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Phytochemical screening, antioxidant activity and estimation of quercetin by HPLC from *Caesalpinia bonducella*

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ABSTRACT

Caesalpinia bonducella is an important medicinal plant for its traditional uses against different types of diseases. The aim of the present study was to evaluate *in vitro* antioxidant activities, phytochemical analysis and HPLC analysis of ethanol extract of leaves of *Caesalpinia bonducella* collected from Bhopal region of Madhya Pradesh. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenolics flavonoids and alkaloids were determined by the well-known test protocol available in the literature. The *in vitro* antioxidant activity of ethanolic extract of the leaves was assessed against DPPH and Nitric oxide scavenging activity using standard protocols. Quercetin was detected in ethanolic extract of *Caesalpinia bonducella* under study by using RP-HPLC analysis. Phytochemical analysis revealed the presence of phenols, flavonoids, tannins, saponins, alkaloids. The total phenolic, flavonoids and alkaloids content of ethanolic leave extract of *Caesalpinia bonducella* was found to be 0.647, 0.941and 0.369mg/100mg respectively. *Caesalpinia bonducella* extracts showed effective DPPH radical scavenging and nitric oxide scavenging activity. Quercetin content was found in *Caesalpinia bonducella* extract, was quantified as 0.285µg/ml. HPLC analysis of plant extracts indicated the presence of the most abundant dietary flavonol, Quercetin. The results of this study revealed that the bioactive compound content differences could be determinant for the medicinal properties of this plant especially for antioxidant activities with potential applications in food and pharmaceutical industries.

Keywords: *Caesalpinia bonducella,* Phytochemical analysis, Antioxidant, HPLC analysis

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INTRODUCTION

Aromatic plants have been used in many domains including medicine, nutrition, flavoring, beverages, dyeing, repellents, fragrances, cosmetics, smoking and other industrial purposes. Since the prehistoric era, herbs have been the basis for nearly all medicinal therapy until synthetic drugs were developed in the nineteenth century[1,2] . Natural products represent a rich source of biologically active compounds and are an example of molecular diversity, with recognized potential in drug discovery and development[3].Medicinal plants contain large amounts of antioxidants such as polyphenols, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. Many of these phytochemicals possess significant antioxidant capacities that are associated with lower occurrence and lower mortality rates of several human diseases[4]. Caesalpinia bonducella 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[5]. The leaves of Caesalpinia bonducella are traditionally used for the treatment of inflammation and toothache[6]. The topical anti-inflammatory activity of Caesalpinia bonducella leaves has been reported[7,8]. It has also been found to posses multiple therapeutic properties like antipyretic, antidiuretic, anthelmintic and antibacterial, anticonvulsant[10], anti-anaphylactic and antidiarrheal, antiviral, antiasthmatic , antiamebic and antiestrogenic, hepatoprotective and antioxidant properties of this plant[9-15]. In the present study, quantification of quercetin by HPLC technique has been made in relation to its antioxidant activity which was not investigated previously.

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 October, 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. Quercetin was kindly provided by Scan Research Laboratories, Bhopal (India). Water used was of HPLC grade water from Merck Ltd, New Delhi, India.

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

200g. of dried plant material were exhaustively extracted with different solvent using maceration method. The extract was evaporated above their boiling points. Finally the percentage yields were calculated of the dried extracts[16].

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[17, 18]. 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.

Total phenol determination

The total phenolic content was determined using the method of Olufunmiso et al[19]. A volume of 2 ml of leaves of Caesalpinia bonducella extracts or standard was mixed with 5 ml of Folin Ciocalteau reagent (previously diluted with distilled water 1:10 v/v) and 4 ml (75g/l) of sodium carbonate. The mixture was allowed to stand for 15 min under room temperature. The blue colour developed was read 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/g).

Total flavonoids determination

The total flavonoid content was determined using the method of Olufunmiso et al[19]. 1 ml of 2% $AlCl_3$ methanolic solution was added to 1 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/g).

Total alkaloids content estimation

The plant extract (1mg) was dissolved in methanol, added 1ml of 2 N HCl and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (40, 60, 80, 100 and 120 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/100mg of extract[20].

Antioxidant activity

DPPH method

DPPH scavenging activity was measured by modified method[19]. 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.

Nitric oxide radical inhibition assay

Nitric oxide was generated from sodium nitroprusside and measured by the Griess reaction. Sodium nitroprosside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitric ions that can be estimated by using Griess reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide. The reaction mixture (6 m) contained sodium nitroprusside (10 mM, 4 ml), phosphate buffer saline (PBS,pH7.4,1ml) and extract or standard (1 ml, 10-200 µg/ml) in DMSO at various concentrations and it was incubated at 25±2ºC for 150min. After incubation, 0.5ml of the reaction mixture containing nitrite ion was removed, sulphanilic acid reagent was added (0.33% w/v, 1ml), mixed well and allowed to stand for 5 min for completion of diazotisation. Then, 1 ml of NEDD was added, mixed and allowed to stand for 30 min in diffused light. A purple colored chromophore was formed. The absorbance was measured at 546 nm[21]. The Nitric oxide radical scavenging ability was calculated using the following equation:

$$\text{Scavenging activity (\%)} = \frac{A_0 - A_t}{A_0} \times 100$$

Where A_0 is the absorbance of the control and A_t is the absorbance of the sample.

Quantification of flavonoid compounds by HPLC technique

For HPLC investigation of flavonoid compounds the ethanolic extracts of Caesalpinia bonducella under study were used as a preliminary assessment of various

compounds. The HPLC apparatus used for analysis was composed of a waters equipped with a UV dual detector and generated data were analyzed using Waters Ace software. For chromatographic separation Thermo C18 column (250X4.6mm, 5µm) was applied. The chromatographic analysis was performed at ambient temperature on a RP-C18 analytical column with a mobile phase composed of Acetonitrile: Methanol (50:50 v/v) and was isocratically eluted at a flow rate of 1 ml/ min. A small sample volume of 20 µl was used for each sample run, being injected into the HPLC system. The chromatogram was monitored with UV detection at a wavelength of 256 nm. Analysis time was 15min for both, standards and samples used for analysis. A quercetin was used as standards. A thermospectronic model of Labindia 3000 + UV/VIS Spectrophotometer with 1cm. matched quartz cells was used for determination of λmax.

RESULTS AND DISCUSSION

The crude extract so obtained after the maceration extraction process, extract was further concentrated on water bath evaporation the solvent completely to obtain the actual yield of extraction. The yield of extracts obtained from different samples using Pet. ether, chloroform, ethyl acetate, ethanol, aqueous as solvents are depicted in the table 1. The results of qualitative phytochemical analysis of the crude powder leave of *Caesalpinia bonducella* are shown in Table 2. The content of total phenolic compounds (TPC) was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: **Y = 0.011X+0.011, R^2= 0.998,** where X is the gallic acid equivalent (GAE) and Y is the absorbance. Total flavonoids content was calculated as quercetin equivalent (mg/g) using the equation based on the calibration curve: **Y=0.040X + 0.009, R^2=0.999**, where X is the absorbance and Y is the quercetin equivalent (QE). Total alkaloid content was calculated as atropine equivalent mg/100mg using the equation based on the calibration curve: **Y=0.007X, R^2=0.999**, where X is the atropine equivalent (AE) and Y is the absorbance. Results were shown in Table 3 and Figure 1-3. Antioxidant activity of the samples was calculated through DPPH assay and Nitric oxide radical scavenging. % 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 10 µg/ml to 100µg/ml. A dose dependent activity with respect to concentration was observed Table 4, 5 & Figure 4,5. The HPLC chromatogram of standard quercetin and ethanolic extract are shown in Figure 6 and the values are expressed in ppm. The retention time for standard and extracts was found to be 2.596 min and 2.423 min respectively. Characteristics parameters for standard quercetin was given in table 6 and results of quantitative estimation of quercetin in ethanolic extracts were given in table 7.

Table 1 % Yield of *Caesalpinia bonducella* (leaves)

S. No.	Extracts *Caesalpinia bonducella* (leaves)	% Yield
1	Pet ether	2.14
2.	Chloroform	3.58
3.	Ethyl acetate	6.28
4.	Ethanol	10.69
5.	Water	7.14

Table 2 Result of phytochemical screening of extracts of *Caesalpinia bonducella*

S. No.	Constituents	Chloroform extract	Ethyl acetate extract	Ethanol extract	Water extract
1.	**Alkaloids**				
	Mayer's Test	-ve	-ve	-ve	-ve
	Wagner's Test	-ve	-ve	-ve	-ve
	Dragendroff's test	-ve	-ve	-ve	-ve
	Hager's test	-ve	+ve	+ve	-ve
2.	**Glycosides**				
	Legal's test	-ve	+ve	+ve	-ve
3.	**Flavonoids**				
	Lead acetate	-ve	+ve	+ve	+ve
	Alkaline test	-ve	+ve	+ve	+ve
4.	**Phenolics**				
	Ferric Chloride Test	-ve	+ve	+ve	-ve
5.	**Proteins and Amino acids**				
	Xanthoproteic test	-ve	+ve	+ve	-ve
6.	**Carbohydrates**				
	Fehling's test	-ve	-ve	-ve	-ve
7.	**Saponins**				
	Froth Test	-ve	+ve	+ve	-ve
8.	**Diterpins**				
	Copper acetate test	-ve	-ve	-ve	-ve

ble 3 Estimation of total phenolic, flavonoids and alkaloid content of *Caesalpinia bonducella*

S. No.	Extracts	Total phenolic content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)	total alkaloid content (mg/ 100 mg of dried extract)
1	**Ethyl acetate**	0.514	0.748	0.154
2	**Ethanol**	0.647	0.941	0.369
3	**Aqueous**	-	0.614	-

Table 4 % Inhibition of ascorbic acid and ethanolic extract using DPPH method

S. No.		% Inhibition	
1	Control	Ascorbic acid	Ethanolic extract
2	10	44.65	37.14
3	20	48.62	41.74
4	40	65.34	42.57
5	60	69.65	48.22
6	80	77.41	51.31
7	100	84.13	53.17
	IC 50	17.68	76.49

Table 5 % Inhibition of ascorbic acid and ethanolic extract using nitric oxide method

S. No.		% Inhibition	
1	Control	Ascorbic acid	Ethanolic extract
2	10	40.92	31.47
3	20	48.70	35.98
4	40	73.48	39.12
5	60	89.04	41.39
6	80	90.20	46.56
7	100	91.35	48.74
	IC 50	14.11	103.90

Table 6 Characteristics of the analytical method derived from the standard calibration curve

Compound	Linearity range µg/ml	Correlation co-efficient	Slope	Intercept
Quercetin	5-25	0.999	94.39	-30.43

Table 7 Quantitative estimation of quercetin in ethanolic extract of *caesalpinia bonducella*

S. No.	Extract	RT	Area	% Assay
1.	Ethanolic extract	2.423	238.674	0.285

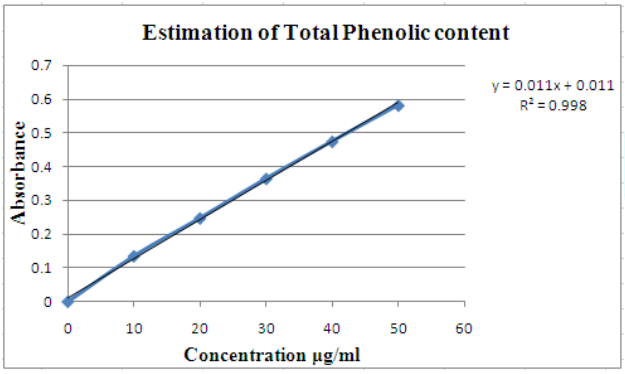


Figure 1 Graph of estimation of total phenolic content

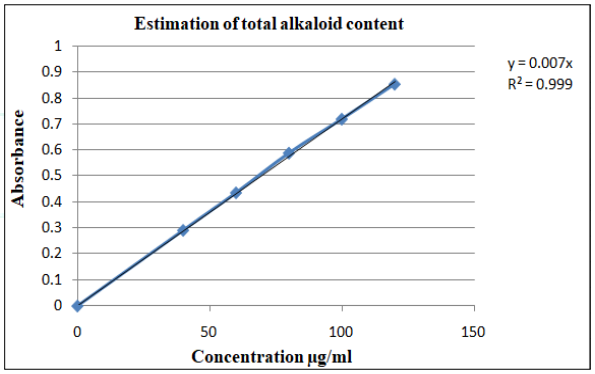


Figure 3 Graph of estimation of total alkaloid content

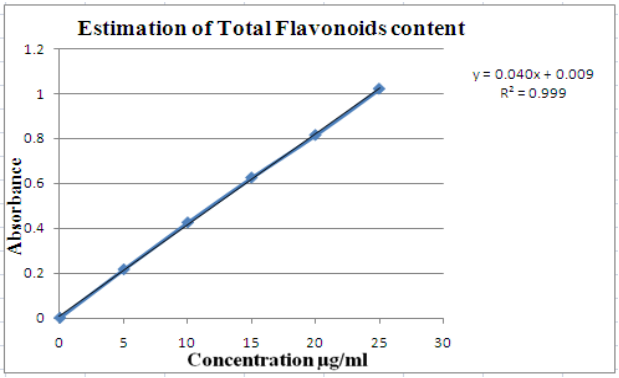


Figure 2 Graph of estimation of total flavonoids content

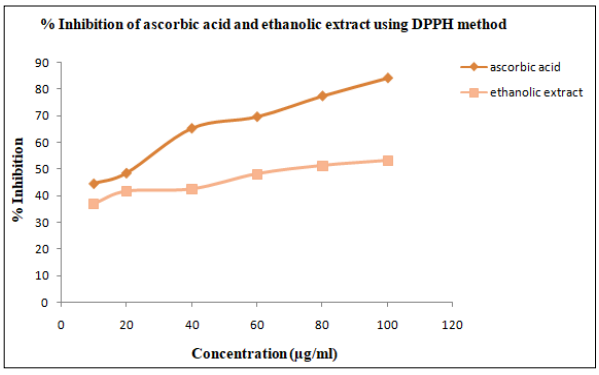


Figure 4 Graph of % Inhibition of ascorbic acid and ethanolic extract using DPPH method

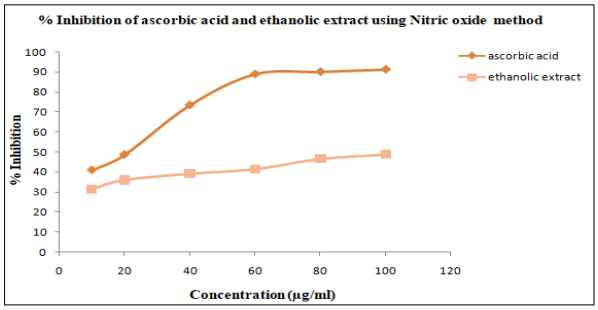
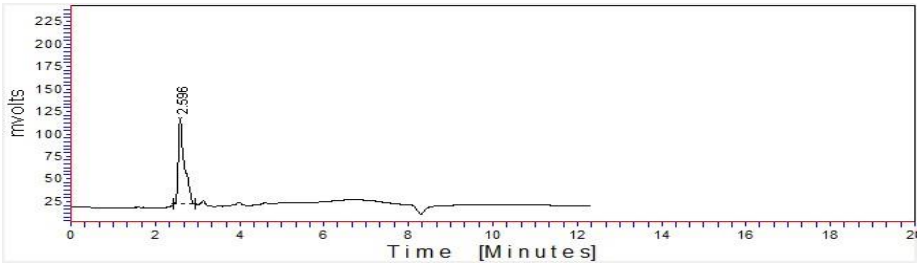
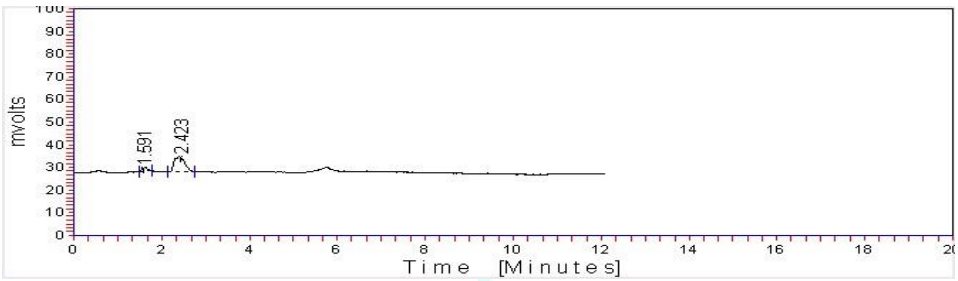


Figure 5 Graph of % Inhibition of ascorbic acid and ethanolic extract using nitric oxide method



(A)



(B)

Figure 6 Chromatogram of (A) standard quercetin (B) ethanolic extract of *caesalpinia bonducella*

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. The study suggests that the leave parts of the plant might be a potential source of natural antioxidants. Thus *Caesalpinia bonducella* can be a good candidate for novel phytomedicine that can be used to treat several diseases. The future study shall be directed towards the identification of bioactive compounds and quality standards for developing a potential drug.

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