



Phytochemical Analysis and Antimicrobial Activity of *Caesalpinia bonducella* leaves

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ABSTRACT

The phytochemical analysis of leaves powder of *Caesalpinia bonducella* was carried out by using extraction method. The standard chemical procedure used to obtain constituents from dried plant tissues was by extracting powdered material in Soxhlet apparatus continuously. *Caesalpinia bonducella* leaves contain Terpenoids & Phenolics - 4.490%, Alkaloids - 0.885%, Q. Alkaloid & N-oxides - 25.745 %, fats and waxes - 8.850 % and Fibers - 60.050%.

The antimicrobial activity of the above extracts was also studied. Antimicrobial studies showed that the extracts had considerable activities against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella aerogenes*, *Aspergillus niger* and *Penicillium chrysogenum*.

Keywords: *Caesalpinia bonducella*, quaternary alkaloids and n-oxides, terpenoids and phenolics, phytochemical, antimicrobial activity.

INTRODUCTION

Only 5% of the total 3, 00,000 plant species has been studied scientifically for their medicinal purposes [1, 2]. More than 30% of all prescription drugs are derived from plant origin in the industrialized countries [3]. Medicinal plants are the main source of pharmaceuticals and healthcare products. Ayurveda, Unani, Homeopathy and Siddha are the traditional systems of medicines which mainly depend on the natural products [4]. People below poverty line in the undeveloped and developing countries of Asia and Africa are suffering from health problems connected with the consumption of mycotoxin contaminated grains and cereals [5].

To prevent illnesses and to maintain human health, phytochemicals are important sources of food and medicine [6]. Medicinal plant products are used as home remedies to treat specific conditions as well as complex preparations to treat life threatening diseases [7]. They are usually made by plant parts like leaves, flowers, bulbs and roots from different plant species in specific proportions based on the desired function of the concoction [8].

A mention has been made in various phytotherapy manuals about the medicinal plants and their uses in the treatment of infectious diseases because of their availability, less side effects and decreased toxicity [9]. Antibiotics which have been effectively used against bacterial infection are very significant things that have been discovered in the twentieth century. But these synthetic drugs are used to treat only one third of the known infectious diseases [10]. It is so because of indiscriminate and continuous use and misuse of antibiotics and consequently the resistant pathogens have emerged [11, 12]. Recently, antibiotic resistance has increased on large scale and it is going to be a big therapeutic problem. The problem can be solved with the help of antibiotic resistance inhibition from plants [13, 14].

To be safe from various pathogens, trees produce different compounds. Plant extracts are expected to be active against drug resistance pathogens [15]. For hundreds of years, various plants have been used to cure various human illnesses in many parts of the world traditionally. So, phytochemicals and biologically active compounds derived from plants which are used in herbal medicines have been focused by researchers [16, 17].

Caesalpinia bonducella is classified under the family of Caesalpinaceae. It is also known as *C. bonducella* Flem and *C. crista* Linn. Commonly it is called as Fever Nut, Bonduc Nut and Nicker Nut also [18]. Leaves, seeds, stem, bark, nuts and roots are useful as herbal medicines. It is found in hotter parts of India especially in west Bengal and the southern states of India. In conventional system of Indian medicine, Ayurveda, *Caesalpinia bonducella* (roxb.) is largely used for its antiperiodic, antipyretic, anti-inflammatory, anthelmintic, antimalarial and also for different diseases like skin diseases, hydrocele, leprosy, convulsions, orchitis, paralysis and analogical nervous complaints. It is also described to have antioxidant, antibacterial, antitumor and antidiabetic activities [19].

The phytochemical analysis of *Caesalpinia bonducella* leaves powder was carried out by using extraction method and leaves extracts were screened for antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella aerogenes*, *Aspergillus niger* and *Penicillium chrysogenum*. It may provide scientific justification for their use in indigenous communities and broaden the scope of existing drug discovery programs.

RESEARCH METHODS

Preliminary Phytochemical Analysis

To identify the phytochemical constituents present in *C. bonducella* leaves, a preliminary analysis of water and ethanol extracts was done by using different testing methods like Frothing test, Mayer's test, Hager's test, Foam formation test, Lead acetate test, Molisch's and Felhing's test and Ferric Chloride test [20, 21, 22].

Phytochemical analysis

Phytochemical analysis was done by using following procedure [23].

Accurately weighed 20 gm of the *Caesalpinia bonducella* leaves powder was continuously extracted in Soxhlet apparatus for 16 hrs. The 500 ml mixture of methanol and water in volume ratio 4:1 was used as extractant. The extract was cooled and filtered by using Whatman filter paper no.41 into a dry and preweighed beaker. From the residue, fats and waxes were separated firstly by using ethyl acetate solvent. The further separation was made by using separating funnel. The filtrate was acidified with the help of 2M H₂SO₄. The acidified filtrate was again extracted by using 150 CC (3 x 50 CC) chloroform in a separating funnel. The chloroform layer obtained was the moderately polar extract. It consists of terpenoids. The aqueous layer obtained was basified to pH-10 with 2M NaOH. It was again extracted with 120 CC (2 x 60 CC) chloroform and methanol in volume ratio 3:1 followed by extraction with 80 CC (2 x 40 CC) chloroform in a separating funnel. The aqueous basic layer was collected in a dry preweighed beaker. The methanol layer contains quaternary alkaloids and N- oxides and the chloroform extract was the basic extract. It consists of alkaloids. Those extracts like methanol crude extract, fats & waxes, terpenoids, quaternary alkaloids & N- oxides, alkaloids were screened for antimicrobial activities.

Procurement of cultures

All the microbial cultures (Table 3 and 4) were procured from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory (NCL) Pune.

Antimicrobial Activity

To carry out antimicrobial activity, agar diffusion method was used and the diameter of growth inhibition zone surrounding the antibiotic disc was measured to determine it. At first the sterilized Muller- Hinton agar medium 20 ml was poured into a sterile petriplate. Then the plate was covered and allowed to gel. The sterile cotton swab was dipped into the culture suspension of bacteria. The agar surface of each plate was inoculated by using the swab and ensuring the even distribution of the organism over the agar surface. The agar surface was allowed to dry for ten minutes. A sterile filter paper disc was picked up by the outer edge with sterile forcep and dipped the opposite edge of the disc in the prepared solution of the extract with concentration 100 µg/ml. The disc was placed near the edge of the agar surface of the inoculated plate. The incubation of the plates was carried out at 37°C for 24 hours. The transparent meter rule was used to measure the zones of inhibition. Chloramphenicol with concentration 10 µg/ml was used as standard [24, 25].

RESULTS AND DISCUSSION

The preliminary phytochemical screening revealed the presence of saponins, tannins, alkaloids, flavonoides, carbohydrates, proteins, phenolic compounds and quinine (Table 1). Saponins were not detected in the ethanol

extract. These constituents present in the leaves extracts have good therapeutic values. Saponins and terpenes have antimicrobial and curative properties against various pathogens [26]. Flavonoids have antifungal as well as antibacterial activity. They also possess anti-inflammatory property. Tannins and saponins are known to have antimicrobial properties [27].

Secondary metabolites of the plants like alkaloids, terpenoids and glycosides play the role of protective agents against different pathogens like insects, fungi or bacteria. They also function as growth regulatory molecules such as hormone like substances. Consequently, they are used as potential anticancer drugs by direct cytotoxic activity against cancer cells or by reducing the tumor development process [28].

Table 1: Preliminary Phytochemical Analysis of extracts of *Caesalpinia bonducella*.

Sr. No.	Phytochemical constituents	Water extract	Ethanol extract
1	Saponins	++	-
2	Tannins	+	+
3	Alkaloids	+	+
4	Flavonoids	+	++
5	Carbohydrates	++	++
6	proteins	++	++
7	Phenolic compounds	+	+
8	quinine	+	++

++ = High concentration; + = Low concentration; - = Absent.

Table 2: Phytochemical Analysis of *Caesalpinia bonducella*.

Sr. No.	Parameters	Net wt. of Content (gm)	% Composition
1	Fats and waxes	1.770	8.850 %
2	Terpenoids and phenolics	0.898	4.490%
3	Q. Alkaloid & N – Oxides	5.149	25.745 %
4	Alkaloids	0.177	0.885%
5	Fibers	12.010	60.05 %

Table 3: Bacterial culture.

Sr. No.	Name	Type	ATCC No.
1	<i>Bacillus subtilis</i>	Gram positive	ATCC 2239
2.	<i>Staphylococcus aureus</i>	Gram positive	ATCC 2178
3.	<i>Escherichia coli</i>	Gram negative	ATCC 25744
4.	<i>Klebsiella aerogenes</i>	Gram negative	ATCC 2239

Table 4: Fungal cultures.

Sr. No.	Name	ATCC No.
1	<i>Aspergillus niger</i>	ATCC 504
2.	<i>Penicillium chrysogenum</i>	ATCC 709

Table 5: Inhibition zones in mm of *Caesalpinia bonducella* against tested Microorganisms.

Sr. No	Compounds Name	Gram Negative bacteria		Gram Positive bacteria		Fungal Species	
		E-coli	K.aerogens	B.subtilis	S.aureus	A.niger	P.chrysogenum
1	Crude Methanol Extract	6.75	7.25	8.5	-	7	7.5
2	Fats & Waxes	7.5	-	-	-	9	9.5
3	Terpenoids	7.5	7.5	8	-	-	-
4	Quaternary Alkaloids & N-Oxides	7	-	10	8	7.5	7.75
5	Alkaloids	6.75	7.25	8.5	-	7	7.5
6	Standard (Chloramphenicol)	10	10	10	10	10	10

The phytochemical analysis study showed the presence of 4.490% terpenoids and phenolics (Table 2). In Pharmaceutical and food industries, terpenes are used as medicines and flavor enhancer because of their potentials and effectiveness. As antibiotic resistant bacteria are being increased globally, terpenes are important [29]. The group of terpenoids exhibits various pharmacological activities like anti-viral, anti-malarial, anti-inflammatory and anti-cancer activities. It also inhibits cholesterol synthesis [30].

The extract showed the presence of 0.885% alkaloids and 25.745 % Q. alkaloid & N-oxides (Table 2). Alkaloids govern plant growth. Different alkaloids show different medicinal properties such as Caffeine is stimulant; Codeine is cough medicine and analgesic; Quinidine is antiarrhythmic; Quinine is antipyretics and antimalarial; Reserpine is antihypertensive etc [31].

8.850 % fats and waxes and 60.050% fibers observed in this plant extract (Table 2). Lipids are useful in nutrition and dietary, food science, cosmetics, pharmaceuticals, paints and varnishes, detergents in human society [32].

In the anti-microbial studies (Table 5) all the extracts exhibited larger zones of inhibition against bacterial species like *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella aerogenes* as well as fungal species like *Aspergillus niger* and *Penicillium chrysogenum*.

CONCLUSION

In conclusion, the results of the present study showed that the crude ethanolic and aqueous extracts contain saponins, tannins, alkaloids, flavonoids, carbohydrates, proteins, phenolic compounds and quinines. The percentage of fats and waxes, terpenoids and phenolics, Q. Alkaloid and N-Oxides was observed good.

It showed good antibacterial and antifungal activities. The highest antibacterial activity was shown by Q-Alkaloid & N-Oxides against *B.subtilis* with zone of inhibition 10 mm followed by alkaloids against *B.subtilis* with zone of inhibition 8 mm. The highest antifungal activity was shown by fats & waxes against *P.chrysogenum* with zone of inhibition 9.5 mm and against *A.niger* 9 mm. It might be an alternate to synthetic antibiotics available in the market. So, its beneficial effect on animals as well as human health may be derived from its antibacterial and antifungal properties to afford protection against various diseases.

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