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PHYTOCHEMICAL ANALYSIS, ANTIOXIDANT, ANTIDIABETIC AND IN VIVO ANALGESIC ACTIVITY OF *CAESALPINIA BONDUCELLA*

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ABSTRACT

Caesalpinia bonducella Fleming (Caesalpiniaceae) plant is well known for its medicinal and therapeutic values in Indian Ayurveda. However, to be clinically useful, more scientific data are needed. Therefore, in the present study, we investigated the antioxidant, antidiabetic and *in vivo* analgesic activities of hydroalcoholic extract of *C. bonducella* leaves. Qualitative analysis of various phytochemical constituents, quantitative analysis of total phenolics (Folins ciocalteau reagent method) and flavonoids (Aluminium chloride method), in vitro free radical scavenging activity (DPPH radical method), in vitro anti-diabetic activity (Inhibition of alpha amylase enzyme) and *in vivo* analgesic activity (Hot plate method) were determined by the well-known test protocol available in the literature. Phytochemical analysis revealed the presence of phenols, flavonoids, tannins, saponins, alkaloids ect. The total phenolics content of hydroalcoholic leaves extract was (1.458mg/100mg), followed by flavonoids (1.025mg/100mg). The results of this study showed the evidence that the hydroalcoholic leaves extracts of *C. bonducella* have considerable antioxidant potential, in vitro enzyme inhibitory and analgesic activity. The results of this study indicate that the hydroalcoholic extract of *C. bonducella* leaves has significant pharmacological properties that may lead to new drug development.

Key words: *Caesalpinia bonducella,* Phytochemical analysis, Antioxidant, Antidiabetic, *In vivo* analgesic activities.

INTRODUCTION:

India is a rich source of medicinal plants and a number of plant derived oils and extracts are used against diseases in various systems of medicine such as Ayurveda, Unani and Siddha. Only a few of them have been scientifically explored. Plant derived natural products such as flavonoids, terpenes and alkaloids (Osawa et al., 1990; Keith et al., 1990) have received considerable attention in recent years due to their diverse pharmacological properties. Inflammation is a defensive response of body against all aggression such as pathogens (bacteria, virus, fungi, parasites) or others stimuli. The inflammatory process involves the release of pro-inflammatory cytokines, prostaglandins and the formation of reactive oxygen species (ROS). Excessive of these inflammatory mediators lead to maintain inflammation and induce a chronic inflammation (Philpott and Ferguson, 2004). Chronic inflammation is considered as a critical factor in many human diseases including cancer, obesity, type II diabetes, cardiovascular diseases, neurodegenerative diseases and aging

(Santangelo et al., 2007). Diabetes mellitus is a complex disease characterized by gross derangement in carbohydrate, fat and protein metabolism due to deficiency in insulin secretion and/or action (Luo et al., 2004). Mammalian α-amylase is a prominent enzyme in the pancreatic juice which breaks down large and insoluble starch molecules into absorbable molecules ultimately maltose (Gupta et al., 2003). α-glucosidase, on the other hand, anchored in the mucosal brush border of the small intestine catalyzes the end step of digestion of starch and disaccharides that are abundant in human diet (Anam et al., 2009). Inhibitors of α-amylase and α-glucosidase delay the breakdown of carbohydrate in the small intestine and decrease the postprandial blood glucose excursion levels in diabetic patients (Rhabasa-Lhoret and Chiasson, 2004). The inhibition of these two prominent enzymes has been found as a useful and effective strategy to lower the levels of postprandial hyperglycemia (Bakırel et al., 2008). Crude extract obtained from the *G. latifolia* was evaluated for its radical scavenging properties and assessed that it could be a rich source of natural oxidants with potential applications (Shukla et al., 2009). *Caesalpinia bonducella* (L.) Roxb. Fever nut; bonduc nut (Family: Caesalpiniaceae) commonly known as Nata Karanja (Hindi), is a prickly shrub found throughout the hotter regions of India, Myanmar and Sri Lanka (Nadkarni, 1954). The leaves of *C. bonducella* are traditionally used for the treatment of inflammation and toothache (Kirtikar and Basu, 1975). The topical anti-inflammatory activity of *C. bonducella* leaves has been reported (Agrawal and Kapadia, 1982; Vijayasarathy et al., 1982). It has also been found to possess multiple therapeutic properties like antipyretic, antidiuretic, anthelmintic and antibacterial (Neogi and Nayak, 1958), anticonvulsant (Adesina, 1982), anti-anaphylactic and antidiarrheal (Iyengar and Pendse, 1965), antiviral [Dhar et al., 1968], antiasthmatic (Gayaraja et al., 1979), antiamebic and antiestrogenic, hepatoprotective and antioxidant properties of this plant (Raghunathan et al., 1982; Gupta et al., 2003). The present study was focused to evaluate the phytochemical analysis, antioxidant, analgesic and antidiabetic activity of *Caesalpinia bonducella* leaves.

MATERIALS AND METHODS

Plant material

The plant material (leaves) for the proposed study was collected from minor forest produce processing & Research center: Vindhya Herbals, Bhopal (M.P.) in the month of Feb, 2018. India. Plant material (leaves) selected for the study were washed thoroughly under running tap water and then were rinsed in distilled water; they were allowed to dry for some time at room temperature. Then the plant material was shade dried without any contamination for about 3 to 4 weeks. Dried plant material was grinded using electronic grinder. Powdered plant material was observed for their colour, odour, taste and texture. Dried plant material was packed in air tight container and stored for phytochemical and biological studies.

Chemical reagents

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). All the chemicals used in this study were of analytical grade.

Extraction Procedure

Defatting of plant material

Caesalpinia bonducella leaves were shade dried at room temperature. The shade dried

plant material was coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place.

Extraction

Dried powdered of leaves of *Caesalpinia bonducella* has been extracted with hydroalcoholic solvent (70:30) using hot continuous percolation process for 48 hrs and dried using vacuum evaporator at 40^0C and stored in an air tight container free from any contamination until it was used. Finally, the percentage yields were calculated of the dried extracts (Mukherjee, 2007).

Qualitative Phytochemical Analysis of Plant Extract

The *Caesalpinia bonducella* leaves extract obtained was subjected to the preliminary phytochemical analysis following standard methods by Khandelwal and Kokate (Khandelwal, 2005, Kokate, 1994). The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavanoids, glycosides, saponins, alkaloids, fats or fixed oils, protein, amino acid and tannins.

Quantification of Secondary Metabolites

Quantitative analysis is an important tool for the determination of quantity of phytoconstituents present in plant extracts. For this TFC are determined. Hydroalcoholic extract obtained from leaves of *Caesalpinia bonducella* plant material of subjected to estimate the presence of TFC by standard procedure.

Total Phenol Determination

The total phenolic content was determined using the method of Olufunmiso et al (Olufunmis et al., 2011). A volume of 2 ml of *Caesalpinia bonducella* leaves extracts or standard was mixed with 1 ml of Folin Ciocalteau reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (75g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using UV/visible spectrophotometer. The total phenolic content was calculated from the standard graph of gallic acid and the results were expressed as gallic acid equivalent (mg/100mg).

Total Flavonoids Determination

The total flavonoid content was determined using the method of Olufunmiso (Olufunmis et al., 2011). 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 60 min at room temperature; the absorbance of the reaction mixture was measured at 420 nm using UV/visible spectrophotometer The content of flavonoids was calculated using standard graph of quercetin and the results were expressed as quercetin equivalent (mg/100mg).

DPPH free radical scavenging assay

DPPH scavenging activity was measured by modified method (Olufunmis et al., 2011). DPPH scavenging activity was measured by the spectrophotometer. Stock solution (6 mg in 100ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance. Decrease in the absorbance in presence of sample extract at different concentration (10-100 µg/ml) was noted after 15 minutes. 1.5 ml of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Three test

samples were taken and each processed similarly. Finally, the mean was taken. Absorbance at zero time was taken for each concentration. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm. The percentage inhibition of free radical DPPH was calculated from the following equation: % inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%. Though the activity is expressed as 50% inhibitory concentration (IC50), IC50 was calculated based on the percentage of DPPH radicals scavenged. The lower the IC50 value, the higher is the antioxidant activity.

In vitro anti -diabetic assays

α-Amylase inhibitory activity

The α-amylase inhibitory activity was determined according to the method described by Jyothi et al (Jyothi et al., 2011). A total of 500 µl of test samples and standard drug (10-50µg/ml) were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5mg/ml) solution and were incubated at 25°C for 10 min. After these, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 dinitrosalicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm. Control represent 100% enzyme activity and were conducted in similar way by replacing extract with vehicle.

***In vivo* analgesic activity**

Animals

Swiss albino male mice (20-25 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2°C, 55-65%). Mice received standard rodent chow and water *ad libitum*. mices were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Acute oral toxicity

Acute toxicity study of the prepared leaves extracts of *Caesalpinia bonducella* was carried out according to the Organization for Economic Co-Operation and Development (OECD) Guidelines-423 (OECD Guideline, 1996) the animals were fasted for 4 h, but allowed free access to water throughout. As per the OECD recommendations, the starting dose level should be that which is most likely to produce mortality in some of the dosed animals; and when there is no information available on a substance to be tested in this regard; for animal welfare reasons, the dose level to be used as the starting dose is selected from one of three fixed levels 5, 50, 300 and 2000 mg/kg body weight. Acute toxicity was determined as per reported method (Jonsson et al., 2013).

Eddy's hot-plate method

The 4 groups of swiss albino mice were prepared with 6 animals in each group. First group received normal saline in 3% tween 80 (2ml/kg) and served as normal control, second group received Diclofenac Na (10 mg/kg), third and fourth group received 100 and 200 mg/kg hydroalcoholic leaves extract The individual animal place on the hot plate maintained at temperature of (55+1)0 C, 15

sec cut-off period considered to avoid the damage to the animal and response recorded for untreated mice such as paw licking or jumping whichever appear first considered at 0 min. (Nahar et al., 2012; Wang et al., 2013), the response time again observed after treatment at 30, 60,120 and 240 min, and changes in the reaction time were noted.

Statistical Analysis

All analysis was performed using graph pad prism for Windows. All statistical analysis is expressed as mean ± standard error of the mean (SEM). Data were analyzed by one-way ANOVA, where applicable $p<0.05$ was considered statistically significant, compared with vehicle followed by Dunnett's test.

Results and Discussions

The yield of *Caesalpinia bonducella* hydroalcoholic leaves extracts was 7.5%w/w. Preliminary phytochemical screening of *Caesalpinia bonducella* hydroalcoholic leaves revealed the presence of various components such as phenolic compounds, carbohydrates, flavonoids, saponins and diterpins among which flavonoids and Phenols were the most prominent ones and the results are summarized in table 1.

Table 1 Result of phytochemical screening of *Caesalpinia bonducella* Leaves

S. No.	Constituents	Hydroalcoholic
1.	Alkaloids	-ve
2.	Glycosides	+ve
3.	Flavonoids	+ve
4.	Diterpenes	+ve
5.	Phenolics	+ve
6.	Amino Acids	+ve
7.	Carbohydrate	+ve
8.	Proteins	+ve
9.	Saponins	+ve

*Present =+ve, Absent -ve

The determination of the total phenolic content, expressed as mg gallic acid equivalents and per 100 mg dry weight of sample. The total flavonoids content of the extracts was expressed as percentage of quercetin equivalent per 100 mg dry weight of sample. TPC and TFC of hydroalcoholic extract of *Caesalpinia bonducella* leaves were found to be 1.458 and 1.025 respectively. Results are provided in table 2.

Table 2 Estimation of total phenolics and total flavonoids content ectract

S. No	Extracts	Total phenolic content (mg/100mg of dried extract)	Total flavonoids content (mg/100mg of dried extract)
1.	Hydroalcoholic	1.458	1.025

Antioxidant activity of the samples was calculated through DPPH assay. % inhibition was calculated as an indicative of antioxidant potency. The higher the % inhibition the better the activity. Ascorbic acid was taken as standard and the values were comparable with concentration ranging from 12.5 µg/ml to 400µg/ml. A dose dependent activity with respect to concentration was observed Table

3 and Fig. 3. The inhibitory action of Hydroalcoholic leaves extract of *Caesalpinia bonducella* were increased as the concentration increases in both the extract and the standard acarbose and results were shown in table 4 and fig. 4. The result of the effect of *Caesalpinia bonducella* on the hot plate method is presented in Table. The result shows that there was no significant difference in the PRT during the pre-drug testing time. After drug and extract administration, comparing the pre and post drug PRT using T-test showed that the reference drug Diclofenac sodium and the extract at the doses of 100 and 200 mg/kg significantly increased the PRT with the extract at the dose of 200mg/kg producing a better effect than the reference drug. The extract at the dose of 100mg/kg did not show any significant increase in the mean PRT table 5 & fig. 5.

Table 3 DPPH activity of Hydroalcoholic extract with reference to ascorbic acid

S. No.	Concentration (µg/ml)	Ascorbic Acid (% Inhibition)	Hydroalcoholic extract (% inhibition)
1	12.5	32.25	23.56
2	25	40.25	41.21
3	50	65.45	60.25
4	100	72.25	72.25
5	200	75.65	79.98
6	400	78.98	85.35
IC $_{50}$ Value		**20.92**	**50.15**

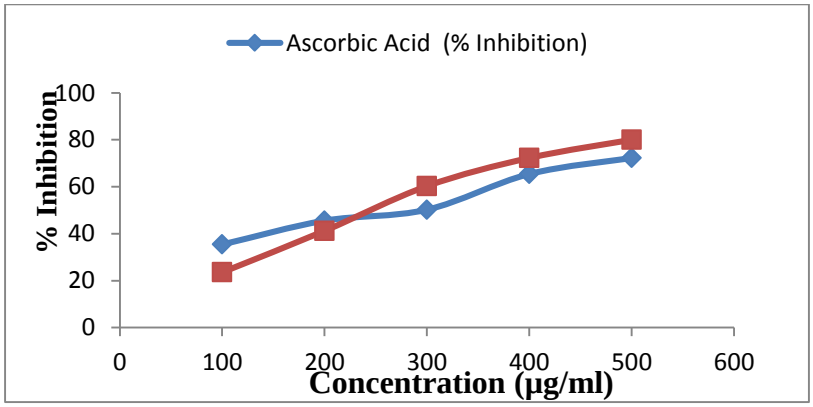

Fig. 3 DPPH Assay

Table 4 Results of *In vitro* antidiabetic studies

S. No	Acarbose		Hydroalcoholic extract	
	Conc.	% Inhibition	Conc.	% Inhibition
1.	100	45.65	100	38.98
2.	200	65.65	200	55.35
3.	300	75.65	300	62.12
4.	400	82.23	400	70.23
5.	500	89.98	500	78.98
IC$_{50}$		**92.76**	**IC$_{50}$**	**134.66**

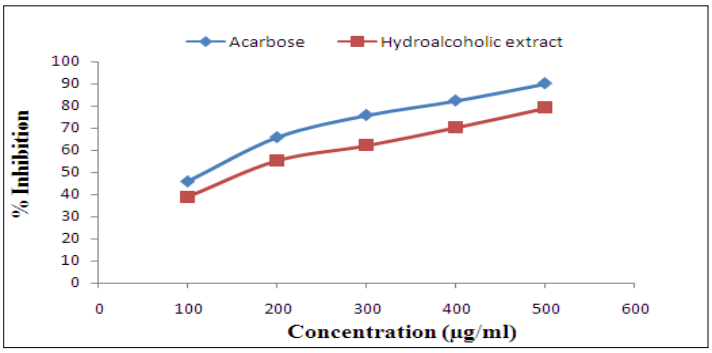

Fig. 4 α-amylase inhibitory activity of extract

Table 5 Results of analgesic activity by hot plate method

Group	Treatment mg/kg	Mean Pre drug reaction Mean ± SD	Mean Post drug reaction Mean ± SD
A	Vehicle 1ml/100gm	2.45±0.25	2.35±0.11
B	Diclofenac sodium	2.65±0.32	4.25±0.36
C	Extract 100mg/kg	2.31±0.65	3.98±0.41
D	Extract of 200mg/kg	1.98±0.41	4.89±0.21

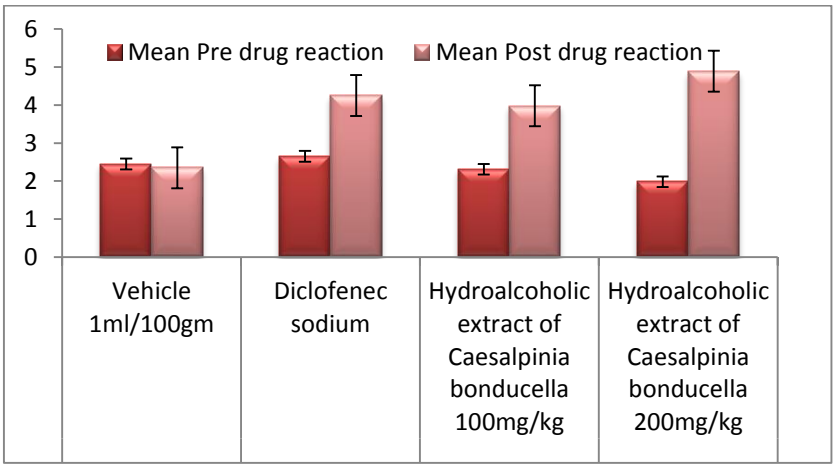

Fig. 5 Graph of Analgesic activity by Hot Plate method

Conclusion

From the result obtained it can be concluded that the leaves of *Caesalpinia bonducella* has medicinal values since it contains more secondary metabolites and its free radical scavenging activity were found to have considerable antioxidant potential. This plant also reveals better in vitro enzyme inhibitory activity (alpha amylase) which is involved in regulation and absorption of carbohydrate and also exhibits good analgesic activity. The

present data, illustrate that the hydroalcoholic extract of *Caesalpinia bonducella* leaves has good medicinal properties and it will be useful in treating various diseases including diabetes and many more.

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