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Phytochemical Extraction and Antibacterial Studies of *Caesalpinia bonducella* Seed Extracts

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Abstract

Caesalpinia bonducella (Linn.) Flem. is an Indian herb belonging to Family Caesalpiniaceae. The seeds contain an alkaloid caesalpinine, bitter principles such as bonducin, saponins and other oils. In the present study, antibacterial activities of the extracts were studied by agar well diffusion method. *C. bonducella* seeds were porously powdered mechanically and were subjected to successive sequential soxhlet extraction using petroleum ether, chloroform, ethanol and water as the solvents. The bacterial cultures *Pseudomonas aeruginosa*, *Shigella dysenteriae*, *Proteus mirabilis*, *Staphylococcus aureus* and *Enterococcus faecalis* were selected for the study. Dry powder of each extract was dissolved in 1 ml of 0.5% DMSO solution to get a concentration of 5, 10, 15 and 20 mg/ml. Each extract was checked for antibacterial activity by introducing 100 µl of each extract into triplicate wells. Controls were maintained with respective solvents. Ciprofloxacin (5 mg/ml) was used as standard antibiotic. Results were subjected to analysis of variance. Chloroform extract have showed good antibacterial activity against *Proteus mirabilis*, *Pseudomonas aeruginosa*

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and *Staphylococcus aureus* followed by ethanol extract which showed good antibacterial activity against *Proteus mirabilis* and *pseudomonas aeruginosa*. Petroleum ether and aqueous extracts have showed good antibacterial activity against only *Proteus mirabilis*. There is no overcoming of growth in the zone of inhibition with prolonged incubation. The studies showed that chloroform extract has significant antibacterial activity.

Keywords: *Caesalpinia bonducella*, seed extracts, soxhlet extraction, antibacterial activity, agar well diffusion method.

Introduction

The toxicity to humans and other animals from antibiotics is generally considered to be low. However, prolonged use of certain antibiotics can have a negative impact on health. The inevitable consequence of widespread and injudicious use of antibiotics has seen the emergence of antibiotic-resistant pathogens, resulting in a serious threat to global public health. The resistance problem demands that a renewed effort be made to seek antibacterial agents effective against pathogenic bacteria resistant to current antibiotics. Possible strategies towards this objective include the increased use of bioactive compounds produced by plants and other sources. Focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. Herbal drugs or medicinal plants, their extracts and their isolated compounds have demonstrated a spectrum of biological activities. Ethnopharmacological studies on such herbs/medicinally important plants continue to be researched.

Caesalpinia bonducella (Linn.) Flem. is an Indian herb belonging to Family Caesalpiaceae. It is found throughout India and other tropical countries of the World [1]. '*Bonducella*' the name of the species is derived from the Arabic word "Bonduce" meaning a "little ball" which indicated the globular shape of the seed [2]. Fruit is also known as a Fever Nut; due to its antipyretic properties. The seeds contain an alkaloid caesalpinine, bitter principles such as bonducin, saponins and other oils. These compounds render the

herb its therapeutic properties [3]. Preparations of the seeds and other plant parts are used to treat a large range of ailments [4, 5]. Triterpenoids, fatty acid triglycerides, and sterols isolated from seeds may possibly explain some of the activity [6, 7]. Seed extracts have been shown to lower blood sugar in laboratory animals [8, 9] and effectively suppress or cure infections of several species of round worms [10]. Antipyretic and analgesic activities of *Caesalpinia bonducella* seed kernel extract [11] had been reported. The seed kernel powder was reported to have hypoglycaemic activity in experimental animals [12]. A new rearranged cassane furanoditerpene, caesalpinin was isolated from the roots of *Caesalpinia bonducella* [13]. Caesaldekalin C was also isolated from the roots of this plant [14].

The present study was carried out with the objective to studying the antibacterial potential of *Caesalpinia bonducella* seed extracts against known human pathogens.

Materials and Methods

Plant source - *Caesalpinia bonducella* seeds were collected from Junagadh Forest, Gir Area (Gujarat, India). The seeds were washed with distilled water thrice and dried on a blotting paper in the laboratory at 37 °C for 24 hrs.

Preparation of extract - After complete drying, the *C. bonducella* seeds were porously powdered mechanically and were subjected to successive sequential soxhlet extraction using petroleum ether, chloroform, ethanol and water as the solvents. 500 gm coarse powder was extracted. Different extracts were made by using standard solvents in increasing order of polarity. Petroleum ether and chloroform were procured from SD Fine Chem Ltd, Mumbai, India and Ethanol from Hong Yang Chemical Corporation, China. Each time before extracting with next solvent, the marc was air dried and then repacked into the soxhlet apparatus. All the three extracts were allowed for complete evaporation of the solvent on water bath and finally vacuum dried. The air dried marc of methanolic extract was mixed with water and kept on orbital shaker for 48 hours at room temperature; followed by heating on water bath and stored in air tight container.

Bacterial source

The bacterial cultures *Pseudomonas aeruginosa* (*P. aeruginosa*), *Shigella dysenteriae* (*S. dysentery*), *Proteus mirabilis* (*P. mirabilis*), *Staphylococcus aureus* (*S. aureus*) and *Enterococcus faecalis* (*E. faecalis*) were collected from hospitals and confirmed based on the colony, morphology, gram staining and biochemical analysis.

Extract Preparation

Dry powder of each extract 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. Ciprofloxacin (5 mg/ml) of concentration was prepared as standard antibiotic.

Assay of antibacterial activity

Antibacterial activities of the extracts were studied by agar well diffusion method [15]. A loopful of grown culture of various strains was inoculated into LB broth in aseptic condition. Cultures were incubated at 37°C for overnight. After incubation, the cultures were used to assess antibacterial activity. The 24 hrs old bacterial test pathogens were swabbed over the entire sterile agar surface to ensure even distribution of the inoculum on Mueller-Hinton agar plates. After allowing the inoculum to dry at room temperature, 6 mm diameter wells were bored in the agar. Each extract was checked for antibacterial activity by introducing 100 µl of each extract into triplicate wells. Controls were maintained with respective solvents. Ciprofloxacin (5 mg/ml) was used as standard antibiotic. The plates were allowed to stand at room temperature for 1 hr for extract to diffuse into the agar and then they were incubated at 37 °C for 24 hrs. Diameters of the zones of inhibition was measured in millimeter and documented.

Statistical analysis

The culture plates were observed periodically and zone of inhibition were recorded at regular intervals. Results were subjected to analysis of variance (*ANOVA*) and mean values were separated according to Duncan's multiple range test at $P = 0.05$.

Results and discussion

The result of antibacterial activity of the extract of *Caesalpinia bonducella* seeds is summarized in table 1. Chloroform extract have showed good antibacterial activity against *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* followed by ethanol extract which showed good antibacterial activity against *Proteus mirabilis* and *Pseudomonas aeruginosa*. Petroleum ether and aqueous extracts have showed good antibacterial activity against only *Proteus mirabilis*. There is no overcoming of growth in the zone of inhibition with prolonged incubation. The bacterial species showed a fairly high degree of sensitivity to chloroform extract. From the values of antibacterial activity, it can be observed that chloroform extract showed significant inhibitory activity. Chloroform extract exhibited more antibacterial activity than other extract. This may also be as a result of their phytochemical constituents.

It has been reported that bioactive natural products present in plants can be used as drugs and biological or pharmacological tools. The variation in activity of the extract showed that bioactive components had varied degree of solubility in the various solvents used for extraction. It is vital to note that extracts that produced no zone of inhibition, had no inhibitory component in them [16], [17], [18]. Studies on the methanolic and ethanolic seed extract respectively on *Garcinia kola* on some selected pathogenic micro organisms including fungi showed that the seed extracts had antimicrobial activity on the pathogenic micro organisms. Vaghasiya [19] found that methanol extract of *M. indica* seeds has potent antibacterial activity. The extraction of the biologically active compounds from the plant material depends on the type of solvents used in the extraction procedure. According to Parekh *et al.* [20], methanol, ethanol and water are the most commonly used solvents for determining the antimicrobial activity in plants.

Table 1: Antibacterial activity of Petroleum ether, Chloroform, Ethanol and Aqueous extracts of *Caesalpinia Bonducella* against some known human pathogens.

Concentration (mg/ml)	<i>Proteus mirabilis</i>	<i>Pseudomonas aeruginosa</i>	<i>Enterococcus faecalis</i>	<i>Staphylococcus aureus</i>	<i>Shigella dysenteriae</i>
Petroleum-ether extract of <i>Caesalpinia bonducella</i> ^b					
5	1.73b	ND	ND	ND	ND
10	1.72b	ND	ND	ND	ND
15	1.93a	ND	ND	ND	ND
20	1.63c	ND	ND	ND	ND
Chloroform extract of <i>Caesalpinia bonducella</i> ^b					
5	2.16a	2.0b	ND	1.53b	ND
10	2.23a	2.3a	ND	1.73a	ND
15	1.86b	2.0b	ND	1.66a	ND
20	1.70c	ND	ND	1.76a	ND
Ethanol extract of <i>Caesalpinia bonducella</i> ^b					
5	2.03a	2.23a	ND	ND	ND
10	2.13a	2.33a	ND	ND	ND
15	2.13a	2.26a	ND	ND	ND
20	2.03a	2.03b	ND	ND	ND
Aqueous extract of <i>Caesalpinia bonducella</i> ^b					
5	1.53b	ND	ND	ND	ND
10	2.03a	ND	ND	ND	ND
15	1.93a	ND	ND	ND	ND
20	1.90a	ND	ND	ND	ND
Standard Drug Ciprofloxacin					
5	4.0	3.5	3.7	3.5	3.6

^aData were collected after 24 hours of incubation and the zone of inhibition was taken in mm; ^bMean values followed by the same letter are not significantly different according to Duncan's multiple range test at $P = 0.05$; ND: not defined.

Conclusion

This study investigated the *in vitro* antibacterial activity of the extracts of *Caesalpinia bonducella* seed extracts. The results showed significant antibacterial activity with low MIC values. The seeds may serve as sources for compounds with therapeutic potency. The present study provides promising baseline information for the potential use of these crude extracts in the treatment of bacterial infections. *C. bonducella* could be a good candidate for

phytochemical and pharmacological investigations to discover new broad spectrum bioactive compounds.

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