

***In vitro* antioxidant studies and phytochemical screening on the seeds of Caesalpinia bonduc**

Jayakrishnan B. M.^{1*}, Nagaraja Perumal¹ and Hashim K. M.²

¹Department of Pharmacology, Karpagam College of Pharmacy, Dr MGR University Coimbatore, Tamilnadu, India

²Uwin Life Sciences, Malappuram, Kerala, India

ABSTRACT

The current scientific investigation deals with the detection of class of compounds present in the seeds of Caesalpinia bonduc. The free radical scavenging activity was also determined using the DPPH assay method and the EC50 value were calculated and found to be 7.5 mg. The seed of the plant is being used by the traditional practitioners and the present work reveals the scientific validation of the usage of the seeds.

Key words: *Caesalpinia bonduc*, Anti-oxidant assay, DPPH, EC 50

INTRODUCTION

Herbal medicines has been enjoying renaissance among the customers throughout the world. However one of the impediments in the acceptance of the Ayurvedha or Siddha formulation is the lack of standard quality control profiles[1]. World Health Organisation (WHO) has defined medicinal plants as plants that contain properties or compounds that can be use for therapeutic purposes or those that synthesize metabolites to produce useful drugs [2]. Among ancient civilisations, India has been known to be rich repository of medicinal plants. The forest in India is the principal repository of large number of medicinal and aromatic plants, which are largely collected as raw materials for manufacture of drugs and perfumery products. About 8,000 herbal remedies have been codified in Ayurva da. The Rigveda (5000 BC) has recorded 67 medicinal plants, Yajurveda 81 species, Atharvaveda(4500-2500 BC) 290 species, Charak Samhita (700 BC) and Sushrut Samhita (200 BC) had described properties and uses of 1100 and 1270 species respectively, in compounding of drugs and these are still used in the classical formulations, in the Ayurvedic system of medicine[3]. The term medicinal as applied to a plant indicates that it contains a substance or substances which modulate beneficially the physiology of sick mammals and that it has been used by man for that purpose[4]. Medicinal Aromatic Plants (MAPs) play a valuable and important role in economic, social, cultural and ecological aspects of local communities the world over[5].

Antioxidant compounds in food play an important role as a health protecting factor. Scientific evidence suggests that antioxidants reduce risk for chronic disease including cancer and heart problems. Primary sources of naturally occurring antioxidants are whole grain, fruits, and vegetables. Plant sourced food antioxidants like Vitamin C, Vitamin E, carotenes, phenolic acids, phytate, and phytoestrogens have been recognized as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. Some compounds such as gallates, have strong antioxidant activity, while others such as the mono phenols are weak antioxidants[6].

The plant was much confused with *Caesalpinia bonducella* (Syn. *C. bonduc*) and was described under the same[7,8,9,10,11,12,13]. Beside this species like *C. Nuga* [6,8,9,14,15] and *C. jayoba* are also sometimes wrongly

designated as synonyms for *C. crista*. In fact, *C. jayoba* is an adulterant of *C. crista*. “Bonducella” the name of the species is derived from the Arabic word “Bonduce” meaning a “little ball” which indicated globular shape of seed [16]. The local name are Kalimarakam, Kazhanji, Kazhanchikkuru and the Great English names are Fever nut, Physic nut, Bonduc nut. It belongs to the Caesalpinaceae family [17].

The other species in this family are *Caesalpinia coriaria*, *Caesalpinia crista*, *Caesalpinia cucullata*, *Caesalpinia decapetala*, *Caesalpinia globulorum*, *Caesalpinia hymenocarpa*, *Caesalpinia mimosoides*, *Caesalpinia pulcherrima*, *Caesalpinia sappan*. The habit is climber. Distribution is Paleotropics. The flowering and fruiting season is March-May. In Kerala it is found in Palakkad, Malappuram, Trichur, Idukki, Alapuzha and Trivandrum. *Caesalpinia bonduc* mainly found in the evergreen and most deciduous forests, also in sacred grooves. *Caesalpinia bonducella* is an Indian herb reported in Ayurveda, the ancient Hindi medicine system of India. Found throughout India and tropical countries of the World [16,17]

Various antioxidant activity methods have been used to monitor and compare the antioxidant activity of foods. In recent years, oxygen radical absorbance capacity assays and enhanced chemiluminescence assays have been used to evaluate antioxidant activity of foods, serum and other biological fluids. These methods need special equipment and technical skills for the analysis. These type of methods are published in literature for the determinations of antioxidant activity of foods involve electron spin resonance (ESR) and chemiluminescence methods. These analytical methods measure the radical scavenging activity of antioxidants against free radicals like the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, the superoxide anion radical (O_2^-), the hydroxyl radical (OH), or the peroxy radical (ROO). The various methods used to measure antioxidant activity of foods can give varying results depending on the specificity of the free radical being used as a reactant. These are the other methods which determine the resistance of lipid or lipid emulsions to oxidation in the presence of the antioxidant being tested. The malondialdehyde (MDA) or thiobarbituric acid reactive substances (TBARS) (1) assays have been used extensively since the 1950's to estimate the peroxidation of lipid in membrane and biological systems. These methods can be time consuming because they depend on the oxidation of a substrate which is influenced by temperature, pressure, matrix etc and may not be practical when large numbers of samples are involved. Antioxidant activity methods using free radicals are fast easy and simple. The ABTS [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation (2) has been used to screen the relative radical-scavenging abilities of flavanoids and phenolics through their properties as electron or H-donating agents [17].

MATERIALS AND METHODS

PREPARATION OF PLANT EXTRACTS AND PHYTOCHEMICAL SCREENING

The dried seed of *Caesalpinia bonduc* subjected for air dried and make up to coarse powder form. Bark powder was extracted successively with methanol using Reflux apparatus. All the extracts were filtered using filter paper. The extract were concentrated. The extract were stored in air tight container. The seed extract of *Caesalpinia bonduc* were analysed for the presence of sterols, alkaloids and amino acids.

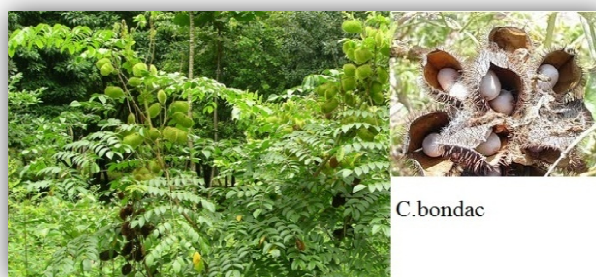


Figure no:-1

PRELIMINARY PHYTOCHEMICAL SCREENING OF THE CAESALPINIA BONDUC

The seeds of *Caesalpinia bonduc* is taken 10g in 50ml methanol and subjected to extraction. The filtrate was subjected to Molisch's test. Formation of reddish brown ring indicated the presence of carbohydrates.

Fehling's test: Dissolve a small portion of extract in water and treat with Fehling's solution [brown color indicated the presence of carbohydrate.]

- Phenols test: The extract was spotted on a filter paper. A drop of phosphomolybdic acid reagent was added to the spot and was exposed to ammonia vapours. Blue coloration of the spot indicated the presence of phenols.

Test for flavonoids :Shinoda test: To 2 to 3ml of extract, a piece of magnesium ribbon and 1ml of concentrated HCl was added .A pink or red coloration of the solution indicated the presence of flavonoids in the drug.

- .Lead acetate test: To 5ml of extract 1ml of lead acetate solution was added. Flocculent white precipitate indicated the presence of flavonoids.

Test for tannins

- Braemer's test: To a 2 to 3ml of extract, 10% alcoholic ferric chloride solution was added. Dark blue or greenish grey coloration of the solution indicated the presence of tannins in the drug.

Test for steroid/terpenoid

- Liebermann-Burchardt test: To 1ml of extract, 1ml of chloroform, 2 to 3ml of acetic anhydride and 1 to 2 drops of concentrated Sulphuric acid are added. Dark green coloration of the solution indicated the presence of steroids and dark pink or red coloration of the solution indicated the presence of terpenoids.

Test for alkaloids

- Dragendorff's test: A drop of extract was spotted on a small piece of precoated TLC plate and the plate was sprayed with modified Dragendorff's reagent. Orange coloration of the spot indicated the presence of alkaloids.
- Hager's test: The extract was treated with few ml of Hager's reagent. Yellow precipitation indicated the presence of alkaloids.
- Wagner's test: The extract was treated with few ml of Wagner's reagent. The reddish brown precipitation indicated the presence of alkaloids.

Tests for Glycosides

- Legal's test: Dissolved the extract [0.1g] in pyridine [2ml], added sodium nitroprusside solution [2ml] and made alkaline with Sodium hydroxide solution. Pink to red color solution indicates the presence of glycosides.

Test for Saponins

- Foam test: 1ml of extract was dilute with 20ml of distilled water and shaken with a graduated cylinder for 15 minutes. A 1cm layer of foam formation indicates the presence of Saponins

Test for Anthraquinones

- Borntrager's test: About 50 mg of powdered extract was heated with 10% ferric chloride solution and 1ml of concentrated HCl. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia. Pink or red coloration of aqueous layer indicated the presence of Anthraquinones.

Test for Amino acids

- Ninhydrin test: Dissolved a small quantity of the extract in few ml of water and added 1ml of ninhydrin reagent. Blue color indicated the presence of amino acids.

Test for fixed oils and fats

Press small quantity of the petroleum ether extract between two filter paper. Oil stains on the paper indicated the presence of fixed oils.

Note: the results for the above experiments can be noted as follows.

- If the response to the test is high it can be noted as +++ which indicates that the particular group is present as the major class.
- If the response is average then note it as ++ indicates the presence in moderate quantity.
- If the response is very small then note it as + indicating the presence of only in traces.
- If no response is then negative.

IN-VITRO ANTIOXIDANT ASSAY

The percentage of antioxidant activity of each concentration was assessed by DPPH free radical assay. The measurement of the DPPH radical scavenging activity was performed according to methodology described by Brand-Williams et al. The samples were reacted with the stable DPPH radical in an ethanol solution. The reaction mixture consisted of adding 0.5 mL of sample, 3 mL of absolute ethanol and 0.3 mL of DPPH radical solution 0.5 mM in ethanol. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in color (from deep violet to light yellow) were read [Absorbance (Abs)] at 517 nm after 100 min of reaction using a UVVIS spectrophotometer (DU 800; Beckman Coulter, Fullerton, CA, USA). The mixture of

ethanol (3.3 mL) and sample (0.5 mL) serve as blank. The control solution was prepared by mixing ethanol (3.5 mL) and DPPH radical solution (0.3 mL). The EC 50 value was determined.

RESULTS AND DISCUSSION

CLASS OF COMPOUND	TESTS PERFORMED	RESULTS
Carbohydrates	Molisch's test Fehlings test	---
Phenols	Phosphomolybdic acid test	---
Flavanoids	Shinoda test lead acetate test	---
Tannins	Braemer's test	---
Sterols	Salkowski's test	+++
Alkaloids	Draggendorf's test	+++
Glycosides	Legals test	---
Saponins	Foam test	++
Anthraquinones	Borntragers test	---
Amino acid test	Ninhydrin test	+++
Fixed oils and fats		---

Table:1 Preliminary phytochemical screening of the seeds of *C.bondac*

Concentration in mg	Percentage Inhibition
0	0.00
5	43.75
10	51.25
15	57.50
20	65.00

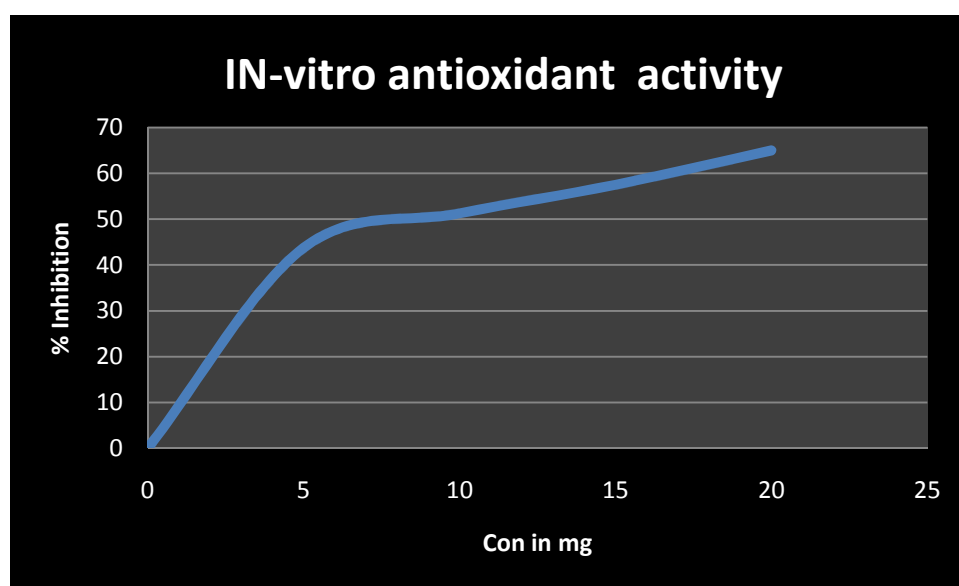


Figure no:- 2 In-vitro anti-oxidant activity of *C.bondac*

The preliminary tests were carried out and from that we came to know that sterols, amino acids and alkaloids were high and saponins in moderate concentration. EC 50 were found to be 7.5mg which is seems to be significant when compared to related species.

CONCLUSION

We conclude that the seed of *Caesalpinia bonduc* is a highly potential drug in terms of biological activity. The plant also contains promising class of compounds like alkaloids and sterols. The EC50 value which we calculated for the free radical scavenging was significant and we recommend further phytochemical studies on the seeds, which may lead to the identification of new potent molecules.

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