



Research Article

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Phytochemical, antibacterial and antioxidant evaluation of *Ceriops decandra* (Griff.) Ding Hou leaf extracts

Nagababu P. and Umamaheswara Rao V.*

Department of Botany and Microbiology, Acharya Nagarjuna University, Nagarjuna Nagar,
Guntur District, Andhra Pradesh, India

ABSTRACT

The present work focuses on phytochemical screening, antibacterial activity and antioxidant activity of various solvent extracts of *Ceriops decandra* leaf material. The preliminary phytochemical screening was done by using standard protocols and the Antibacterial activity was carried out by using agar well diffusion method. The phytochemical screening study revealed the presence of the important chemicals namely Tannins, Steroids, Terpenoids, Flavonoids and Alkaloids in different solvent extracts of *C. decandra* leaves. Ethyl acetate and Chloroform extracts showed antibacterial activity against most of the Gram positive and Gram negative bacteria tested. However, acetone extract exhibited relatively larger zones of inhibition against the susceptible bacteria than any other solvent extract. The MIC and MBC studies of acetone extract against different bacteria revealed the antibacterial potential of the *C. decandra* leaf material. Acetone extract at 100 µg/ml and 200 µg/ml concentrations showed more free radical scavenging activity than the standard ascorbic acid. The results of this study suggesting the medicinal importance of this plant.

Key words: *Ceriops decandra*, Phytochemicals, Antibacterial activity, antioxidant activity, MIC, MBC.

INTRODUCTION

Medicinal plants are the richest bio-resources of folk medicines and traditional systems of medicine; and food supplements, nutraceuticals, pharmaceuticals and chemical entities for synthetic drugs[1-2]. The use of plants or their extracts for the treatment of human disease predates the earliest stages of recorded civilization, dating back at least to Neanderthal period [3-4]. Pathogenic bacteria have developed resistance against existing antibiotics due to indiscriminate use of antimicrobial drugs to treat the infectious diseases and also more toxic for human being during long term therapy. The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Mangroves are the intertidal salt tolerant group of plants which have wide applications in folk medicine since ages. They are found mainly in the tropical and subtropical intertidal zones of the world largely confined to the region between 30° north and south of the equator. Their ability to survive under very stressful condition has led them to unique morphology and unusual physiological processes[5-6]. Mangrove plants are the best choice to isolate potent bioactive compounds against many pathogenic bacteria. In recent years, there has been a great interest in finding natural antioxidants from plant materials to replace synthetic antioxidants, which are being restricted due to their carcinogenicity. *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.

The present study was undertaken to investigate the phytochemical constituents and evaluate the antibacterial and antioxidant potential of various solvent extracts of the *C. decandra* leaves.

EXPERIMENTAL SECTION

Preparation of the plant extracts

Ceriops decandra (Griff.) Ding Hou plant was collected from Koringa Mangrove forest, near Kakinada, Andhra Pradesh, India (Fig. 1). Leaves of this plant were 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.



Figure-1. Geographical location map of plant collection

Preliminary phytochemical screening

Phytochemical screening was carried out by the following tests [7].

Test for flavonoids

a) 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.

b) 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.

c) 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.

d) Leadacetate test

Extract of 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 gms 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 H₂SO₄. Yellow coloration indicates the presence of soluble starch.

Test for Saponins

Crude powder of 0.5 g 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. H₂SO₄. A change in color from pink to violet confirms the presence of terpenoids.

Test for steroids

a) Salkowskii test

In 2 ml of chloroform, 0.2 g of extract was dissolved and added the conc. H₂SO₄. The development of reddish brown color at inter phase indicates the presence of steroids.

b) Keller-Killiani test

To 0.5 ml of test solution, 2 ml of 3.5% FeCl₃, small amount of glacial acetic acid and 2 ml of conc. H₂SO₄ were added carefully. Appearance of reddish brown ring at inter phase is a positive indication for the presence of steroids.

c) Liebermann-Burchard test

To 0.2 g of each extract, 2 ml of acetic acid was added and the solution was cooled well in ice followed by the addition of conc. H₂SO₄ 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

a) Molisch's test

Two ml of *Molisch's* reagent was added to the extract dissolved in distilled water and 1 ml of conc. H₂SO₄ 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.

b) 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.

c) Fehling's test (for Combined Reducing Sugars)

Extract of 0.5 g 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.

d) Barfoed's test (for monosaccharide)

In distilled water, 0.5 g 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 g 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.

a) Borntrager's Test:

Extract of 0.2 g 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.

b) Phlorotannins test:

To 0.2g 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 including *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 including *Alcaligenes 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 [8]. 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 10µg/ml DMSO. A minute quantity of sterile agar suspension was added to the well. 100 µl of the sample which was prepared by dissolving 100mg 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° C for 24 hrs. After incubation, the diameter of the zone of the inhibition was measured. For each sample and bacterial species, triplicates were maintained.

Determination of MIC and MBC

Minimal inhibitory concentration (MIC) was determined by using broth dilution method. Minimal Inhibitory Concentration and Minimal Bactericidal Concentration (MBC) were determined at different concentrations viz., 12.5mg, 25 mg, 50mg, 75mg and 100mg 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[9]. 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 concentrations 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

$$\text{DPPH scavenging activity (\%)} = [(A_0 - A_s) / A_0] \times 100,$$

Where, A_0 -- absorbance of the control, A_s -- absorbance of the plant sample

RESULTS AND DISCUSSION

Phytochemical screening

The phytochemical screening study of the *C. decandra* leaves showed the presence or absence of various phytochemicals in different solvent extracts (Table-1). Flavonoids were present in all the solvent extracts except the chloroform. Hexane, benzene, acetone and methanol extracts were found positive for tannins. Steroids, terpenoids and cardiac glycosides were present in hexane, benzene and chloroform extracts only. Alkaloids were found in benzene and acetone extracts. The free anthraquinones were present in benzene, acetone and methanol extracts. But saponins were not found in any solvent extract. The healing properties of medicinal plants are possibly due to the presence of various phytochemical constituents such as phenolics, flavonoids, alkaloids, terpenoids and phytosterols etc. Phytochemical analysis of plant extracts revealed the presence of constituents which are known to show medicinal properties in addition to physiological activities [10-11]. Several classes of phenols and polyphenols such as phenolics, flavonoids and tannins contribute to plant defense mechanism in resisting pathogenic microorganisms [12].

Table-1. Phytochemical analysis of *Ceriops decandra* Leaf extracts in different 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

Antibacterial activity

The antibacterial activity results of different solvent extracts of *C. decandra* leaves are given in figures 2 and 3. Among the solvent extracts tested for the antibacterial activity ethyl acetate, chloroform and acetone extracts unveiled better results against majority of bacteria used in the study. Ethyl acetate extract showed activity against all the test organisms, except *P. aeruginosa*. Whereas, chloroform solvent extract exhibited antibacterial activity against all the test organisms except *S. enterica* and *P. aeruginosa*. Though acetone extract had shown antibacterial activity only on five Gram positive and two Gram negative bacteria, when compared to ethyl acetate and chloroform, the zone of inhibition caused by acetone extract was larger than other extracts. And *M. luteus* was found to be more susceptible to acetone extract than other bacteria. Benzene extract was active against *M. luteus*, *R. rhodochrous*, *B. subtilis*, *L. acidophilus*, *A. faecalis* and *P. vulgaris*. Methanol extract showed antibacterial activity against *M. luteus*, *A. protophormiae*, *B. subtilis*, *E. faecalis*, *S. mutans*, *A. faecalis* and *P. vulgaris*. In our study, *R. rhodochrous* and *E. aerogenes* were found resistant to the positive control, streptomycin. But, benzene, chloroform, ethyl acetate and acetone extracts were effective against *R. rhodochrous*, and chloroform and ethyl acetate extracts exhibited activity on *E. aerogenes*. Of all the extracts, hexane extract was found to be not so potential as showed the activity only on *S. mutans*. The present study results showed that ethyl acetate, chloroform and acetone extracts of *C. decandra* leaves have antibacterial activity. This antibacterial activity of the plant is in agreement with an earlier study that reported the antibacterial activity of *C. decandra* leaf, root, bark and wood extracts but on only four bacteria [13]. The leaf extract of *C. decandra* also known to have an ability to prevent the DMBA (Dimethylbenz[a]anthracene) induced carcinoma [14]. In the present study, many phytoconstituents were found in the extracts of *C. decandra* leaves, which could possibly be the reason for the better antibacterial activity against various pathogens. Therefore, the beneficial medicinal effects of plant material may result from the combination of secondary products present in the plant. Secondary metabolites play a role in the plant's defense through cytotoxicity towards microbial pathogens and this could prove the usefulness of these as antimicrobial medicines for humans.

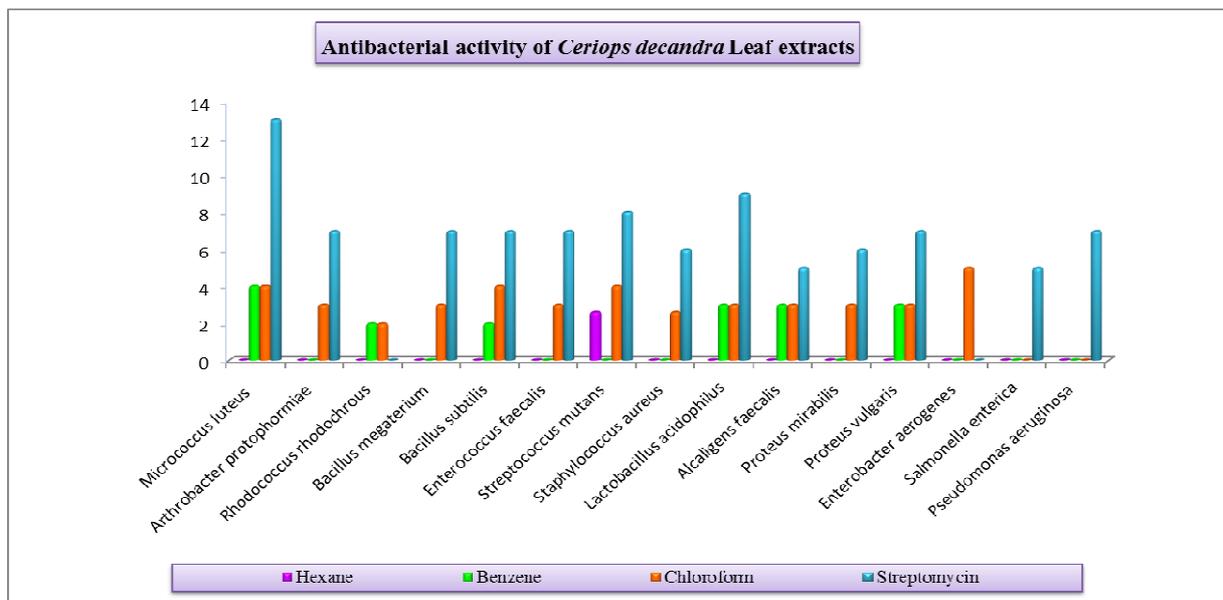


Figure-2. Antibacterial activity of *Ceriops decandra* leaf extracts

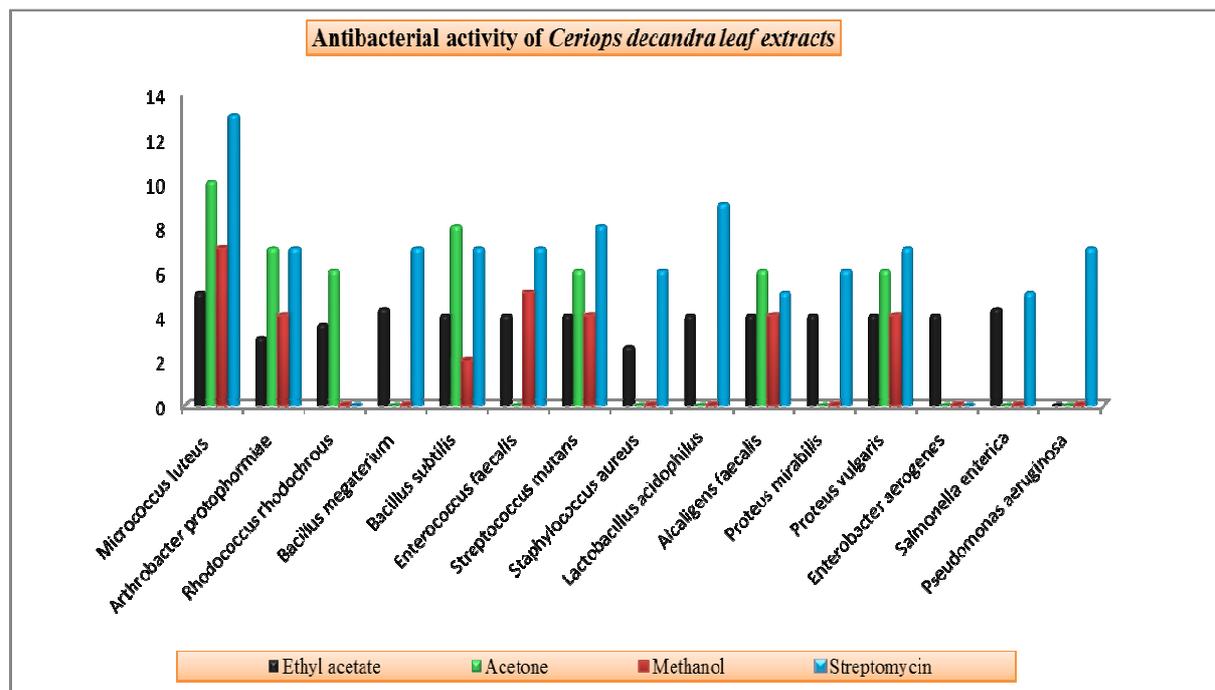


Figure-3. Antibacterial activity of *Ceriops decandra* leaf extracts

MIC and MBC

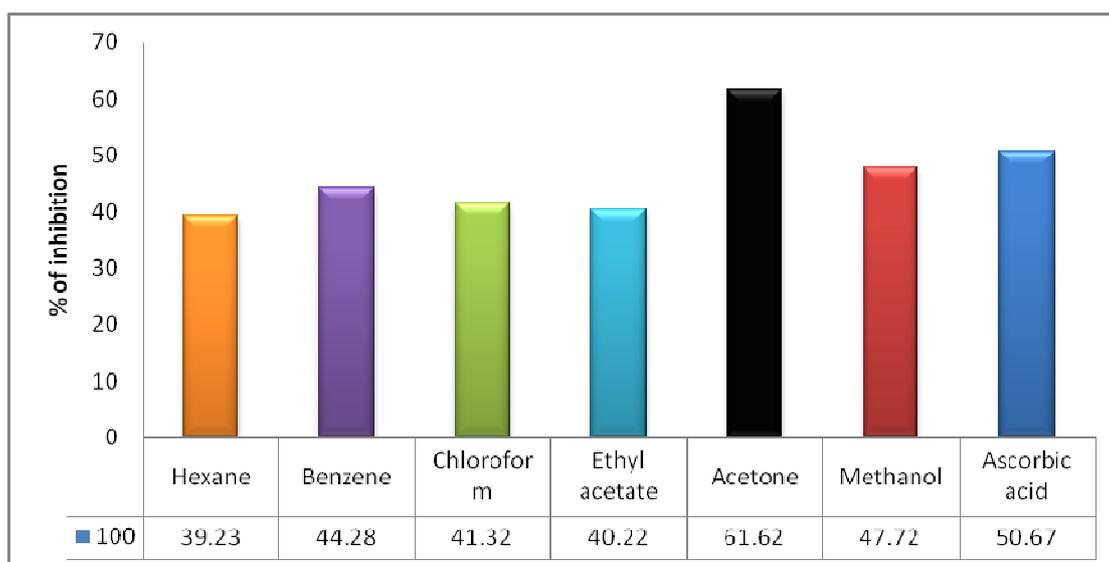
The MIC and MBC studies were done for the acetone extract which has shown significant and larger zones of inhibition against susceptible bacteria and values are tabulated (Table-2). The MIC values for *M. luteus* and *R. rhodochrous* were 25 mg and MBC values were 50 mg. MIC values for *A. protophormiae*, *B. subtilis*, *S. mutans* and *P. vulgaris* were 50 mg and MBC values were 75 mg. For *A. faecalis*, the MIC and MBC values were 75 mg and 100 mg, respectively.

Table-2. MIC and MBC values of *Ceriops decandra* leaf extract

Test organisms	Acetone (mg/ml)	
	MIC	MBC
<i>Micrococcus luteus</i> MTCC 106	25	50
<i>Arthrobacter protophormiae</i> MTCC 2682	50	75
<i>Rhodococcus rhodochrous</i> MTCC 265	25	50
<i>Bacillus subtilis</i> MTCC 441	50	75
<i>Streptococcus mutans</i> MTCC 497	50	75
<i>Alcaligenes faecalis</i> MTCC 126	75	100
<i>Proteus vulgaris</i> MTCC 426	50	75

DPPH Free radical scavenging assay

The DPPH Free radical scavenging assay results revealed that the *C. decandra* leaf extracts were found to have good antioxidant activity (Figures 4 -8). DPPH radical scavenging activity of *C. decandra* was increased with an increase in concentration of the plant extracts. All the extracts at different concentrations exhibited considerably good antioxidant activity. Acetone extract, in particular, showed more radical scavenging activity at 100 μ g/ml, 200 μ g/ml concentrations than the standard ascorbic acid. DPPH is a free radical and accepts electron or hydrogen radical to become a stable diamagnetic molecule [15]. A freshly prepared DPPH solution exhibits a deep purple colour with an absorption maximum at 517 nm. This purple colour generally fades when an antioxidant is present in the medium. Thus, antioxidant molecules can quench DPPH (by providing hydrogen atom or by electron donation) and convert it to a colourless product resulting in decrease in absorbance at 517 nm [16]. Phenolic compounds from plants were responsible for the radical scavenging activity [17]. Phenolic compounds that contain phenyl ring are the diverse group of molecules containing flavonoids, tannins, flavanols and anthocyanines etc. and these phenolic compounds are present in most of the mangrove plants. In the present study, the phytochemical screening revealed the maximum positive results for phenolic compounds and that may be the reason for the free radical scavenging activity of all the extracts tested for antioxidant assay.

**Figure-4:Antioxidant activity (%) of *C.decandra* leaf extracts at 100 μ g/ml concentration**

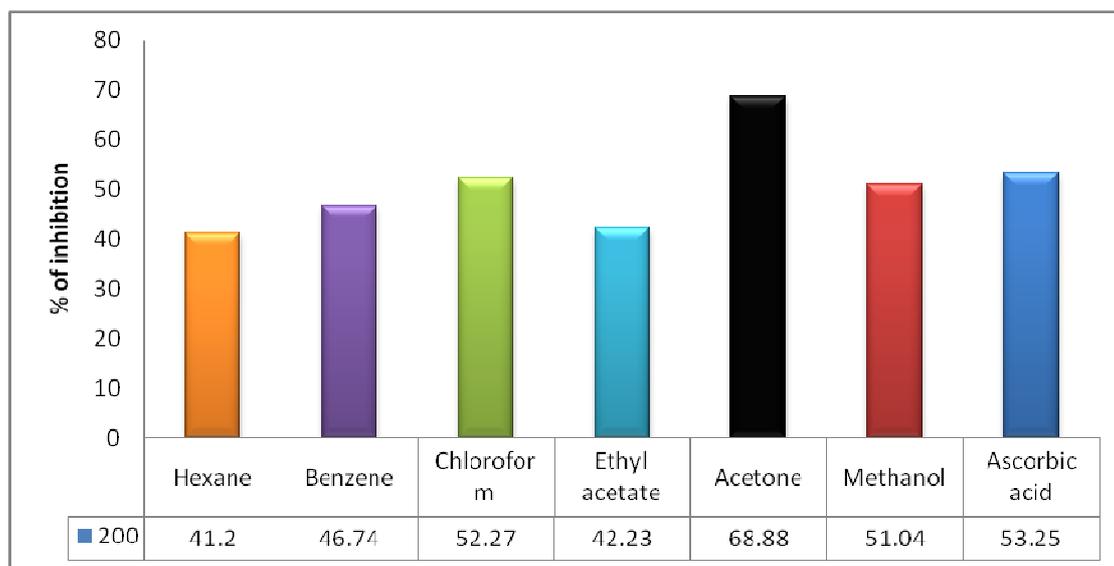


Figure-5: Antioxidant activity (%) of *C. decandra* leaf extracts at 200 µg/ml concentration

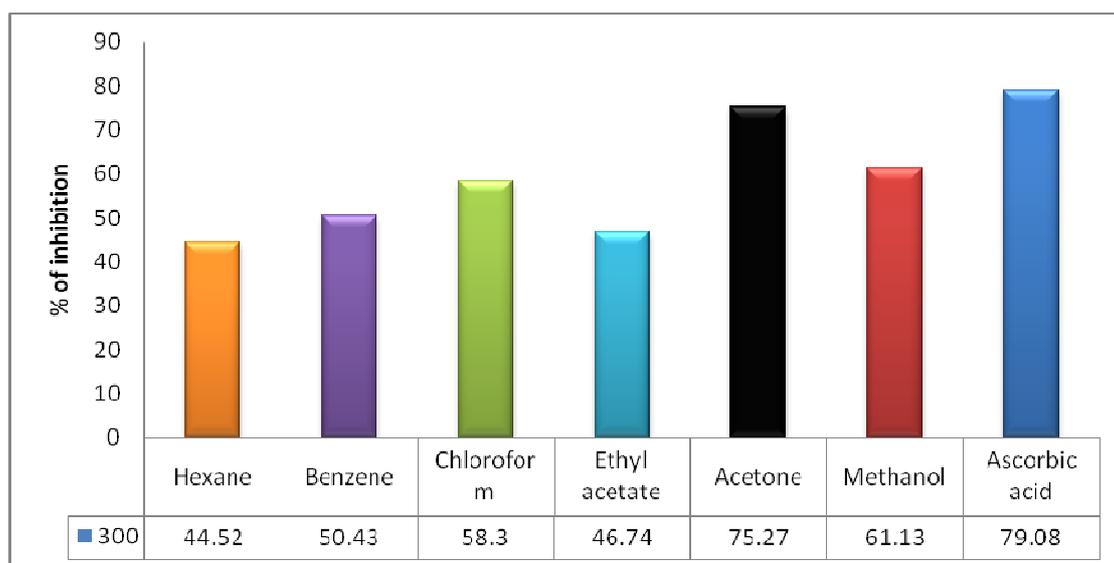


Figure-6: Antioxidant activity (%) of *C. decandra* leaf extracts at 300 µg/ml concentration

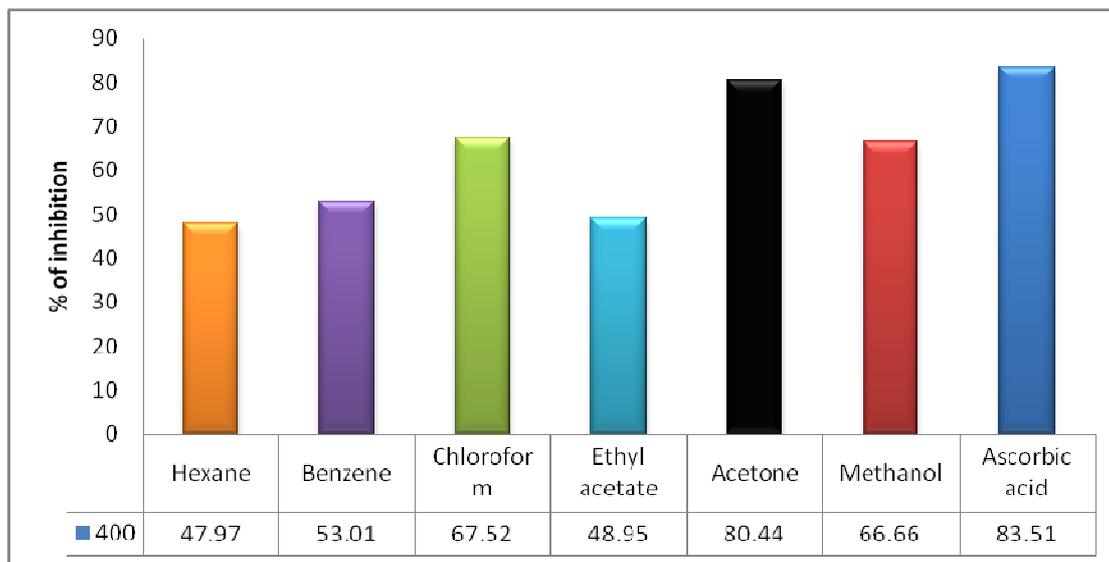


Figure-7: Antioxidant activity (%) of *C. decandra* leaf extracts at 400 µg/ml concentration

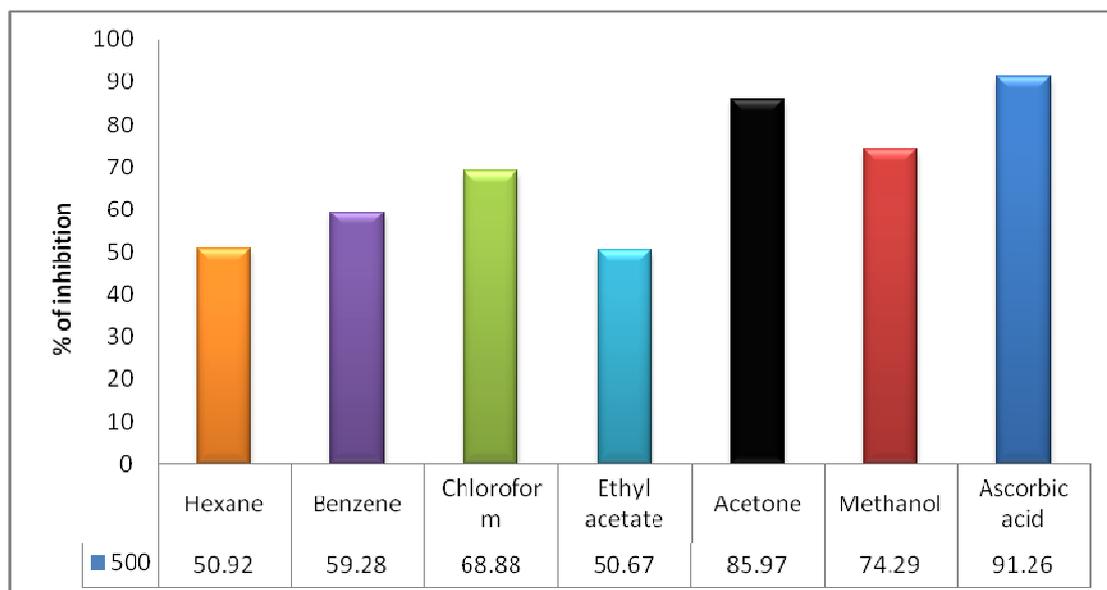


Figure-8: Antioxidant activity (%) of *C. decandra* leaf extracts at 500 µg/ml concentration

CONCLUSION

The present study indicates that the ethyl acetate, chloroform and acetone extracts of *C. decandra* leaves possess a potential and promising antibacterial and antioxidant activities. Further studies on characterization of bioactive principles and their cytotoxic activities on cell lines are required for various useful applications of this plant against infectious diseases in humans.

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