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Phytochemical and Pharmacological Screening of *Caesalpinia Bonduc*

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Subject: Pharmacology

Abstract
This study was carried out with an objective to investigate the Antipyretic activity of rhizomes of *Caesalpinia bonduc*. The aim of the study is to assess Antipyretic activity. In the present study, the Antipyretic activity of aqua's extracts of rhizomes of *Caesalpinia bonduc* was selected. Preliminary phytochemical evaluation of the EECB revealed the presence of tannins, carbohydrates, sterols, flavonoids, glycosides, alkaloids, saponins and proteins. Acute oral toxicity studies indicate no mortality recorded. The antipyretic activity was determined in the extracts using photochemical analysis, acute toxicity studies, statistical analysis and pharmacological screening in albino rats. The Aqueous extract of *Curcuma Amada* has antipyretic effect supporting the ethno pharmacological use as antipyretics.

Keywords: *Caesalpinia bonduc*,Physicochemical, Protein denaturation,*Caesalpinia*, terpenoids, phenolic derivatives, biological activity.

Introduction
Plants were the mainstay of medicine and credited with mystical and almost supernatural powers of healing. The practice of herbal medicine dates back to the very earliest periods of known history. There is evidence of herb having been used in the treatment of diseases and for revitalizing body systems in almost all ancient civilizations. Medicinal plants were existing even before human beings made their appearance on the earth. It is therefore often said that where ever we are born, we have around us herbs, shrubs &plants which are useful for us.[1]

The use of plants, plant extracts or plant derived pure chemicals to treat disease is therapeutic modality, which has stood the test of time. Indeed,many pharmacological classes of drugs including a natural product prototype. Aspirin, Atropine, Ephedrine, Digoxin, Morphine, Quinine, Reserpine and Tubocurarine are a few examples of drugs, which were originally discovered through the study of traditional cures and folk knowledge of indigenous people. There is a revived interest in herbal products (botanicals) at a goal level and conventional medicine is now beginning to accept the use of botanicals once they are scientifically validated. Isphagula, Garlic, Ginseng, Ginger, Ginkgo, St. John's Wort and a Saw palmetto are a few examples of botanicals which are gaining popularity amongst modern physicians and this trend is likely to continue, partly due to high cost involved in the development of patentable chemical drugs. There is growing evidence to show that medicinal plants contain synergistic and/or side-effects neutralizing combinations. Ethnopharmacology has already played an important role in the development of conventional medicine and is likely to play more significant role in the years to come. [2]

During the later part of this century the practice of herbalism has become mainstream throughout the world. This is due in part to the recognition of the value of traditional medicinal systems, particularly of Asian origin, and the identification of medicinal plants from indigenous pharmacopoeias that have been shown to have significant healing power, either in their natural state or as the source of new pharmaceuticals. Generally, these formulations are considered moderately in efficacy and thus less toxic than most pharmaceutical agents. In the western world, in particular, the developing concept that 'natural' is better than 'chemical' or 'synthetic' has led to the evaluation of Neo-western herbalism that is the basis of an ever expanding industry. In the U.S, often used as food or food supplements, known as

neutraceuticals, these formulations are readily available for those that wish to self medicate.[3]
Caesalpinia bonduc *has* documented *to possess* antioxidant, Antidiabetic Activity, but the effect of Caesalpinia bonduc as an Anti-Pyretic agent is still not reported. Hence it was thought worthwhile to screen extract of *Caesalpinia bonduc for* its Antipyretic activity. [4, 5]

Materials and Methods

Animals: The experiment was carried out on albino rabbits. They were 13-15 months old, of both sexes, weighing between 1.5 and 1.6 kg. Considering the group, the rabbits were kept in iron cages to adjust to the environment, and fed with cauliflower, cabbage, banana, and tap water for 40 days before the experiment. Food and water were withdrawn 6 h prior to the experiment.[6,7]

Plant collection and identification: The medicinal plants used for the experiment were leaves of *Caesalpinia bonduc collected* from the hilly regions nearby Koilkonda, Telagana State.. It was shade dried and authenticated by the botanist of Dr. K Madhava Chetty, Tirupati. Shade dried leaves were powdered with the help of electric grinder and Passed through sieve for coarse powder. This powder was used for the preparation of different extracts successively using various solvents.

Extraction: The plant material was dried and seeds were broken so as to separate the kernel and the seed coat. the seed coat was coarsely powdered and extracted with 95% ethanol in a soxhlet extractor; further, the extract was filtered and concentrated on rota-evaporator to get a sticky reddish brown extract .[8]

Phytochemicals Analysis [9,10,11,12]

The extracts prepared were analyzed for the presence of alkaloids, saponins, tannins, steroids, flavonoids, anthraquinones, cardiac glycosides and reducing sugars based on the protocols available in the literature.

1) Tests For Alkaloids

A) Mayer's Test (Potassium Mercuric Iodide): Fraction of the extract was treated with Mayer's reagent and observed in the formation of cream-colored precipitate.

B) Dragendroff's Test: Fraction of the extract was heated with Dragendroff's reagent and observed for the formation of a reddish orange-colored precipitate.

C) Wagner's Test: Fraction of the extract was treated with Wagner's reagent and observed in the formation of a reddish brown -coloured precipitate.

D) Hager's Test: Fraction of the extract was treated with Hager's reagent and observed in the formation of a yellow -colored precipitate.

2) Tests for Carbohydrates

A) Molisch's test: Fraction of extract was treated with a solution of 2-napthol and few drops of sulphuric acid was added through the sides of test tube and observed in the formation of a violet ring between the junction show the presence of carbohydrates.

B) Fehling's Test: Fraction of the extract was treated with Fehling's A solution and B and they are heated on a water bath for a few minutes and observed in the formation of a red -colored precipitate.

C) Barfoed's Test: Fraction of the extract was treated with Barfoed's reagent and observed in the formation of a red -colored precipitate.

D) Benedict's Test: Fraction of the extract was treated with Benedict's reagent and in boiling water bath for a few minutes and observed in the formation of a orange red -colored precipitate.

3) Test for Glycosides

A) Legal test: To the sample 1 ml of pyridine and a few drops of sodium nitroprussside solution was added and then it was made alkaline with sodium hydroxide solution. Appearance of pink color shows the presence of glycoside.

B) Kiddes Test: Cardenolides give blue or violet with firs reagent which fades after 1-2 hours. This reagent is prepared by mixing equal volume of 0.21 solution of 3, 5 di nitro benzoic acid in 100 ml of 0.5 N KOH solution on 50% methanol.

C) Keller killiani test:1 gm of powdered drug extracted with 10 ml of 70% alcohol for a few minutes and filtered. To 5 ml of filtrate add 10 ml of hydrogen peroxide and 0.5 ml of strong solution of lead acetate was added. Precipitate thus obtained was filtered. The filtrate is shaken with 5 ml of chloroform and the layer is separated and to this 1 m l of mixture of volume of 5% ferric sulfate and 99 volumes of glacial acetic acid was added.To this mixture 1-2 drops of conc. Sulphuric acid is added. Appearance of blue colour confirms the presence of deoxy sugars.

i) Antimony trichloride test: Solution of the extract is heated with antimony trichloride and tri chloro acetic acid to obtain. Blue or violet colour. Both Cardenolides and bufadienolides give this test.

ii) Borntrager's Test: The extract was treated with chloroform and chloroform layer was separated. To this equal quantity of dilute ammonia solution was added ammonical layer acquires rose pink colour shows the presence of a glycoside.

4) Test for Fixed Oils

A) Small quantity of extract was separately passed between two filter paper. Appearance of stain on the paper indicates the presence of fixed oil.

B) Few drops of 0.5 alcoholic KOH were added a small quantity of extract along with drops of phenolphthalein. Then the mixture was heated on a water bath for 1-2 hours. Formation of soap neutralization of alkali indicates the presence of fixed oil and fats.

5) Tests for Tannions And Phenolic Compounds

A) Ferric chloride test: Fraction of the extract was treated with ferric chloride solution and observed for the formation of brownish colorization.

B) Lead acetate test: To the extract adds 10% lead acetate solution and observed for the formation of white precipitate.

C) Gelatin solution test: To the extract add 1% solution gelatin containing sodium chloride solution and observed in the formation of white precipitate.

6) Test for Saponins

A) Foam test: The extract was diluted with 20 ml of distilled water and it was agitated on a graduated cylinder for 15 minutes. The formation of 1 cm layer of foam shows the presence of saponins.

7) Test for Proteins

A) Millon's Test: To the extract add little amount of water and milon's reagent. The appearance of red colour shows the presence of proteins.

B) Ninhydrin test: To the extract adds little amount of Ninhydrin reagent. Appearance of purple colour shows the presence of proteins.

8) Test for Flavoniods

A) Aqueous NaOH Test: To the extract adds little amount aqueous sodium hydroxide solution and observed in the formation of color.

B) Conc. H_2SO_4 Test: To the extract adds little amount of conc. Sulphuric acid and observed inthe formation of color.

C) Schinodo's test: To a small amount of extract adds a piece of magnesium followed by conc., hydrochloric acid and heated slightly, and then observe the color changes.

Pharmacological Screening : Depends up on the presence of active constituents in the various extract pharmacological activities were planned.

Experimental Procedure: Number of rabbits in each group was 6.

a.Experimental groups: 4 groups; 2 groups receiving Chloroform-acetone fraction (2 doses; 100 and 200 mg/kg) and the other 2 groups receiving Aqueous fraction (2 doses; 100 and 200 mg/kg).

b.Control groups were: i.Aspirin group (+Ve Control): Receiving standard antipyretic agent aspirin.

ii.Solvent group (-Ve Control): receiving solvent (used).

Acute toxicity study: Acute toxicity study was carried out by graded doses of each fraction in albino mice. Both petroleum ether and ethyl acetate fractions were administered intraperitoneally in graded doses (200 to 1000 mg/kg body weight). They were observed continuously for the first 2 h for toxic symptoms and up to 24 h for mortality.[13]

Treatment protocol: Before the experiment, rectal temperatures of the rabbits were recorded by inserting a well lubricated bulb of a thermometer in to the rectum. Care was taken to insert it to the same depth each time (about 6 cm). Milk was collected from local cattle. Rabbits were injected with boiled milk at room temperature at the dose of 0.5 ml/kg body weight to induce pyrexia. Induction of fever took about 1 to 2 h.Then the solvent (2 ml) was given on the negative control group, the known antipyretic agent aspirin solution (2 ml) was given on the positive control group and each sample solution (2 ml) was given to the corresponding experimental group (Table).Intraperitoneal route was used to administer boiled milk, aspirin solution, solvent, and sample solutions. Finally,rectal temperatures were recorded at 1 h intervals up to 3 h.[14,15]

Statistical analysis: Data were presented as mean ± standard error (Mean± SE). Student's t-test was used for comparison between the experimental and control groups. $P < 0.05$ was considered to be statistically significant.

Results and Discussion

Preliminary Phytochemical Screening

EECB was subjected for phytochemical screening and found to contain tannins, sterols, falvonoids, glycoside, and alkaloids in aqueous extract. The Phytochemical constituent of various extracts of Caesalpinia bonduc was shown table 1.The phytochemical analysis of the fractions showed the presence of tannins and flavonoids.

Table 1: Phytochemical Evaluation of different extract of rhizomes of *Caesalpinia Bonduc*

S.NO.	TESTS	ETHANOL
1.	ALKALOIDS	-Ve
2.	CARBOHYDRATES	-Ve
3.	GLYCOSIDES	-Ve
4.	FIXED OILS	-Ve
5.	TANNINS	+Ve
6.	STEROLS	-Ve
7.	SAPONINS	-Ve
8.	PROTEINS	-Ve
9.	FLAVONIODS	+Ve

+Ve Indicates Present,-Ve Indicates Absent

Pharmacological Investigation

Acute Oral Toxicity: The Rabbits Treated with EECB at a dose of 2000 mg/kg ,p.o. Prohibited normal behavior, without any signs of passivity, stereotypy, and vocalization. Their motor activity and secretary signs were also normal and no signs of symptoms. EECB at a dose of 2000 mg/kg, Body weight did not produce any Behavioral symptoms and mortality. So 1/5th dose used in the present study. In acute toxicity study, it was found to be safe and no mortality was observed.

Anti-Pyretic activity in Rabbits:

Caesalpinia bonduc:EECB at a dose of 200 mg/kg has exhibited a significant reduction in body temperature in rabbits at different time intervals. Aspirin (10mg/kg) was used as standard reference and it has significantly body temperature by 33.3 % at 1st hr, 92.6 % at 2nd hr, and 92.6 % at 3rd hr, which was found to be a time dependent effect as shown in figure 1-2 and table 2-3.

It was also observed that the extract have no effect on the reduction of pyrexia of rabbit.

The antipyretic activity exhibited that the ethanol extract of leaf possess a significant antipyretic effect in maintaining normal body temperature and reducing boiled milk induced elevated rectal temperature in rabbits and their effect are comparable to that of standard antipyretic drug aspirin.

Such reduction of rectal temperature of testing animals by the extract at 200 mg/kg appears to be due to the presence of a single bioactive principles or mixture of compounds in them.The antipyretic activity observed can be attributed to the presence of flavonoids have been reported to exhibit antipyretic effect.[18,19]The present study, therefore, supports the claims of traditional medicine practitioners as an antipyretic remedy.

Table 2: Antipyretic Effect of EECB on Rectal temperature in Milk induced Pyrexia in rabbits

Groups	Dose	Rectal temperature (ºC)		Rectal temperature after treatment (ºC)		
		Normal	3 h after boiled milk admin.	1 h (C1)	2 h (C2)	3 h (C3)
Solvent	2 ml/rabbit	38.44 ± 0.31	40.16 ± 0.19	40.05 ± 0.12	40.00 ± 0.54	39.88 ± 0.07
Aspirin	10 mg/kg	38.61 ± 0.14	40.11 ± 0.31	39.61 ± 0.32	38.72 ± 0.56	38.72 ± 0.62
EECB	200 mg/kg	38.50 ± 0.09	40.16 ± 0.17	40.13 ± 0.15	39.76 ± 0.23	39.5 ± 0.11
EECB	100 mg/kg	38.55 ± 0.09	40.33 ± 0.40	40.14 ± 0.13	39.89 ± 0.11	39.89 ± 0.07

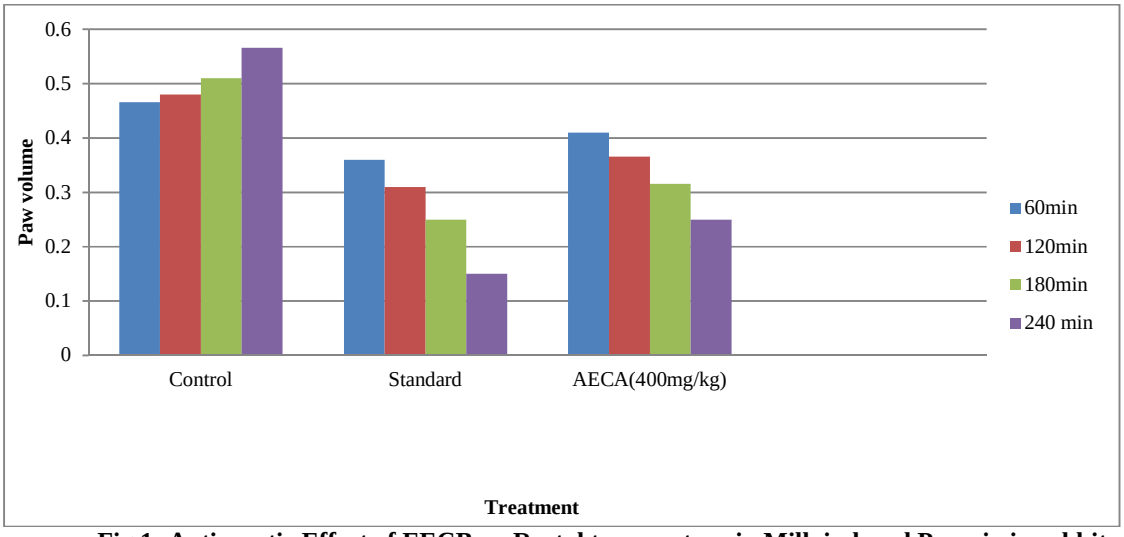

Fig.1: Antipyretic Effect of EECB on Rectal temperature in Milk induced Pyrexia in rabbits

Table 3: Percentage reduction of rectal temperatures in Milk induced Pyrexia in Rabbits

Groups	Dose	% Rectal temperature after treatment (ºC)		
		1 h (C1)	2 h (C2)	3 h (C3)
Solvent	2 ml/rabbit	6.4 ± 0.27	9.3 ± 0.12	16.3 ± 0.74
Aspirin	10 mg/kg	33.3 ± 0.13	92.6 ± 0.71	92.6 ± 1.52
EECB	200 mg/kg	32.5 ± 0.54	84.4 ± 0.63	89.4 ± 0.64
EECB	100 mg/kg	14.3 ± 0.15	40. 2 ± 0.35	43.4 ± 0.34

% reduction = B-C_n÷ B-A× 100; where n = 1, 2 and 3.

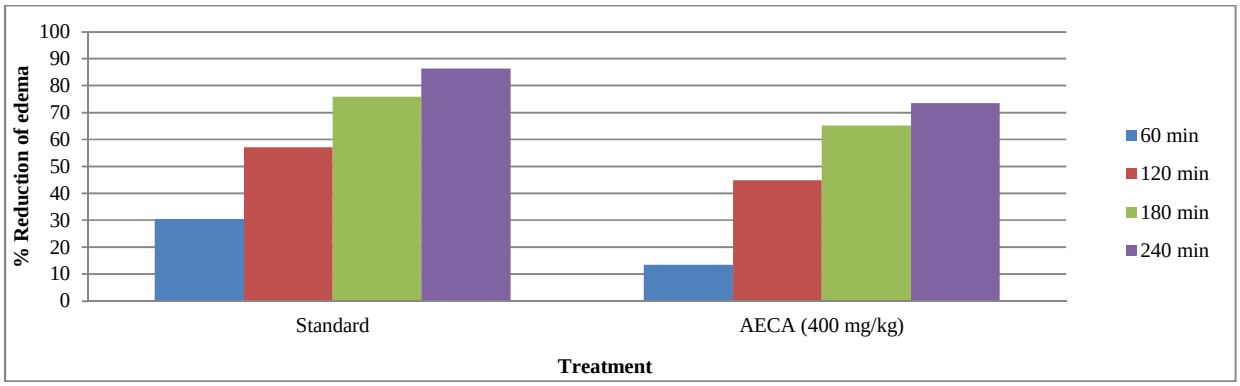

Fig .2. Percentage reduction of Rectal temperatures in Milk induced Pyrexia in Rabbits

Conclusion

In conclusion, the present study indicated a significant effect of the Ethanolic extract of *Caesalpinia bonduc and* supports its traditional usage as an Antipyretic agent.Preliminary Phytochemical evaluation of EECB revealed the presence of tannins, carbohydrates, sterols, flavonoids, glycosides, alkaloids. Acute oral toxicity studies indicate no mortality recorded. Antipyretic activity confirmed with EECB in experimental animals. Further, studies are required for the detailed studies in isolation of the compounds and pharmacological investigations of constituents, which have many pharmacological activies reported in traditionally and its exact mechanism of action.

"Cite this Article"

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