity

The data on antibacterial activity of *Ceriops decandra* against selected test organisms including both Gram positive and Gram negative bacteria are given in figures-1 and 2. Zone of inhibition of *Ceriops decandra* stem extract in Acetone against *Micrococcus luteus* was greater than the positive control. Then, the next greater zone was seen for Chloroform extract, Benzene extract and Methanol extract. Hexane and Ethyl acetate extracts of *C. decandra* stem have shown relatively lesser zones of inhibition.

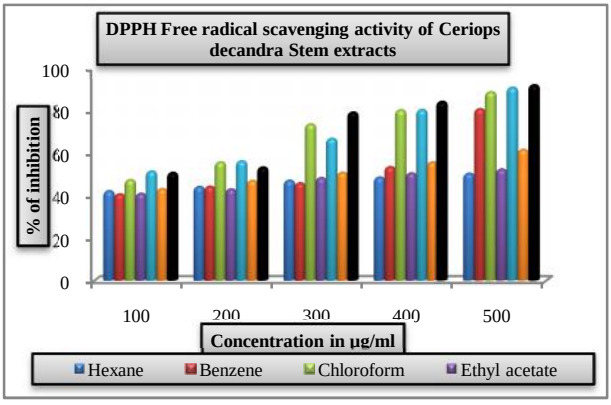
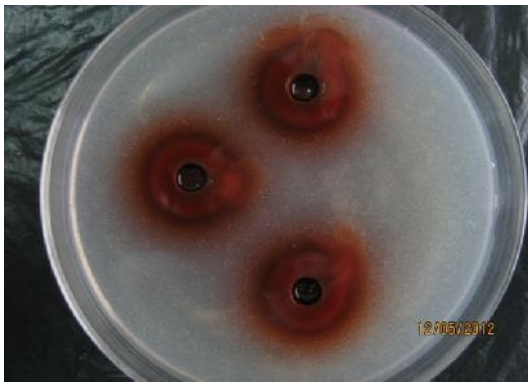


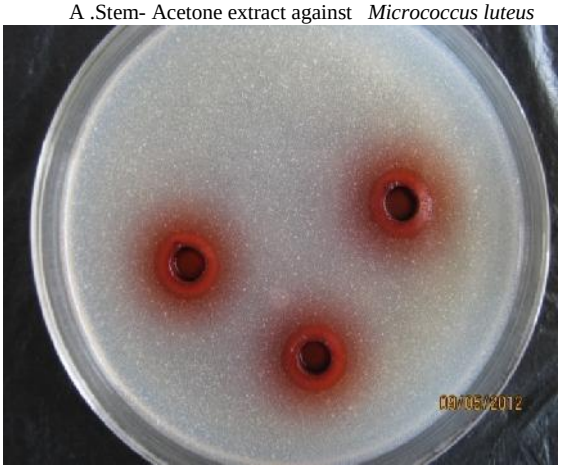
Figure3 Antioxidant activity of different solvent extracts of *C.decandra* stem

C. decandra stem extracts of all the solvents have shown zones of inhibition against *Rhodococcus rhodochrous*, but the positive control did not show any zone of inhibition. Whereas, extracts of Benzene, Chloroform, Ethyl acetate and Acetone have displayed activity against *Enterobacter aerogenes* which was found totally resistant to positive control. All the solvent extracts tested against *Alcaligens faecalis* have shown zones of inhibition and Acetone extract exhibited an equal zone of inhibition as that of positive control. Hexane and Methanol extracts have exhibited larger zones of inhibition against *Enterococcus faecalis* than the positive control. Chloroform and Hexane extracts of *C. decandra* stem gave larger zones of inhibition against *Salmonella enterica* than the positive control. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world.

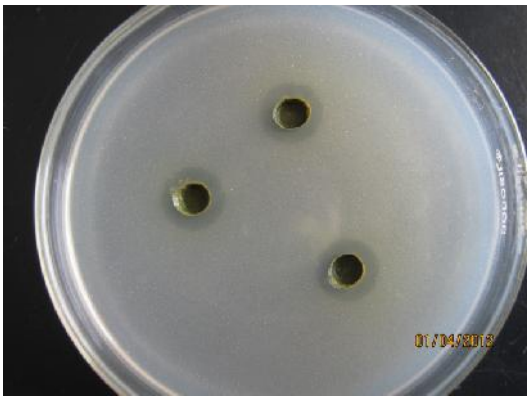
The World Health Organization estimates that plant extract or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population. Mangrove plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, etc, which have been found in vitro to have antimicrobial properties. In the present study each of the solvent extract of plant displayed the antibacterial activity on bacterial strains tested, however with some differences among the extracts. These differences could be due to the differences in qualitative and quantitative chemical composition of these extracts, as the secondary metabolites of plants have many effects including antibacterial activity (Doriane E Djeussi *et al*., 2013, Noumedem *et al*., 2013, Cowan, 1999).



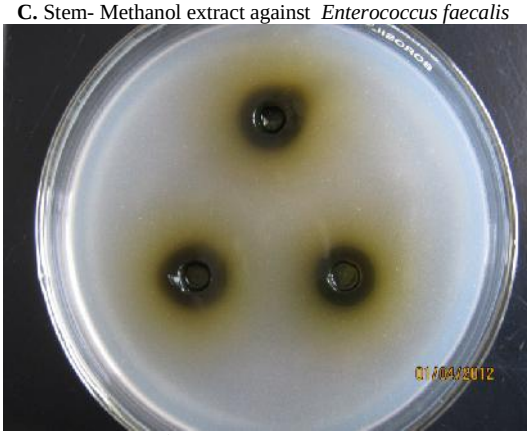
A .Stem- Acetone extract against *Micrococcus luteus*



B. Stem- Acetone extract against *Salmonella enteric*



C. Stem- Methanol extract against *Enterococcus faecalis*



D. Stem- Chloroform extract against *Micrococcus luteus*

MIC and MBC

Minimum Inhibitory Concentration and Minimum Bactericidal Concentration values are presented in table-2. The results showed significant MIC values between 25mg/ml to75mg/ml and MBC between 50mg/ml to 100mg/ml. The lowest MIC value of 25mg/ml was observed with Chloroform extract against *Salmonella enterica* and Acetone extract against *Micrococcus luteus* and the MBC value is 50mg/ml. Hexane extract against *Bacillus megaterium*, *Enterococcus faecalis* and *Salmonella enterica*, Benzene extract against *Micrococcus luteus*, Chloform extract against *Micrococcus luteus*, *Enterococcus faecalis*, *Lactobacillus* acidophilus, *Proteus vulgaris* and Acetone extract against *Proteus vulgaris* displayed 50mg/ml MIC value and the MBC value of 75mg/ml. Determination of the MIC is important in diagnostic laboratories because it helps in confirming resistance of micro-organism to an antimicrobial agent and it monitors the activity of new antimicrobial agents.

Antioxidant activity

DPPH radical scavenging activity is one of the most widely used method for screening the antioxidant activity of plant extract. Figure-3 represents the antioxidant activity of different solvent extracts of *Ceriops decandra* stem. Higher % of inhibition indicates better scavenging activity or antioxidant potential and % of inhibition was gradually increased with the concentration. Acetone extract showed moderate to high scavenging activity at different concentrations when compared to other extracts and standard Ascorbic acid. The highest DPPH scavenging activity was observed in Acetone extract (90.03%) followed by Chloroform (87.94%), Methanol (61%), Benzene (53.38%), Ethyl acetate (51.9%) and Hexane (49.93%). Total phenolics and flavonoids possess a broad spectrum of chemical and biological activities including radical scavenging properties. Oxygen-centered free radicals and other reactive oxygen species (ROS) can be generated as byproducts during oxidative progresses of living organisms (Halliwell *et al*., 1985). Antioxidants are necessary to supplement the natural antioxidant defenses of the body to cure these diseases. However, the synthetic antioxidants might be unsafe, therefore, more attention is being paid for searching the natural antioxidants from plants to prevent oxidative damage (Christen, 2000).

CONCLUSION

In conclusion, the results of this present study clearly indicate that the bioactive phytochemical constituents from *C. decandra* stem can be extracted with different solvents. The Acetone extract displayed good antibacterial and antioxidant activity followed by Chloroform extract. These extracts need to be explored to obtain purified form of compounds that may act as potential medicinal drugs for various ailments and that part of work is in progress.

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