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Preliminary phytochemical and antifungal screening of various organic extracts of *Caesalpinia bonducella* seeds

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Abstract

Ceasalpinia bonducella Fleming (Caesalpiniaceae) plant is well known for its medicinal value in Indian Ayurveda. However, more experimental data need to be collected in order to scientifically prove its efficacy. In this regard, the phytochemical screening conducted on various seed extracts of C. bonducella revealed the presence of several bioactive molecules that include oils, sterols, saponins, alkaloids, glycosides, phenols, tannins, flavonoids and resins. Besides, the ethyl acetate and aqueous extracts of C. bonducella seeds exhibited high to moderate antifungal effect against the tested fungal species of Aspergillus niger, Candida albicans, Fusarium oxysporum and Alternaria solani. The results obtained in this study indicate that C. bonducella possesses a potential value to control certain important fungal pathogens.

Keywords: *Ceasalpinia bonducella*, Phytochemicals, Filamentous fungi, Ethyl acetate extract

Introduction

Fungi are significant destroyers of grain and foodstuffs during storage, rendering them unfit for human consumption by diminishing their nutritive value and often by producing mycotoxins [1]. A significant proportion of the agricultural products in Brazil and all over the world become unfit for human consumption due to mycotoxins contamination. More than 25% of the world cereals are contaminated with known mycotoxins and more than 300 fungal metabolites are reported to be toxic to man and animals [2]. A sizeable portion of the world population living below poverty line in the developing and underdeveloped countries of Asia and Africa are suffering from health problems associated with the consumption of mycotoxin contaminated grains and cereals [3]. Even though the effective control of fungi in seeds can be achieved by the use of synthetic chemical fungicides, the same can not be applied to grains for reasons of pesticide toxicity [4]. Thus, there is a need to search for alternative compounds that are environmentally friendly, sustainable and that do not present health risks to humans, and help preserve the nutrient characteristics of food during storage. Plant extracts of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory trials [5]. Plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and present little danger to consumers compared to the synthetic pesticides [6].

C. bonducella, commonly known as Nata Karanja, a prickly shrub found throughout the hotter parts of India, Myanmar and Sri Lanka, has grey, hard, globular shaped seeds with a smooth shining surface. Seeds consist of a thick, brittle shell with a yellowish white bitter fatty kernel [7]. *C. bonducella* is reported to have multiple therapeutic properties like antipyretic, antidiuretic, anthelmintic and antibacterial [8], anti-anaphylactic and antidiarrheal [9], antiviral [10], antiasthmatic [11], anti-amoebic and anti-estrogenic [12]. Further, it has also been observed that *C. bonducella* has been traditionally used for the treatment of tumor, inflammation and liver disorders [13]. Additionally, the aqueous solution of the outer shell of the seeds of *C. bonducella* has also been used traditionally by the tribal people of Andaman and Nicober Islands for the relief of the symptoms of diabetes mellitus. Blood sugar lowering activity of *C. bonducella* has been primarily evaluated with significant results in rabbit and rat models [14].

However, there are only few reports available in the literature on the antifungal activity of some specific extracts of *C. bonducella* seeds. Therefore, the present study was undertaken to assess the antifungal activities of the various extracts derived from the seeds of *C. bonducella* in relation with their folklore medicinal properties.

Materials and Methods

Microorganisms

The fungi *Candida albicans, Aspergillus niger, Aspergillus flavus, Alternaria solani* and *Fusarium oxysporum* were originated from the local culture collection of Department of Botany, Laboratory of Microbiology, Dr. Hari Singh Gour University, Sagar, India. The cultures of the tested fungi were maintained on potato-dextrose agar (PDA) medium at 28 ± 2^{o}C and stored at 4^{o}C until use. A disc of fungal inoculum was removed from the previous cultures of all the fungal strains for antifungal screening.

Plant material

Seeds of *C. bonducella* were collected in March 2006 from Sagar District, Madhya Pradesh, India. Further taxonomic identification was conducted by Herbarium in charge of Department of Botany, Dr. Hari Singh Gour University, Sagar, MP, India. A voucher specimen was deposited in the Herbarium at the Laboratory of Ecology under the voucher specimen number (Bot/H/2692).

Preparation of the extract

The air-dried seeds of *C. bonducella* (50 g) were extracted with 500 ml of each solvent such as petroleum ether, benzene, chloroform, acetone, ethyl acetate and aqueous extracts separately using Soxhlet apparatus. The crude extracts were filtered and evaporated under reduced pressure to give a viscous dark mass of petroleum ether (7.2%), benzene (6.0%), chloroform (5.0%), acetone (6.4%), ethyl acetate (6.6%) and aqueous (7.2%) extracts. Griseofulvin was used as a reference standard antibiotic.

Preliminary phytochemical screening

To identify the phytochemical constituents present in *C. bonducella* seed extracts, a preliminary screening was carried by the application of various testing methods of Draggendorff's and Mayer's test, Liebermann-Burchard test, Foam formation test, Lead acetate test, Molisch's and Felhing's test and Ferric Chloride test for determining the presence of alkaloids, terpenes, steroids, saponins, flavonoids, polysaccharides and tannins, respectively [15], [16].

Antifungal activity assay

The *in vitro* antifungal activity was determined by agar diffusion assay for the determination of fungal mycelium growth inhibition percentage [17]. Aliquots (100 µl) of test solutions were added to each potato dextrose agar (PDA) plates containing 20 ml of agar. Discs (5 mm diameter) of the test species were cut from 7 day old culture on PDA plates and placed mycelial surface down, on opposite edges of the test plates on the center of dishes. The plates were then incubated in dark at 28 ± 1^0C. The extension diameter (mm) of hyphae from the center to the side of dishes was measured every 24 h for 5 days. Mean growth measurements were calculated from 3 replicates of each of fungal species. PDA plates treated with distilled water without extracts (negative control) and 100µl griseofulvin (1 mg/ml) served as reference positive control.

Growth inhibition of treatment against control was calculated as percentage of inhibition using the following formula: % Inhibition = C-T/C×100, where C is an average of 3 replicates of radial growth (mm) of control and T is an average of 3 replicates of radial growth (mm) of treatment.

Statistical analysis

Data were expressed as the mean ± standard deviation (S.D.) values of three independent experiments using Sigma Stat.

Results and Discussion

Phytochemical screening

C. bonducella seed extracts contained various phytochemical substances such as oils, sterols, saponins, alkaloids, glycosides, phenols, tannins, flavonoids and resins, depending on the solubility of the compound and the solvent used (Table1). The sensitivity of a specific test organism to the seed extracts may be due to the presence of specific active phytochemical in the individual extract. As reported by others, these phytochemicals may act as potent antifungal agents and their mode of antifungal action may involve the effect on membrane integrity, resulting in the lyses of fungal cell wall followed by the loss of intracellular dense material on the surface of treated cells, ultimately leading to cell death [18].

Table 1. Phytochemical screening of various organic solvent extracts obtained from *Caesalpinia bonducella* seeds

Constituents	Organic solvent extracts[a]					
	PEE	**BZE**	**CFE**	**ATE**	**EAE**	**AQE**
Fixed oil and fats						
Spot test	+	-	-	-	-	-
Saponification	+	-	-	-	-	-
Phytosterols						
Liebermann Test	+	-	+	-	-	-
Libermann-Burchard Test	+		+			
Saponins						
Foam Test	-	-	-	-	+	+
Alkaloids						
Dragendorff's Reagent	-	-	-		+	+
Mayer's Reagent	-	-	-	-	+	+
Wagner's Reagent	-	-	-	-	+	+
Tannic acid	-	-	-		+	+
Carbohydrates and Glycosides						

Molisch Test	-	-	-	-	+	+
Fehling Solution	-				+	+
Benedict Solution	-	-	-	-	+	+
Liebermann-Burchard Test	-	-	-	-	+	+
Legal test	-	-	-	-	+	+
Keller-Killani Test	-	-	-	-	+	+
Phenolic compounds and tannins						
Ferric Chloride Solution	-	+	-	+	-	-
Gelatin Solution	-	+	-	+		
Lead -Acetate Solution	-	+	-	+	-	-
Chlorogene Test	-	+	-	+	-	-
Gambin Flourescent Test	-	+		+	-	-
Flavonoids						
with ethyl acetate	-	-	+	-	-	+
with ammonia solution	-		+			+
Gum	-	-	-	-	+	-
Resins						
With H_2SO_4	+	-	-	+	-	-
Color reaction with HCl and Acetone	-	-	-	+	-	-

[a]PEE: Petroleum ether extract, BZE: Benzene extract, CFE: Chloroform extract, ATE: Acetone extract, EAE: ethyl acetate extract, AQE: Aqueous extract.
[*](+): Presence of the phytochemicals; (-): Absence of the phytochemicals.

Antifungal activity

Certain plant extracts possessing essential phytochemicals act in many ways on various types of disease complex, and may be applied in food, agriculture and medicine industries to control filamentous fungi and yeast. In the present study, among the tested seed extracts of *C. bonducella*, ethyl acetate, aqueous and petroleum extracts showed maximum antifungal effect as a radial growth inhibition percentage against *A. niger* (58.09%), *C. albicans* (44.73%) and *A. niger* (45.71%), respectively (Table 2). Also the aqueous extract exhibited a moderate antifungal effect against *A. solani* and *C. albicans*. However, insignificant inhibition was observed against all tested fungal species using benzene, chloroform and acetone seed extracts of *C. bonducella* (Table.2). Earlier papers on the analysis and antifungal properties of plant extracts have shown that they have a varying degree of growth inhibitory effects against some filamentous fungi and yeast [19]. Fungal cell wall may be considered to be a prime target for selectively toxic antifungal agents because of its chitin structure. Petroleum ether and ethyl acetate seed extracts of *C. bonducella* showed potent results of antifungal activity against *A. niger* which might be due to the presence of steroids and saponin types of phytochemicals in *C. bonducella* seeds, and these findings are in strong agreement with the previous findings of other researchers [19]. Besides, the traditional use of *Tribulus terrestris* documented that saponins exert antifungal activity by inhibiting fungal hyphae and destroying the ultra structure of fungi in particular [20].

Table 2. Percentage of mycelial growth inhibition in fungi treated with potential antifungal extracts obtained from *Caesalpinia bonducella* seeds[a]

Fungal species	Radial growth inhibition (% inhibition)						
	PEE[1)]	BZE	CFE	ATE	EAE	AQE	GF
Aspergillus niger	11.4 ± 0.17[2)] (45.71)	18.0 ± 0.06 (14.28)	22.4 ± 0.15 (29.0)	20 ± 0.62 (4.76)	8.8 ± 0.20 (58.09)	18.0 ± 0.15 (14.28)	21.0 ± 0.95 (100)
Aspergillus flavus	18.0 ± 0.44 (29.13)	20.6 ± 0.82 (18.89)	22.8 ± 1.14 (10.23)	22.2 ± 0.61 (12.59)	15.0 ± 1.00 (40.94)	19.8 ± 0.49 (22.04)	25.4 ± 0.21 (100)
Alternaria solani	8.2 ± 0.40 (24.07)	7.9 ± 0.35 (26.85)	9.5 ± 1.30 (12.03)	9.0 ± 0.86 (16.66)	6.8 ± 0.21 (37.03)	6.5 ± 0.67 (39.81)	10.8 ± 0.45 (100)
Fusarium oxysporum	15.0 ± 1.04 (31.81)	19.5 ± 0.70 (11.36)	20.0 ± 1.32 (9.0)	20.4 ± 1.06 (7.27)	12.0 ± 0.86 (45.45)	18.0 ± 0.20 (18.18)	22.0 ± 0.55 (100)
Candida albicans	22.2 ± 0.66 (26.97)	20.6 ± 1.05 (32.23)	28.6 ± 0.44 (5.92)	22.8 ± 2.10 (25.0)	18.2 ± 0.17 (40.13)	16.8 ± 0.98 (44.730	30.4 ± 0.66 (100)

[1)] PEE: Petroleum ether extract, BZE: Benzene extract, CFE: Chloroform extract, ATE: Acetone extract, EAE: ethyl acetate extract, AQE: Aqueous extract, GF: Griseofulvin (control, tested volume 100 µl/plate).
[2)] Mean ± SD (% inhibition).
[*]Growth inhibition of treatment against control was calculated as percentage of inhibition using the following formula: % Inhibition = C-T/C×100, Where C is an average of 3 replicates of radial growth (mm) of control and T is an average of 3 replicates of radial growth (mm) of plates treated with (aliquots of 100 µl/20 ml PDA/plate) extract solutions.

Conclusion

The ethyl acetate and petroleum ether extracts of *C. bonducella* seeds effectively inhibited the growth of all tested fungal pathogens with activity comparable to that of griseofulvin used as a reference compound. Also the aqueous extract exhibited moderate antifungal effect against *A. solani* and *C. albicans*. The phytochemical screening revealed the presence of alkaloids, glycosides and flavonoids in ethyl acetate, petroleum ether and aqueous extracts of *C. bonducella* seeds, which might be responsible for a wide range of antifungal activities, however they do not account for the overall activity and deserve further studies for their antifungal potential.

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