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ORIGINAL ARTICLE **OPEN ACCESS**

Evaluation of Antimicrobial and Antioxidant activities of *Caesalpinia bonducella* seed extract

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ABSTRACT
In Indian Ayurveda, Caesalpinia bonducella plant has its own medicinal value. But experimental data's are required to be collected to prove its efficacy scientifically. Therefore in order to identify the Antimicrobial and Antioxidant properties in the seed extract of Caesalpinia bonducella, an attempt was made. In this study antimicrobial activity of ethanol, methanol, aqueous and petroleum ether crude extracts of Caesalpinia bonducella was determined by disc diffusion method against E.Coli, Klebsiella, Proteus and Staphylococcus aureus bacteria and Candida albicans fungi. Antioxidant potential of seed extract was determined by reducing power assay, hydrogen peroxide and DPPH assay. The different seed extracts of Caesalpinia bonducella possess significant antimicrobial activity. The significant antibacterial inhibition was observed at 90µg/µl of methanol extract in Proteus Species and antifungal inhibition was observed at 30µg/µl of ether extract in Candida albicans. The ethanol extract of the seed showed good antioxidant activity, free radical scavenging and reducing power. The result of this study revealed, it was thought worthwhile, since this plant seed has potential value to control different bacterial and fungal diseases and hence, the use of these plants in the indigenous system of phytomedicine is justified.
Keywords: *Caesalpinia bonducella, Antioxidant activity, antimicrobial activity and phytomedicine*

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INTRODUCTION

In different countries prospective source of antimicrobial agents are plants [1].In developing countries about 60-90% population use plant-derived medicine. Herbal medicine such as crude plant extracts are used for the treatment of human infectious diseases [2]. To cure various diseases of human and animals' plants have been used since ancient times.

In Ayurveda classics *Caesalpinia bonducella* is an important plant and its different parts like nuts, root, bark and leaves are used by health workers in Colic fever, intermittent fever, malaria, menstrual complaints, pneumonia, skin diseases, swelling, fever, pulmonary tuberculosis and edema [3]. Belonging to the family Fabaceae / caesalpiniaceae, its widely distributed all over the world specially, in India, Sri Lanka and Andaman and Nicobar Islands, in India it's found especially tropical regions and it's a prickly shrub [4].

Ayurveda *C. bonducella* (Roxb.) is mostly used for its antiperiodic, antipyretic, anti- inflammatory, anthelmintic and antimalarial properties during the conventional system of Indian medicine, In the conventional system of Indian medicine. Ayurveda is described to have antioxidant, antibacterial, antitumor, and antidiabetic activities [5]. Standardization of selected medicinal plants of potential therapeutic significance have been a rapid increase in the recent years. Proper control of stating material is utmost essential to ensure reproducible quality of herbal. To asses physiochemical, phytochemical and antimicrobial activities of the various extracts which is derived from the leaves extracts of *Caesalpinia bonducella* in relation with their ancient medicinal properties, the present study was undertaken.

MATERIAL AND METHODS

PLANT SOURCE:

Seed sample of *Caesalpinia bonducella* was collected from Viramathi (Latitude:10°10& #39; 31.29& quot;N and Longitude: 78°39& #39;43.71& quot;E), a small village in Tamil Nadu, India. At Rapinat Herbarium of St. Joseph's College, Tiruchirappalli the plant seed was authenticated. At room temperature to prevent the denaturing of some active principle the seed was subjected to air drying. It was ground to increase the surface area after dying. In ethanol the powdered sample was soaked at seed material to solvent ratio 1:10 w/v with frequent manual agitation for 72 h at room temperature.

PREPARATION OF EXTRACT

C. bonducella seeds were porously powdered mechanically after complete drying, and using petroleum ether, chloroform, ethanol and water as the solvent were subjected to successive soxhlet extraction. Coarse powder of 500 gm was extracted. In increasing order of polarity different extracts were made by using standard solvents. From SD Fine Chem Ltd, Mumbai, India Petroleum, Ether and Chloroform were procured and Ethanol from Hong Yang Chemical Corporation, China. The marc was air dried each time before extracting the next solvent and the soxhlet apparatus is then repacked. On a water bath complete evaporation of all the three extracts were allowed and finally the vacuum dried [6]. Marc of air dried Methanolic extract was mixed with water and was kept on orbital shaker at room temperature of 48 hours; it was followed by heating on a water bath and was stored in an air tight container.

MICROBIAL SOURCE

From Arun hospital, (Tiruchirappalli) the microbial cultures *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Staphylococcus aureus* and *Candida albicans* were collected and confirmed based on the colony, morphology, gram staining and biochemical analysis.

PLANT EXTRACT PREPARATION FOR ANTIMICROBIAL ACTIVITY

Each extract as dry powder was dissolved in 1 ml of 0.5% DMSO solution to get a concentration of 5 mg/ml, 10 mg/ml, 15 mg/ml and 20 mg/ml.

ANTIBACTERIAL ACTIVITY:

Four different bacterial strains (*Staphylococcus aureus, E.coli, Klebsiella pneumoniae, and Proteus sp*.) using disc diffusion method were plated with *Caesalpinia bonducella* at different extracts of solutions ranging (30µg/ml, 60µg/ml, 90µg/ml and 120µg/ml) [7]. The growth inhibition zones of the Cb after 24 h showed that selective antibacterial activity towards Proteus species, was exhibited by the drug with a maximum inhibition zone of 16 mm whereas limited antibacterial activity with a least zone of inhibition around 2.12 mm for Klebsiella species was shown by the other bacterial strains[8].

STUDY OF ANTIFUNGAL ACTIVITY

To inhibit *Candida albicans* the ability of antifungal activity was evaluated. The drug concentrations were plated following the similar method above [9]. The growth inhibition zones of the test spores were determined after 24 h interestingly revealed that the drug exhibited selective anti-fungal activity with a maximum inhibition zone of 18.70 mm at 30µg/ml in ether concentration. Minimum inhibition zone of 10.4 mm was shown by *Caesalpinia bondcella*. Around the well diffusion there was a presence of fungal spore growth.

2,2-DIPHENYL-1-PICRYLHYDRAZYL (DPPH) RADICAL SCAVENGING ASSAY

To analyze the antioxidant property of the given sample the DPPH radical scavenging assay was used. Different concentrations of the samples (100 µl) were added to the ethanolic solution of 0.9 ml DPPH (2.5 mg/100 ml), and in the dark the reactants were incubated at the room temperature for 30min. Butylatedhydroxytoluene (BHT) of different concentrations were used as a standard, and the solvent (distilled water) was used in control instead of extract. Using a spectrophotometer, the absorbance was measured at 515 nm after 30 min and the radical scavenging activity of the extract was calculated and expressed on a percentage basis [10].

REDUCTIVE ABILITY

Caesalpinia bonducella seed extracts reducing ability was determined according to the method [11]. Seed powder of Eethanolic extract (0.08-0.4 mg) was dissolved in distilled water of 1 ml and then 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide [K 3 Fe (CN) 6] (1%) were added. At 50°C the mixture was incubated for 20 min, after that 2.5 ml of trichloroacetic acid (10%) was added to the mixture, and it was then centrifuged at 3000 rpm for 10 min. Upper layer of the solution measuring 2.5 ml was mixed with 2.5 ml of distilled water and 0.5 ml of FeCl 3 (0.1%). At 700 nm the absorbance was measured. As a reference compound BHT was used. Analysis pf all was performed in the triplicate.

FREE RADICAL SCAVENGING ACTIVITY (FRSA) USING HYDROGEN PEROXIDE

As suggested bythe hydrogen peroxide FRSA of the ethanolic extract was done [12]. 2ml of hydrogen peroxide (43 m/mol) and 1.0 ml of ethanolic sample [20-100 µl of ethanolic extract (4 mg/ml) of plant in

methanol] followed by 2.4 ml of 0.1 M phosphate buffer (pH 7.4) were added. The absorbance was recorded at 230 nm of the resulting solution which was kept for 10 min. Three times all the readings were repeated. Without adding hydrogen peroxide blank reading was prepared and without sample control was prepared. As a standard compound Ascorbic acid was used. Free radical scavenging activity of hydrogen peroxide (%) was calculated as FRSA (%) =[(V 0 -V 1)/V 0] ×100, where,V 0 = absorbance of control and V 1 = absorbance of sample.

STATISTICAL ANALYSIS

By one-way ANOVA Statistical analysis of *Caesalpinia bonducella* seed extract was done followed by Student' s t test. P<0.05 was considered as significant.

RESULTS

ANTIBACTERIAL ACTIVITY

Using agar well diffusion method, four different bacterial strains (*Staphylococcus aureus, E.coli, Klebsiella pneumoniae, and Proteus sp*) were plated with *Caesalpinia bonducella* at different extracts of solutions ranging (30µg/ml, 60µg/ml, 90µg/ml and 120µg/ml). After 24 h, the growth inhibition zones of the *Caesalpinia bonducella* showed that the drug exhibited selective antibacterial activity towards *Proteus*, with a maximum inhibition zone of 16.4 mm whereas the other bacterial strains showed limited antibacterial activity with a least zone of inhibition around 2.12 mm.(Figure:1)

FIGURE:1 Antibacterial activity of *Caesalpiniabonducella*seed against *Proteus*

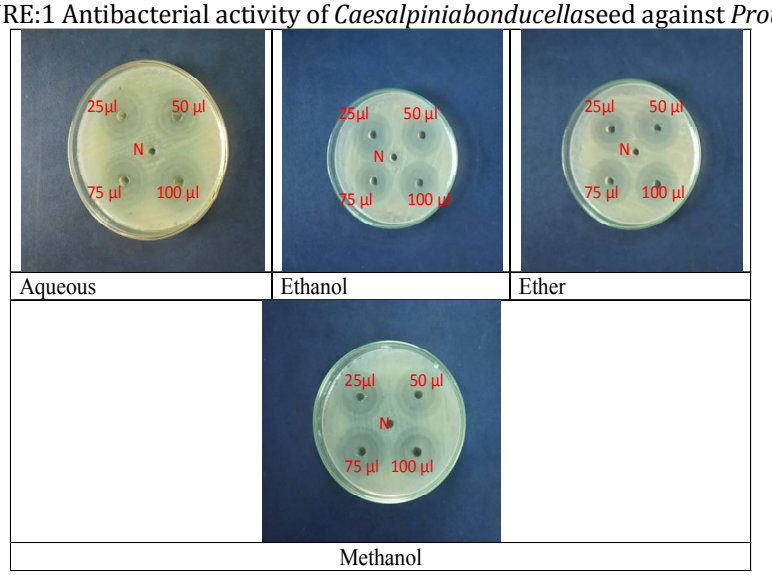

Table 1 Shows the antibacterial activity.

TABLE:1 Antibacterial activity of *Caesalpinia bonducella* seed

Different extract	Different concentration (µg/ml)	Zone of inhibition (mm)			
		Escherichia coli	***Klebsiella pneumoniae***	***Proteus* sp.**	***Staphylococcus aureus***
Aqueous	30	4.10±1.60	7.06±1.22	6.12±0.39	7.04±1.00
	60	6.00±1.03	5.66±1.05	5.01±2.16	6.66±1.08
	90	6.02±1.33	6.23±2.12	7.01±2.08	7.17±3.00
	120	2.16±0.50	3.00±1.33	5.00±1.00	6.01±2.01
Ethanol	30	6.00±2.13	2.12±1.20	7.00±1.10	6.60±1.10
	60	7.00±0.07	9.05±0.15	10.0±0.22	10.5±0.20
	90	6.15±0.26	7.22±0.12	6.04±0.17	8.00±0.19
	120	9.00±0.17	9.55±0.20	11.5±0.25	12.0±0.23
Ether	30	6.05±0.25	8.00±0.23	9.11±0.17	10.0±0.18
	60	9.66±0.10	10.8±0.25	11.5±0.30	12.6±0.17
	90	10.6±0.35	6.00±0.30	10.1±0.21	10.2±0.21
	120	13.0±0.47	13.4±0.46	14.0±0.40	15.4±0.45
Methanol	30	10.2±0.12	5.10±0.36	9.00±0.29	11.2±0.30
	60	13.1±-0.22	11.6±0.42	15.4±0.35	13.7±0.77
	90	15.3±0.16	6.11±0.49	16.4±0.35	12.2±0.30
	120	4.00±1.30	11.6±0.22	8.19±0.23	15.5±0.33

ANTIFUNGAL ACTIVITY

The ability of antifungal activity was evaluated to inhibit *Candida albicans*. Following the similar method above, the drug concentrations were plated. Interestingly, after 24 h, the growth inhibition zones of the test spores were determined which revealed that the drug exhibited selective anti-fungal activity with a maximum inhibition zone of 18.70 mm at 30 µg/ml in ether concentration. The *Caesalpinia bonducella* showed minimum inhibition zone of 8.01 mm. Also, there was a presence of fungal spore growth around the well diffusion.(Figure:2) Table 2 shows the antifungal activity.

FIGURE:2 Antifungal activity of *Caesalpiniabonducella*seed against *Candida albicans*

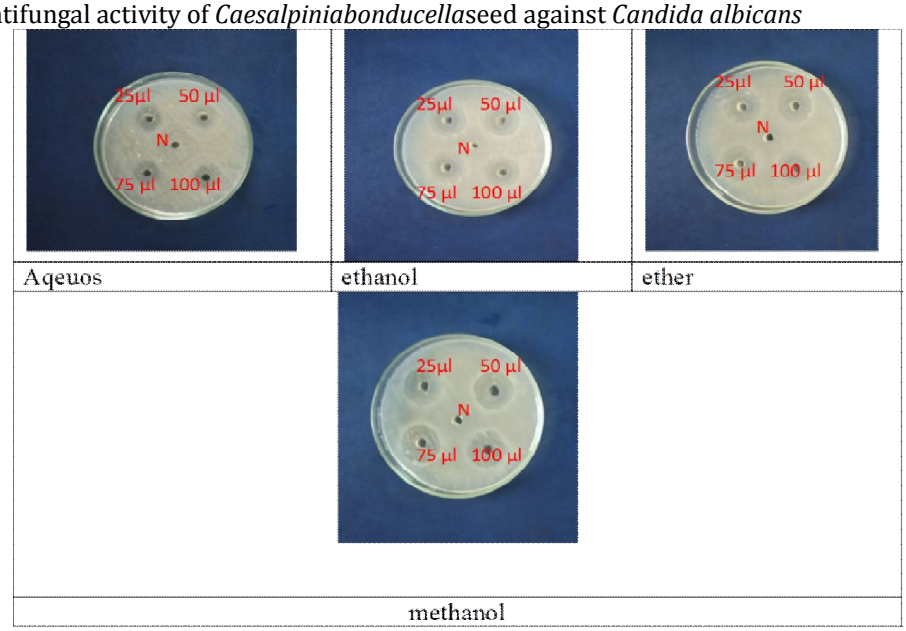

TABLE:2 Antifungal activity of *Caesalpiniabonducella*seed

Different extract	Different concentration (µg/ml)	*Candida albicans* Zone of Inhibition(mm)
Aqueous	30	18.12±0.15
	60	12.3±0.15
	90	12.10±0.27
	120	11.24±0.23
Ethanol	30	11.6±0.37
	60	10.32±0.15
	90	15.10±0.12
	120	11.5±0.20
Ether	30	18.70±0.15
	60	10.04±0.37
	90	15.5±0.30
	120	15.12±0.26
Methanol	30	12.91±0.21
	60	16.89±0.23
	90	18.00±0.25
	120	14.19±0.13

ANTIOXIDANT PROPERTIES

By measuring their DPPH FRSA, reducing ability and hydrogen peroxide FRSA, the antioxidant properties of *Caesalpinia bonducella* have been evaluated contents using crude methanolic extract of seed parts of these plants. As Mean ± Standard Deviation (SD) all data were expressed of the number of experiments (n =3)[13]. Using linear regression analysis of the percent inhibition obtained using different concentrations the antioxidant properties of *Caesalpinia bonducella* have been evaluated. The concentration required to produce 50% effect (IC50) was calculated and the regression equation [14] was obtained.

Total reducing power activity and hydrogen peroxide activity along with the results of the antioxidant characteristics of methanol extracts of the tested plant seed by the DPPH scavenging activity are presented in Table:3.

TABLE 3: Determination of antioxidant activity of potential Seed Extract of *Caesalpinia bonducella* by standard methods

Concentration (µg/µl)	Percentage of activity (%)					
	Reducing power assay		Hydrogen peroxide		DPPH assay	
	Standard (ascorbic acid)	Seed Extract of *Caesalpinia bonducella*	Standard (ascorbic acid)	Seed Extract of *Caesalpinia bonducella*	Standard (VitaminC)	Seed Extract of *Caesalpinia bonducella*
100	20.14±3.03	29.84±3.05	50.09±4.24	57.01±5.50	90.0±4.07	96.8±6.26
200	29.08±3.98	37.85±3.17	56.02±5.36	60.55±6.33	91.5±5.99	97.5±7.08
300	36.05±4.06	43.81±4.18	59.32±5.38	63.30±6.12	91.9±5.12	97.9±7.19
400	50.62±4.68	65.21±5.09	60.35±5.44	69.02±7.19	95.0±6.05	98.0±8.11
IC_{50}	**12.5**	**13.9**	**13.0**	**14.9**	**31.40**	**24.8**

FREE RADICAL SCAVENGING ACTIVITY BY DPPH METHOD

At different concentrations (100,200, 300 and 400µg/ml) the DPPH radical scavenging ability of the crude ethanolic extract of the seed of *C.bonducella* with the reference standard ascorbic acid it was compared [15]. It was found to be dose dependent by the activity of its extract. From low to high dose the percentage of antioxidant activity of extract was 96%, 97%, 97.5% and 98% with inhibition percentage being maximum at dosage 400 µg/ml which recorded 85% while that of ascorbic acid is 95% at the same concentration. The antioxidant property of DPPH radical scavenging ability is due to its hydrogen-donating ability. Standard ascorbic acid exhibited 90%, 91.5%, 91.9% and 95% of antioxidant activity at concentrations of 100, 200, 300 and 400 µg/ml respectively.

SCAVENGING OF HYDROGEN PEROXIDE

With the results of H_2O_2 scavenging, it was reported that the seeds of *Caesalpinia bonducella* possessed reliable hydrogen peroxide scavenging potential. H_2O_2 scavenging of *Caesalpinia bonducella* seed was examined to be in the range of 57% to 69%, and it was closely related to that Ascorbic acid. The IC 50 values for Ascorbic acid and *Caesalpinia bonducella* seed were recorded to be 14% and 13%. The seeds were efficient in scavenging H_2O_2 correlative with the standard Vitamin C as it was suggested by its data. In vivo the formation of hydrogen peroxide occurs by the reactions of superoxide dismutase and its catylisation. H_2O_2 can damage a number of compounds as a result of its oxidizing ability as it possesses the potential of crossing the membrane. Many cellular energy- producing systems are targeted by Hydrogen Peroxide. Metabolic perturbations happens because of excess H_2O_2. Hydrogen peroxide which is dose- dependent and its scavenging activity reveals that *Caesalpinia bonducella* seeds are effectual free radical scavengers, and it acts as an essential antioxidant.

REDUCING POWER ESTIMATION

As it can be seen from Table 1, it had been shown that the plant extract shows reducing activity in terms of Absorbance at 700 nm in the range from 29-65. In the presence of plant extract thus Fe3+ was transformed to Fe2+. A strong capacity of donating electrons from *Caesalpinia bonducella* has been indicated though it has low polyphenols content.

CONCLUSION

Enormous Therapeutic Potential is being possessed by Plant Based Antimicrobials and they do not show any side effects while they serve its purpose since they are often in association with the synthetic antimicrobials. This study hopes that it will lead to the establishment of results which could be used for investigation which further helps to isolate the active compounds from the extracts which is promising and it could be used to formulate more useful and potent Antioxidant and Antibacterial drugs which is new and it is the extract from Nature.

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CONFLICT OF INTEREST

The author's declared that they have no conflict of interest.

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