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Original Article

Evaluation of Pharmacological Properties of *Caesalpinia bonducella* Seed and Shell Extract

Vigasini Subbiah, Pannaga Nagaraja, Priya Narayan*, Holenarasipur Gundu Rao Nagendra

ABSTRACT

Background: *Caesalpinia bonducella* L. is a medicinal plant belonging to the family *Caesalpiniaceae*. It is a prickly shrub widely distributed all over the world especially in Indian tropical regions such as Kerala, Andaman and Nicobar Islands and Sri Lanka. There are claims that its leaves or seeds/ seed kernel possess antipyretic, antidiuretic, antibacterial, antiviral, antiestrogenic and antidiabetic activities. Due to the above properties several preparations of the plant were used in folk medicine. **Materials and Methods:** The aqueous extract of *Caesalpinia bonducella* nut containing the seed and the shell, has been evaluated for qualitative analysis of secondary metabolites (tannis, flavonoids, alkaloids, saponins, coumarins, quinone and phenols), *in-vitro* anti-inflammatory, anti-diabetic assay, antioxidant, antimitotic and antimicrobial activity. The studies were carried out using HRBC membrane stabilization, inhibition of alpha amalyse enzyme, DPPH method, green gram growth inhibition, agar diffusion method respectively. **Results:** Our results indicate the presence of Alkaloids, Flavanoids and Saponins. We report in our study the antidiabetic, anti-inflammatory, anti-oxidant, anti-microbial and anti-mitotic activity of *Caesalpinia bonducella.*

Key words: Anti-inflammatory, Anti-diabetic, Anti-mitotic, Anti-oxidant, *Caesalpinia bonducella.*

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INTRODUCTION

Caesalpinia bonducella L. also known as "fever nut" Bonduc nut and Nicker nut belongs to the family of *Caesalpiniaceae* and has been reported in Folklore medicine and ancient Ayurveda scriptures.[1-3] *C. bonducella* has been known to be used by Siddha practitioners in Malabar regions for psoriasis treatment.[1] *C. bonducella* is a large prickly shrub known to be a native of South India, Burma and Ceylon, particularly along the sea coast and up to 2500 ft. in hilly regions.[4] It is reported in literature that most parts of the plant has therapeutic properties, but much has been studied with the seed and shell.[5-6] The alkaloids in *Caesalpinia bonducella* L are known to be found in shell, seed and twigs, the predominant one being Natin. The active molecule, Bonducin is reported to be present in the seed as a powerful glycoside. Saponins and terpenoids are also known to be found in seed.[7] The shell is known to contain fatty oil, starch, sucrose, phytosterols, stearic, palmitic, oleic, linoceric, linolenic and a mixture of unsaturated acid of low molecular weights. The protein and amino acid content varies from 7.430 to 25.346%.[8] The seeds are reported to have anti-diabetic properties. Type 2 diabetes, a chronic metabolic disorder affects people of all ages across the globe. This disease is characterized by increase in the blood glucose level which may be multifactorial. The primary cause is the decrease or lack of insulin production. The treatment regimen for Type 2 Diabetes is mainly to prevent breakdown of carbohydrates to glucose and preventing its diffusion into the intestinal membrane into blood stream. The abundance of natural resources in India and the rising numbers of Diabetes patients will pave the way for newer medications/adjunct therapies to manage the disorder.[9] Inflammation is often associated with pain and involves the increase of vascular permeability, increase of protein denaturation and membrane alteration. When the cell undergoes injury, inflammation of tissue becomes a defensive response characterized by redness, pain, heat and swelling and loss of function in the injured area. The management of inflammation related diseases is of concern and may have to be addressed using plant extracts.[10] The plant is also known to possess antioxidant,[11] antifilarial activity,[12] anticonvulsive activity[13] and anti-microbial activity,[14] antimalarial activity[15] antitumor activity[16] anti-ulcer activity[17] immunomodulatory activity[18] and anticataract activity.[19] With the rising problems of Diabetes, inflammation related diseases and cancers; there is a need to address the issues using alternate therapy. As there is limited study on the aqueous extract of *Caesalpinia bonducella* L, shell and seed we have evaluated their pharmacological properties.

MATERIALS AND METHODS

Plant Material

Nuts of the *C. bonducella* were collected from the local market in Bengaluru, Karnataka, India. The

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seed and shell extract was prepared by following the method of Shukla *et al.*[20] The nuts were shade dried, coarsely powdered and sieved to get a uniform powder. The sample was extracted in water using Soxhlet extractor. The crude extract obtained was evaporated and concentrated.

Phytochemical analysis

Phytochemical analysis was carried out for saponins, flavonoids, quinones, alkaloids and tannins were performed as described by Maria Shabbir *et al.*[21] Wagner's reagents was used for alkaloid, foam test for saponins, lead acetate test for flavonoids, Braemer's test for tannins, Sulphuric acid test for quinones. All these experiments were carried out for water extract for seed and shell individually.

In-vitro Anti Inflammatory Assay

The activity was carried out by the method of Gandhisan *et al.*[22] The blood was collected from healthy volunteers and mixed with equal proportion of Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl). The sample was centrifuged at 3,000 rpm and cells washed with saline.

Extract concentrations of 200, 400, 600, 800 and 1000µg/ml was prepared using distilled water. To this 1 ml of extract, 1 ml of phosphate buffer, 2 ml hypo saline and 0.5ml of HRBC suspension were added. It was incubated at 37°C for 30 min and centrifuged at 3,000 rpm for 20 min. The percentage of HRBC membrane stabilization or protection was calculated by using the following Formula with Aspirin (1 mg/ml) as reference standard drug

% protection = 100- (Optical density of drug treated sample/ Optical density of control) x100

Anti-Diabetic Assay

The anti-diabetic assay was carried out using 100 µl of (500,1000µg/ml) plant extracts and 200 µl of amylase and incubated at 37°C for 20 min. To the reaction mixture 1% starch (100 µl) was added and incubated at 37°C for 10 min. The reaction was arrested by adding 200 µl DNSA and keeping in a boiling water bath for 5 min. The reaction mixture was diluted with 2.2 ml of water and absorbance read at 540 nm against blank.[23]

Determination of DPPH free radical scavenging activity

The antioxidant property was assessed using Ascorbic acid as the standard and DPPH (1,1-diphenyl-2-picrylhydrazyl) as control. 100µl of the extract was taken with 3ml of DPPH solution and incubated for 30 min. The absorbance was read at 517nm and the ability of the DPPH to scavenge free radical was calculated using the formula

$$\text{DPPH scavenged (\%)} = \{(Ac - At)/Ac\} \times 100$$

Where Ac is the absorbance of the control reaction and At is the absorbance in presence of the sample. The antioxidant activity of the extract was expressed as IC_{50} which is the concentration in mg of dry material per ml (mg / ml) that inhibits the formation of DPPH radicals by 50%. Each value was determined from regression equation.[24]

Anti-mitotic activity

Green gram seeds of equal weight were germinated in 500 µl of (10, 20, 30, 40 mg/ml) plant extracts in a 24-well microtiter plate. Seeds germinated in distilled water served as the control and that in drug doxorubicin as the standard (Kumar and Singhal, 2009). For the morphological study, the length of the radical was observed. Experiment was performed in triplicates.[25]

Anti-Microbial Activity

The antimicrobial activity of the extract was assessed using *Staphylococcus aureus, Candida albicans* and *Mycobacterium smegmatis* by the agar diffusion method. The agar diffusion method was used to evaluate the anti-microbial activity of the plant extract. 30 ml of nutrient agar was poured into petri plates containing 100µl of microorganisms (McFarlands Number 5). After 24 h the zone of inhibition was measured and compared using Streptomycin and Candid B as the standards for bacteria and fungi respectively.

RESULTS

Phytochemical analysis

The qualitative analysis of the secondary metabolites like tannins, flavonoids, alkaloids etc., was done for water extract of *C. bonducella* and the results tabulated in Table 1. Our results indicate the presence of flavanoids and alkaloids in both parts of the nut. The seed and shell was rich in saponins indicating its therapeutic value.

In-vitro Anti Inflammatory Activity

The HRBC membrane stabilization method was used to study the anti-inflammatory activity. The prevention of hypo tonicity induced HRBC membrane lysis was taken as measure in estimating the anti-inflammatory property. The % protection is indicated in Figure 1 and depicted in Table 2. The maximum anti-inflammatory activity of seed and shell was found to

Table 1: Phytochemical analysis of water extract of seed and shell. '+' indicates the presence of the metabolites and '-'indicates the absence of the metabolites. '+++' indicates higher concentration of metabolites.

	Aqueous Extract	
	Seed	Shell
Tannins	-	-
Flavanoids	+	+
Alkaloids	+	+
Saponins	+++	++
Coumarins	-	-
Quinones	-	-
Phenols	-	-

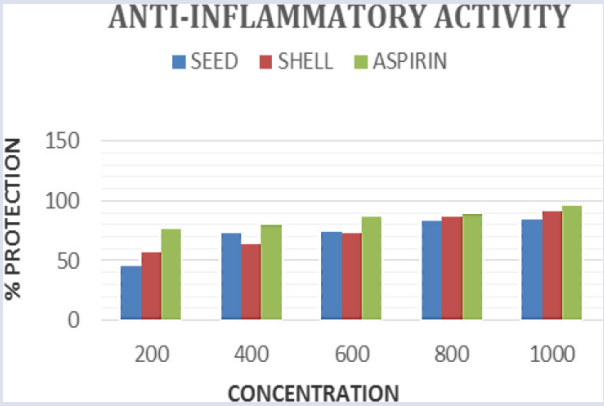

Figure 1: *In-vitro* Anti- inflammatory activity of *C. bonducella* aqueous extract.
(source: Personal collection)

Table 2: *In vitro* Anti- inflammatory activity of *C. bonducella* aqueous extract.

% Protection	200 µg/ml	400 µg/ml	600 µg/ml	800 µg/ml	1000 µg/ml
Seed	44.927 ± 4.03	72.463 ± 2.366	73.913 ± 4.81	82.608 ± 4.098	84.37 ±2.19
Shell	56.521 ± 6.072	63.768± 1.366	72.463 ±3.414	86.956 ± 3.56	91.304± 4.054
Aspirin	76.811 ± 0.682	79.71 ± 1.18	86.956 ± 4.381	88.405± 4.782	95.652 ± 4.652

Table 3: Inhibition of α-amylase by aqueous extract of *C. bonducella*.

% Inhibition	500µg/ml	1000µg/ml
Shell	31.818%	78.947%
Seed	-	47.36%
Glycomet GP2(Standard drug)	59.20%	80.97%

Table 4: % antiradical activity of *C. bonducella* aqueous extract.

% Anti -radical activity	200 µg/ml	400 µg/ml	600 µg/ml	800 µg/ml	1000 µg/ml
Shell (in %)	41.17 ± 6.355	55.88 ± 1.385	58.82 ± 1.3	61.76 ± 1.385	61.76 ± 1.385
Seed (in %)	-	-	-	-	-
Standard (in %)	74.28 ± 2.75	80 ± 2.696	85.71 ± 1.16	87.14 ± 1.78	88.571 ± 2.34

be 84.37% and 91.304% respectively at 1000 µg/ml and the % protection of shell was close to that of standard drug aspirin.

Anti-Diabetic Assay

Inhibition of α-amylase is considered a strategy for the treatment of disorders in carbohydrate uptake such as diabetes and obesity. α-amylase activity can be measured *in-vitro* by hydrolysis of starch in presence of α-amylase enzyme. Glycomet GP2 was used as the standard drug. The results of the Anti- diabetic activity is tabulated in Table 3. The maximum activity was at a concentration of 1000µg/ml and shell showed a better antidiabetic activity compared to the seed. It is also observed that the antidiabetic activity of the shell extract is close to the standard value.

Determination of Antioxidant Efficacy by DPPH Method

1,1-Diphenyl-2-picrylhydrazyl is a stable free radical with red colour (absorbed at 550nm). If free radicals have been scavenged, DPPH will change its colour to yellow. This assay uses this character to show free radical scavenging activity. The seed did not show any anti-oxidant activity. % antiradical activity of the sample is indicated in Table 4. The IC_{50} value was calculated and represented in Figure 2.

Anti-Mitotic Assay

Seeds of equal weight were taken in each well and 500µl of the extract of various concentrations were added. The dry weight of the seeds was taken after 24h and 48h. Doxorubicin (1mg/ml) was used as the standard drug and it showed 20% inhibition after 24h and 51.1% inhibition after 48hrs. Shell did not show any anti-mitotic activity. The results are tabulated in Table 5. Maximum inhibition of growth was found at 40mg/ml of seed extract as seen in Figure 3 and 4.

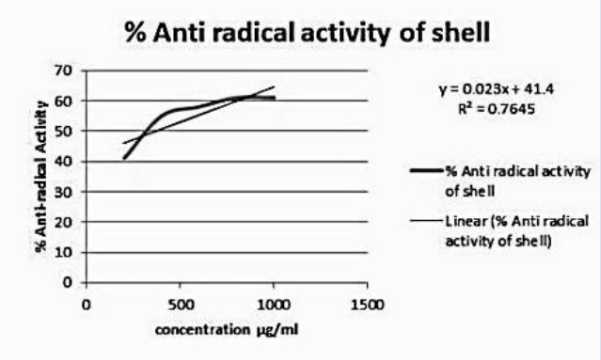

Figure 2: IC_{50} value of the aqueous extract of shell. The IC_{50} was found to be 350.638 µg/ml for the shell extract.
(source: Personal collection)

Figure 3: A: Seed germination at 24 h; B: Seed germination at 48 h.
(source: Personal collection)

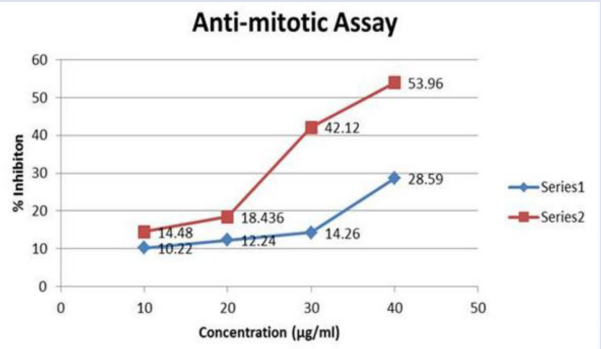

Figure 4: Antimitotic assay for *C. bonducella* aqueous extract.
(source: Personal collection)

Table 5: % inhibition of seed growth, shell extract did not show inhibition of seed growth.

Seed	10 mg/ml	20 mg/ml	30 mg/ml	40 mg/ml
24 h	10.22%	12.24%	14.26%	28.59%
48 h	14.48%	18.436%	42.12%	53.96%

Table 6: Zone of inhibition of the *C. bonducella* crude extract.

Zone of inhibition	Crude extract of shell	10mg/ml of shell extract	Crude extract of Seed	10mg/ml of seed extract	standard
Staphylococcus aureus	11mm	-	-	-	12mm (Streptomycin)
Candida albicans	10mm	-	-	-	10mm (Candid B)
Mycobacterium smegmatis	11.5mm	-	-	-	12mm (Streptomycin)

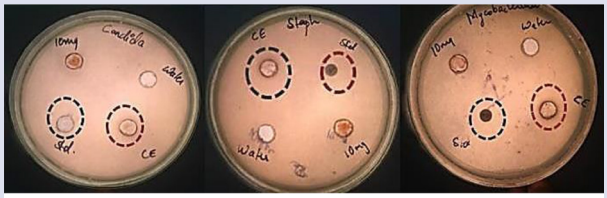

Figure 5: A: Zone of inhibition for *Candida albicans* B: Zone of inhibition for *Staphylococcus aureus* C: Zone of inhibition for *Mycobacterium smegmatis*.
(source: Personal collection)

Anti-Microbial Studies

The antimicrobial activity was assessed for the aqueous extract of shell using the agar diffusion method. The zone of inhibition was measured using streptomycin and Candid B as the standard for bacteria and fungi respectively. The results are indicated in Table 6 and Figure 5. Our results indicate that the crude extract of the sample showed a better antimicrobial activity, indicating its use as a topical application.

DISCUSSION AND CONCLUSION

Many herbal remedies have been employed in various medical systems for the treatment and management of different diseases. The plant *Caesalpinia bonducella* (syn: *Caesalpinia crista* Linn.) has been used in different system of traditional medication for the treatment of diseases and ailments of human beings.[26] Phytochemicals are a class of molecules found predominantly in tea, grapes, berries, cocoa and other plants. These are known to have diverse pharmacological properties.[27] Though they do not have any nutritive value the protective and disease preventing properties have been well explored. It is in this context that the study of the pharmacological properties of *Caesalpinia bonducella* was conceived.[28] Flavonoids are found in fruits, nuts, grains and vegetables and used extensively to study their effect on heart diseases and cancer. Flavanoids are known to exhibit anti-inflammatory and anti-oxidant and anti-microbial properties. This is in accordance with our results wherein the flavonoid content in the seed and shell extract are moderately high. Taken together, these results indicate the anti-oxidant and anti-inflammatory properties of Bondoc nut. Our results reveal the antimitotic activity of the seed extract and hence may be exploited further for the treatment of cancer. Our experimental results also reveal the inhibition of α amylase activity, indicating that the plant may be used in anti-diabetic therapy. Further, our results indicate the presence of Alkaloids, Flavanoids and Saponins. We report in our study the antidiabetic, anti-inflammatory, anti-oxidant, anti-microbial and anti-mitotic activity. Taken together our results indicate the use of *C bonducella* as an adjunct therapy for inflammation and diabetes.

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CONFLICT OF INTEREST

The author declare no conflict of interest.

ABBREVIATIONS

DPPH: 1,1-diphenyl-2-picrylhydrazyl.

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GRAPHICAL ABSTRACT

SUMMARY

- The study reported the presence of various Alkaloids, Flavonoids and Saponins in the seed/shell of *Caesalpinia bonducella* L. Due to the presence of these bioactive compounds, the seed and the shell extracts showed antidiabetic, anti-inflammatory, anti-oxidant, anti-microbial and anti-mitotic activity. *C bonducella* can thus be used as an adjunct therapy for inflammation and diabetes. The use of *C bonducella* seed extract for the treatment of cancer requires further research.

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