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Antimicrobial and Free Radical Scavenging Activity of *Cordia obliqua* W. (Boraginaceae) Fruit Extracts

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The present study was carried out to evaluate the free radical scavenging activity and antimicrobial activity of *Cordia obliqua* against bacteria and fungi. Results of the current study disclosed that *Cordia obliqua* exhibited antibacterial effect against Gram positive *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and Gram negative *Escherichia coli*. It also showed inhibition effect against *Aspergillus niger* and *Candida albicans*. Further, *Cordia obliqua* showed free radical scavenging ability against the DPPH radicals (IC50 <100 μg/ml).

Key words: Antibacterial activity, *Cordia obliqua*, Free radicals, Phytoconstituents.

Plants are the premise of the numerous ancient medication systems all through the globe for thousands of years and still give humans with new remedies. Secondary metabolites' from plants are used as a foremost source for medicine as they found to own a reservoir of chemical agents[1] The process of drug discovery is multi- and interdisciplinary. Apart from the core disciplines associated with pharmaceutical research, ethno botany is currently become an integral part of drug discovery from medicinal plants. The plant based indigenous knowledge was passed down from generation to generation in diverse components of the globe throughout its history and has considerably contributed to the event of various ancient systems of medicine[2] Antibiotics are one of the most significant weapons in fighting bacterial infections and have substantially availed the health connected quality of human life since their introduction. However, over the past decades, these health benefits are under threat as several commonly used antibiotics became less effective against certain illnesses not only because of toxic reactions, but also due to the emergence of resistant's against drugs[3].

Antioxidants are the substance that controls the free radicals generated during the cellular respiration and other external causes. These free radicals cause damage in proteins, lipids and nucleic acids that leads to cancer, diabetes, atherosclerosis and inflammatory joint diseases[4] Recently abundant attention has been shown on the plant based antioxidant due to the side effect caused by chemical derivatives[5] Therefore, the medication derived from natural sources play a big role within the hindrance and treatment of human diseases[6].

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Cordia obliqua Willd (Boraginaceae) found in India and Bengal commonly known as "Large sebesten". It's a medium size deciduous wood tree, grows upto 10.5 meters. Traditionally the fruit has been used for the treatment of coughs, chronic fever, to remove joint pains and spleen diseases[7], the fruit was reported to posses Hesperetin 7-rhamnoside, a glycoside[8]. However as per our literature knowledge no reports available on the free radical scavenging and antimicrobial effects of *Cordia obliqua* fruit extract. Hence the present study was under taken to evaluate the antibacterial and free radical scavenging potential of the selected medicinal plants.

MATERIALS AND METHODS

Plant materials and extraction

Fruits of *Cordia obiqua* were collected from Chennai, Tamil Nadu, India during April 2013, authenticated by a Botanist and the voucher specimen was deposited. The fruits were directly used for extraction. The fruits were (500g) extracted by maceration with methanol. The solvent was removed by vacuum distillation in a rotary evaporator at reduced pressure which yielded brownish green extract of 17.9 g approximately. The extracts were stored at 4°C for further use.

Phytochemical analysis

The methanol extracts of *C.obliqua* was tested for the presence of the phyto constituents like flavonoids, terpenoids, phlobatannins, glycosides, steroids and saponins[9]

Microorganisms

The selected Bacterial strains, i.e. *Bacillus subtilis* (MTCC 121), *Staphylococcus aureus* (MTCC 96), *Staphylococcus epidermidis* (MTCC 435), *Escherichia coli* (MTCC 433), *Klebsiella pneumoniae* (MTCC 1320) and fungi, *Aspergillus niger* (MTCC 1344), *Candida albicans* (MTCC 227) and *Curvularia lunata* (46/01) were used for the experiment. The cultures were procured from Microbial Type Culture Collection (MTCC), IMtech, Chandigarh, India. Bacterial and fungal cultures were maintained at Muller Hinton Agar and Sabouraud Dextrose Agar (SDA) respectively under laboratory conditions.

Antimicrobial susceptibility study

The antimicrobial activity of methanol extract of *Cordia obiqua* was performed according to the standard broth micro dilution method[10] *Cordia obiqua* extracts were dissolved in water together with 1% dimethyl sulfoxide (DMSO). The initial test concentration (500 µg/ml) was serially diluted two-fold, that were added to each medium in 96 well plates. The Each well was inoculated with 5 µL of inocullum. The antibacterial agent streptomycin and antifungal agent ketoconazole were used as positive controls. The plates were incubated for 24h at 37°C for bacteria and for fungi 72h at 27°C for 5 days. MIC was determined as the lowest concentration inhibiting the visual growth of the test cultures. Three replications were maintained to confirm the antimicrobial activity.

DPPH radical scavenging activity

In this assay, 1 ml of varied concentrations (100, 200, 300, 400 and 500 µg/ml) of the methanolic extracts of *Cordia obiqua* was blended with 1 ml of methanol solution of DPPH (0.2 mM). The mixture was vortexed and incubated for 30 min. The optical densities of the solutions were measured at 517 nm using Hitachi 2050 spectrophotometer, using BHA as the standard reference[11]

$$\text{Radical scavenging activity (\%)} = \frac{\text{Test absorbance}}{\text{Control Absorbance}} \times 100$$

RESULTS AND DISCUSSION

Natural products are on rise in drug industries recently replacing synthetic products. However they are not new to mankind, as they have been used up for many for medicinal purposes, since thousands of years ago. The use of natural products in novel drug designing is preferable as it is comparatively safe for those who get benefits of it and for environment too.

In our study, it was found that the methanol extract of *C.obiqua* is effective against the tested bacteria and fungi. The antimicrobial potentials in terms of MIC for the *Cordia obiqua* are shown in Table 1 and 2. The methanol extract of *C. obiqua* exhibited MIC value against *B.subtilis* (156.25 µg/ml), *S. aureus* (312.5 µg/m) and *E.coli* (156.25 µg/m). No inhibition was observed against *S. epidermidis* and *K. pneumoniae*. It was noted that *C.obiqua* showed good antifungal effect against *A.niger* (250 µg/ml) and *Candida albicans* (>250 µg/ml), but the extract showed no inhibitory effect on *C.lunata*. Plant secondary metabolities

constitutes of different phytochemical constituents like alkaloids, flavonoids, terpenoids, steroids, tannins, phenols and saponins that serve a defense mechanism against microbes, insects and from herbivores[12] In our study the methanol extract of *C. obiqua* exerted the presence of glycoside, flavonoids and steroids. The antimicrobial property by *C.obiqua* may be due the presence of phyto chemicals and it has been evidenced that flavonoids, steroids are associated with the antibacterial properties, by disturbing the membrane lipid of the bacterial cell and cause leakages from liposomes[13, 14] Previously it was reported that the bark extracts of *Cordia dichotoma* showed antimicrobial and antifungal activities[6] The radical scavenging ability of *C. obiqua* was evaluated by DPPH assay (Fig 1). The result showed that *C. obiqua* exhibited significant radical quenching ability against DPPH. In DPPH assay, the generated radicals are reduced by the donation of protons by the extracts, leading to the colour change, which could be quantified by its decrease in absorbance at 517 nm. DPPH may be a stable radical; this stable radical becomes unstable once it receives electrons from reducing agents. This assay is employed to show herbs free radical scavenging activity. Similarly, *Cordia macleodii* was found to posse's antioxidant and hepatoprotective activity[15] The present study indicates that *Cordia obiqua* possesses good antibacterial effect and antioxidant effect. Further study is needed to reveal the active principle by isolation and characterization of their constituents.

Table 1. Minimum inhibitory Concentration of *Cordia obliqua* against bacteria

Bacteria	Minimum inhibitory Concentration (µg/ml)	
	C. obliqua	Streptomycin
Bacillus subtilis	156.25	12.5
Staphylococcus aureus	312.5	12.5
Staphylococcus epidermidis	-	25
Escherichia coli	156.25	12.5
Klebsiella pneumoniae	-	12.5

- : no activity; Experiment performed in triplicates

Table 2. Minimum inhibitory Concentration of *Cordia obliqua* against fungi

Bacteria	Minimum inhibitory Concentration (µg/ml)	
	C. obliqua	*Ketoconazole*
Aspergillus niger	250	<25
Candida albicans	>250	25
Curvularia lunata	-	<12.5

- : no activity; Experiment performed in triplicates

Table 3. Phytochemical analysis of *Cordia obliqua*

Plant	Flavonoids	Terpenoids	Phlobatannins	Glycosides	Steroids	Saponins
Cordia obliqua	+	-	-	+	-	+

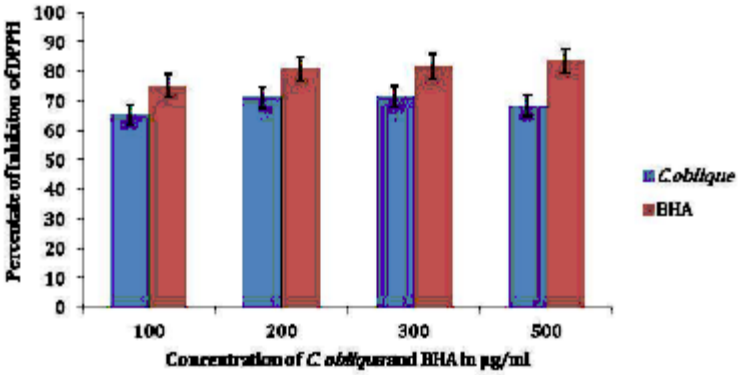

Fig. 1. Scavenging activity of *C. obliqua* on DPPH free radicals; (Mean ± SEM) (n = 3)

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Conflict of interest statement

We declare that we have no conflict of interest.

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