

Pharmacognostical Screening and Radical Scavenging Activity of Nicker Bean seed

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Abstract

The utilization of botanicals has gained more significance because of its natural origin and high therapeutic significance. The plant Nicker Bean botanical known as *Caesalpinia bonducella* has been used in diverse system of traditional medication for the treatment of diseases and ailments of human beings. The Plant has been reported as anti-asthmatic, antidiabetic, anti-inflammatory, anti-oxidant, anti-bacterial, anti-filarial, anti-tumor, immunomodulatory, hypoglycemic, activity. The work deals with preliminary Pharmacognostical studies of *Caesalpinia bonducella* seed, further seed extracts (ethanolic and aqueous) were evaluated for radical scavenging activity.

Keywords: *Caesalpinia bonducella*, Physiochemical parameters, phytochemical screening & radical scavenging.

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INTRODUCTION

The therapeutic worth of many indigenous plants, for various diseases has been described by traditional herbal medicinal practitioners. Natural products are the source of synthetic and traditional herbal medicine [1]. Ancient Indian literatures incorporate a extremely broad definition of medicinal plants to be potential sources of medicinal substances [2]. More than 500 species of *Caesalpinia* has been identified has medicinal benefits based on their pharmacological activity. One of the medicinal plants from this genus is *Caesalpinia bonducella* Linn. (*C. bonducella*) an Indian herb belonging to Family *Caesalpiniaceae*[3]. The name of the species "Bonducella" is derived from the Arabic word "Bonduce" meaning a "little ball" which indicated the globular shape of the seed [4]. The reactive oxygen species (ROS) stir up superoxide anionic radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH) are implicated in oxidative damage to various cellular macromolecules. Increasing number of confirmation suggested that oxidative stress induced biochemical changes are crucial etiological factors in several chronic human diseases such as diabetes mellitus, cancer, atherosclerosis, arthritis, inflammation and neurodegenerative diseases. There are many synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are available which are renounced unsafe. Therefore, in recent year's considerable attention has been directed to recognize

the natural antioxidants (plant derived) that may be used for human consumption⁵. In the present investigation we have attempted to investigate preliminary phytochemical screening along with antioxidant potential in ethanolic and aqueous extract of seed of *Caesalpinia bonducella*.

MATERIALS AND METHODS

Collection & Authentication

Herb was collected from Moolchand and phoolchand shop, jummerati, bhopal (M.P). The plant was collected in the month of August- September 2019 & authenticated. Seeds were separated and were made completely clean and dust free.

Drying and Size Reduction of Plant Material

The plant material was dried under shade. It was pulverized to coarse powder with the help of mixer grinder. The coarse powder was passed through sieve No.20 to maintain uniformity and packed into airtight container and stored in cool and dry place. This material was used for the further study.

Preparation of Crude Extract

500 gram of powdered plant material was extracted using a Soxhlet apparatus with 500 ml 90% ethanol and 500 gram with distilled water at 60–70°C for 35 complete cycles. The extracts were dried at 30–40°C.



Fig-1: Extraction process

PHARMACOGNOSTICAL SCREENING

Organoleptic evaluation

Various outer morphological characteristics like color, shape, size taste etc. were identified.

Screening of seed powder (Physiochemical analysis) [6-8]

Loss on Drying

10 gm of the powdered drug was weighed in a tarred petridish. It was dried at 105°C for 1 hour in hot air oven and then reweighed. Loss on drying was determined by calculating the initial and final weight.

Total Ash value

About 5 gm accurately weighed powdered drug was incinerated in a silica dish at a temperature not exceeding 450°C until free from carbon in muffle furnace. It was then cooled and weighed. The % w/w of ash with reference to the air-dried drug was calculated.

Acid insoluble ash value

1 gram ash was boiled for 5 minute with 25ml hydrochloric acid by covering the crucible with a watch-glass on water bath then cooled. The watch-glass was rinsed with 5 ml of hydrochloric acid and this liquid was added in to the crucible. Then the content was filtered on a previously weighed filter paper and filtrate was dried and weighed. Acid insoluble ash value was determined by calculating the % content remaining after deducting the weight of filter paper.

Water soluble ash value

1 gram ash was boiled for 5 minute with 25ml distilled water by covering the crucible with a watch-glass on water bath then cooled. The watch-glass was rinsed with 5 ml of distilled water and this liquid was added in to the crucible. The % of remaining content was deducted from initial % of ash taken (i.e. 100%) to determine the water soluble ash value.

Foaming Index

1 gm coarse powder was weighted and transferred to a 500 ml conical flask containing 100 ml of water. It was maintained at moderate boiling for 30 minute on water bath. It was cool and filtered in to a 100 ml volumetric flask. Volume was diluted by adding

sufficient amount of water. The decoction was poured in test tube, and then shaken in a lengthwise motion for 15 seconds. They were allowed stand for 15 minutes and the height of foam was measured to determine the foaming index.

Screening of crude extracts (Qualitative Phytochemical Analysis)

The crude extracts obtained by solvent extraction were subjected to various qualitative tests to detect the presence of common chemical constituents as:

Tests for Alkaloids

Dragendorff's test

To the 1 ml of extract, add 1 ml of Dragendorff's reagent (potassium bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.

Mayer's test

To the 1 ml of extract, add 1 ml of Mayer's reagent (Potassium mercuric iodide solution). Whitish yellow or cream coloured precipitate indicates the presence of alkaloids

Tests for Glycosides

Legal's test

Dissolve the extract in pyridine and add sodium nitroprusside solution to make it alkaline. No formation of pink to red colour shows absence of glycosides.

Baljet's test

To 1ml of the test extract, add 1ml of sodium picrate solution and the yellow to orange color reveals the presence of glycosides.

Tests for carbohydrate

Benedict's test

To 5ml of Benedict's reagent, add 1ml of extract solution and boil for 2 minutes and cool. Formation of red precipitate shows the presence of sugars.

Molisch's test

A small fraction from the respective extracts was taken in ethanol separately and a few drops of 20% w/v solution of α -naphthol in ethanol (90%) were added to it. After shaking well, about 1 ml of concentrated sulphuric acid was allowed to flow carefully by the side of the test tube. A reddish violet ring at the junction of the two layers indicated the presence of carbohydrates.

Tests for steroids

Salkowski test

The extract was dissolved in chloroform and equal volume of conc. H_2SO_4 was added. Formation of bluish red to cherry color in chloroform layer and green

fluorescence in the acid layer represents the steroidal components in the tested extract.

Liebermann-Burchard test

A small portion from each extract was taken with about 1 ml of acetic anhydride and dissolved by warming. The contents were cooled and a few drops of concentrated sulphuric acid were added in each case by the sides of the test tube. Appearance of blue colour indicated the presence of sterols.

Test for Proteins

Biuret test

Add 1ml of 40% sodium hydroxide solution and 2 drops of 1% CuSO₄ solution till a blue color is produced, and then add to the 1ml of the extract. Formation of pinkish or purple violet color indicates the presence of proteins.

Tests for Saponins

A little fraction from the various extracts were boiled with about 1 ml of distilled water and shaken. Appearance of a characteristic foam formation indicated the presence of Saponins. Aqueous and alcoholic extracts were tested directly.

A little fraction from various extracts was taken with about 2 ml of distilled water. A small quantity of sodium carbonate was added to each and shaken. The characteristic foam formation indicated the presence of Saponins. Aqueous and alcoholic extract were tested directly.

Tests for Tannins

A small fraction of the residue from each extract was dissolved in about 2 ml of distilled water separately and filtered. The filtrate was tested with the Ferric chloride solution. Appearance of a blue to bluish green or bluish-black colour indicated the presence of tannins

Tests for Flavonoids

Shinoda test

To the test solution add few magnesium turnings and concentrated hydrochloric acid dropwise, pink scarlet, crimson red or occasionally green to blue colour appears after few minutes.

Alkaline reagent test

To the test solution add few drops of sodium hydroxide solution, intense yellow color is formed which turns to colorless on addition of few drops of dilute acid indicate presence of Flavonoids.

ANTIOXIDANT ACTIVITY

DPPH Radical Scavenging Activity

Ascorbic acid and EtOH * Aqueous extract were weighed (25 mg each) and dissolved in 250 ml of methanol to get 500µg/ml stock solutions separately. Lower concentrations of ascorbic acid and extracts (2,

4, 6, 8, 10 µg/ml and 2, 4, 6, 8, 10 µg/ml respectively) were prepared by serially diluting stock solutions. The stable DPPH radical was used for determination of free radical-scavenging activity of the extracts. The 0.1 mM solution of DPPH in methanol (22.2 mg in 1000ml) was freshly prepared. Different concentrations of extracts were added at an equal volume (2ml) to methanolic solution of DPPH. After 30 min at room temperature, the absorbance was recorded at 517 nm. IC₅₀ values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals. Radical scavenging activity was calculated by the following formula [9].

$$\% \text{ Radical Scavenging Activity} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} * 100$$

Where, A_{control} = Absorbance of control A_{sample} = Absorbance of sample

The inhibition curve was plotted for duplicate experiments and represented as % of mean

Inhibition ± standard deviation

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RESULT AND DISCUSSION

The remedial properties of medicinal plants are possibly due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols etc. The macroscopic characteristics of *C. Bonducella* seed was tabulated in table 1. The phytochemical parameters were tabulated in table 2. The successive extraction seed of *C. Bonducella* were carried out in ethanol & water. The result was tabulated in table 3. The successive extraction was carried out by hot percolation method and % yield was determined (table 4). The reveals that ethanolic extract showed high % as compared with aqueous extract. The preliminary phytochemical tests revealed the presences of flavonoids, saponins & glycosides in both the extracts. (Table 5). Thus, the preliminary screening tests may be helpful in the detection of the bioactive principles and consequently may lead to the drug discovery and development. Antioxidant activities of both the extracts were carried out by DPPH by using ascorbic acid as standard. DPPH is a purple-coloured stable radical of organic nitrogen with a maximum absorbance at 517 nm and it is widely used to study radical scavenging activities of extracts and pure compounds. When the odd electron becomes paired off in the presence of a free radical scavenger to form

hydrazine, the absorption reduces and the DPPH solution is decolourised from deep violet to light yellow. The degree of reduction in absorbance measurement is indicative of the radical scavenging (antioxidant) power of the extract. IC₅₀ (concentration required to obtain a 50% antioxidant capacity or is the concentration of substrate that brings about 50% loss of the DPPH) is typically employed to express the antioxidant activity and to compare the antioxidant capacity of various samples [2, 4]. In the present study, the IC₅₀ values of ethanolic and aqueous extract were found to be 19.1 and 21.46 µg/ml. The results were tabulated in table 6 and the % inhibitions vs. concentration graph were showed in fig.2, 3 & 4.

CONCLUSION

The present study deals with macroscopic, phytochemical analysis and antioxidant activity of *C. Bonducella* seed. These studied will provide referentially information for correct identification and for standardization. The preliminary phytochemical screening of aqueous & ethanolic extracts showed presence of flavonoid in both the extracts. Free radical creation in animal cells can either be accidental or deliberate. With the increasing acceptance of free radicals as common place and important biochemical intermediates, they have been implicated in a large number of human diseases. The scavenging activity of flavonoids present in aqueous and ethanolic extract had been determined by DPPH.

Table-1: Organoleptic Seed characteristic

S.No.	Characteristics	Observation
1.	Colour	The seed color was dark Green.
2.	Odor	Characteristic
3.	Taste	Acrid
4.	Size	2-2.5cm length, 0.5-1cm width
5.	Texture	Soft
6.	Fracture	Tough.

Table-2: Physiochemical analysis

S.No.	Parameters	Observations
1	Loss on drying	0.1%
2	Foaming index	Less than 20 %
3	Total Ash value	2.95%
4	Acid insoluble ash value	1.27%
5	Water soluble ash value	1.19%

Table-3: Extractive values of *Ceasalpinia Bonducella*

Plant	Ethanol	Water
<i>Ceasalpinia Bonducella</i>	0.89 %	0.7%

Table-4: % yields of Successive Solvent extracts of *Ceasalpinia Bonducella* in following solvents

S. NO.	Extracts	Percentage yield
1	Ethanolic	1.3%
2	Aqueous	1.8%

Table-5: Phytochemicals Screening of *Ceasalpinia Bonducella* seeds.

S.No.	Phytochemicals	Test	Ethanolic extract	Aqueous extract
1	Alkaloids	Mayer's test, Dragendorff's test	-	-
2	Tannins	Ferric chloride test	-	+
3	Flavonoids	Shinoda's Test	+	+
4	Glycosides	Legal's test ,Baljet's test	+	+
5	Carbohydrates	Benedict's test	-	+
6	Amino acid	Biuret test	-	-
7	Fixed oil & Fats	Spot test	-	+
8	Steroid	Salkowski test	-	-
9	Saponins	By shaking the extract in test tube	+	+

(+ Present, - Absent)

Table-6: 50% inhibition (IC₅₀) of Aqueous & ethanolic extract of *C. Bonducella* seeds by DPPH method

S.No	Sample	IC ₅₀ (µg/ml)
1.	Ascorbic acid	3.17
2.	Aqueous extract	19.1
3.	Ethanolic extract	21.46

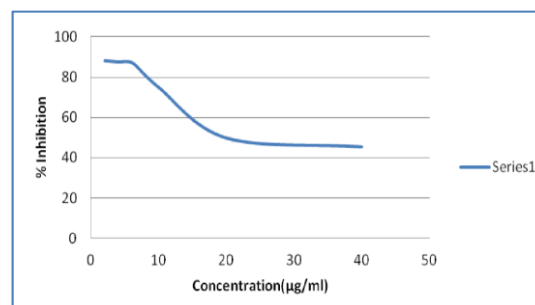


Fig-2: Antioxidant activity of Aq. Extract

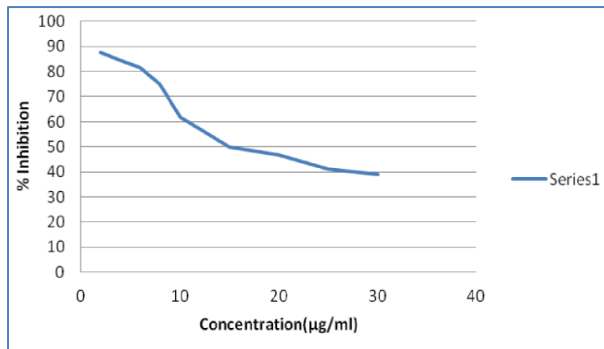


Fig-3: Antioxidant activity of ethanolic extract

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