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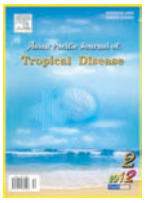

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In Vitro Free Radical Scavenging Activity of Gallic Acid Isolated From *Caesalpinia Decapetala* Wood

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ABSTRACT

Objective: Antioxidant capacities and phenolic contents of Gallic Acid isolated from plant *Caesalpinia decapetala* were investigated. *Caesalpinia decapetala* is a plant used to treat burns, biliousness and stomach disorders. **Methods:** The antioxidant activity was estimated by the ABTS and DPPH methods. The total phenolic was measured using Folin-Ciocalteu assay. Identification of isolated Gallic Acid was also performed by reverse-phase high-performance liquid chromatography (RP-HPLC) using Cosmosil C18 column (150mm x 4.6mm, 5μm particle). The mobile phase was a mixture of ethyl acetate: ethanol: water (1:5:4, v/v/v) delivered at a flow rate of 1.0 mL min-1. **Results:** The total phenolic content was found to be 4.31% (w/w). Isolation of Gallic Acid with optimum yield was performed using a mixture of solvents (Ethanol: Water 65:35). Isolated Gallic Acid showed significant in vitro free radical scavenging activity in both model; but in ABTS assay significant % inhibition of free radical was observed as compared to DPPH assay. **Conclusions:** Research concluded that *Caesalpinia decapetala* extract can be used as potent antioxidant which can play vital role against the diseases like neurodegenerative disorders, inflammation, viral infections and gastric ulcer.

1. Introduction

Polyphenolic compounds are commonly found in both edible and inedible plants, and they have been reported to have multiple biological effects, including antioxidant activity [1]. *Caesalpinia decapetala* is a thorny climber or shrub up to 25 m in height, commonly found wild in the sub- Himalayan tract and planted in hedges throughout India. It is planted in gardens for its large racemes of bright yellow flowers. It is an excellent hedge-plant. A bath with decoction of the plant is useful in treatment of jaundice. The leaves are used to treat the burns, biliousness and stomach disorders. In Maharashtra and South India, the bark is used for tanning [2]. It is used as laxative, tonic, carminative and antipyretic [3]. Leaves and root of *Caesalpinia decapetala* act as a purgative and emmenagogue [4]. The leaves of *Caesalpinia decapetala* contain cassane diterpenoid, caesaldecan, spathulenol, 4, 5-epoxy-8(14)-caryophyllene, squalene, lupeol, resveratrol, quercetin, astragalin and stigmasterol [5].

There is evidence concerning the participation of reactive oxygen species in the etiology and pathophysiology of human diseases, such as neurodegenerative disorders, inflammation, viral infections, autoimmune pathologies and digestive system disorders such as gastrointestinal inflammation and gastric ulcer [6]. Drugs with multiple mechanisms of protective action, including antioxidant properties, may be one way forward in minimizing tissue injury in human disease [7]. Many indigenous drugs are known to possess significant free radical scavenging properties [8-11]. Thus present study was aimed to isolate and investigate antioxidant effects of Gallic-Acid present in *Caesalpinia decapetala*; to establish Gallic Acid as a main antioxidant component of plant.

2. Materials and methods

ABTS, DPPH, were purchased from Sigma-Aldrich. All reagents and solvent were used of analytical and HPLC

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grade.

2.1. Plant Material

The plant wood of *Caesalpinia decapetala* was collected from Nashik region in the month of October and dried wood was milled with the help of pulveriser, sieved with 60 mesh and stored in air-tight container at 25℃.

2.2. Extraction procedure

Dried wood powder was extracted by using mixture of different solvents. The major process parameters of extraction were optimized. These include solvents (Ethanol: Water 65:35); temperature (50-55℃) and time (36 hr). Gallic acid is soluble in ethanol and water therefore these solvents were selected for extraction. *Caesalpinia decapetala* wood powder was put into 100 ml conical flask and Ethanol-Water mixture was added in it and then kept aside for selected temperature. After extraction, the extract was concentrated, vacuum evaporated and were kept in desicator.

2.3. Estimation of total phenolic

The total phenolic content of Ethanol: Water extract of *Caesalpinia decapetala* was determined by using the Folin Ciocalteu Assay. A stock solution (1 mg/ml) of the extract was prepared in methanol. From the stock solution, 1 ml of the extract of different concentrations ranging from 20 to 100 μg/ml were taken into 25 ml volumetric flasks and 10 ml of water and 1.5 ml of Folin Ciocalteau reagent were added. The mixture was kept for 5 min and then 4 ml of 20% sodium carbonate solution was added and made up to 25 ml with double distilled water. The absorbance was recorded after 30 min. Percentage of total phenolics was calculated from calibration curve of gallic acid plotted by using the above procedure [12, 13].

2.4. Measurement of gallic acid by HPLC

The separation and identification of Gallic Acid was performed by HPLC using Jasco's Binary Type High Performance Liquid Chromatography; comprising two PU-1550 pumps with 20 μl loop and a Jasco multi wavelength detector MD-1510. A Cosmosil C18 column (150mm x 4.6mm, 5 μm particle) was used for the analysis. The mobile phase was a mixture of ethyl acetate: ethanol: water (1:5:4, v/v/v) delivered at a flow rate of 1.0 mL min-1. Peak of Gallic Acid in sample was identified by comparison with retention time of standard Gallic Acid [14, 15].

2.5. Antioxidant activity

Free radical-scavenging ability by the use of a stable DPPH radical.

The DPPH radical-scavenging activity was determined; DPPH (0.1 mM) was dissolved in pure ethanol (96%). The radical stock solution was prepared freshly. The DPPH solution (1 ml) was added to different concentrations of isolated sample with 3 ml of ethanol. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 10 min. The decrease in absorbance of the resulting solution was monitored at 517 nm. The results were corrected for dilution and expressed in % inhibition. Equal volume of ethanol & DPPH was used as control. All determinations were performed in triplicate [16]. (Results are shown in Table 1).

Free radical-scavenging ability by the use of a stable ABTS radical cation:

The free radical-scavenging activity was also determined by ABTS radical cation decolorization assay [17]. The ABTS radical cation was generated by reacting 7 mM ABTS and 2.45 mM potassium persulfate after incubation at room temperature in the dark for 16 h. The ABTS solution was diluted with 80% ethanol to an absorbance of 0.700±0.005 at 734 nm. The ABTS solution (3.9 mL; absorbance of 0.700 ±0.005) was added to 0.1 mL of different concentrations of isolated sample and mixed thoroughly. The reactive mixture was allowed to stand at room temperature for 6 min, and the absorbance was immediately recorded at 734 nm. All determinations were performed in triplicate. (Results are shown in Table 1).

2.6. Statistical analysis

Table 1

Results of free radical-scavenging activity of extract (% Inhibition by DPPH and ABTS assay).

S.No.	% Inhibition			
	DPPH assay		ABTS assay	
	Concentrations(μg mL^{-1})	% Inhibition	Concentrations(μg mL^{-1})	% Inhibition
1.	10	41.8±0.69	10	49±0.32
2.	20	52.94±0.67	20	61.45±0.44
3.	30	72.37±0.49	30	80.29±0.56
4.	40	81.19±0.77	40	91±0.68
5.	50	91.99±0.59	50	95.22±0.71

Note: Data are the mean±SD of three measurements.

The results are expressed as mean values±S.E.M. (standard error of mean). Statistical comparison was carried out by analysis of variance (ANOVA).

3. Results

The amount of total phenolics in Ethanol: Water (65:35) extract of *Caesalpinia decapetala* wood was found to be 4.31% (w/w) by using Folin Ciocalteu Assay. Separation and identification of Gallic Acid in sample extract was performed by HPLC using Cosmosil C18 column (150mm x 4.6mm, 5 μm particle) and mobile phase a mixture of ethyl acetate: ethanol: water (1:5:4, v/v/v), a well resolved separate peak of Gallic Acid was identified at retention time of 8 min. which was found to be comparable with the retention time of standard (Figure 1 & 2).

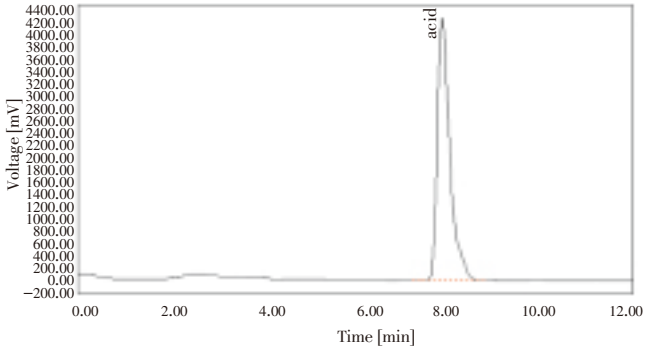

Fig. 1 Chromatogram of standard Gallic-Acid

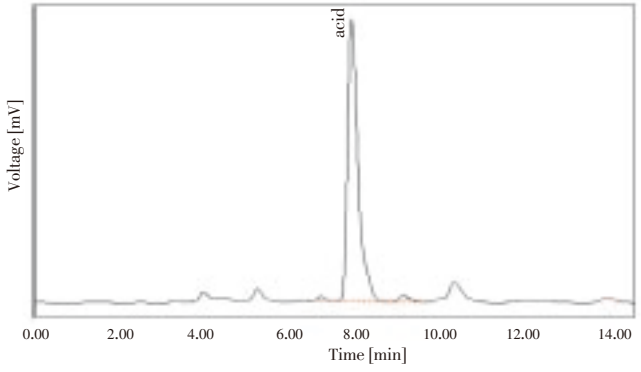

Fig. 2 Chromatogram of Gallic-acid in sample

The total antioxidant capacity was estimated by the ABTS and DPPH methods; results of in vitro antioxidant activity reveals that sample extract showed marked inhibition in the both model but in ABTS assay more inhibition of free radical was observed as compared to DPPH assay (Figure 3). It was found that Gallic Acid isolated from *Caesalpinia decapetala* extract showed potent free radical scavenging activity even in low concentration (Table 1).

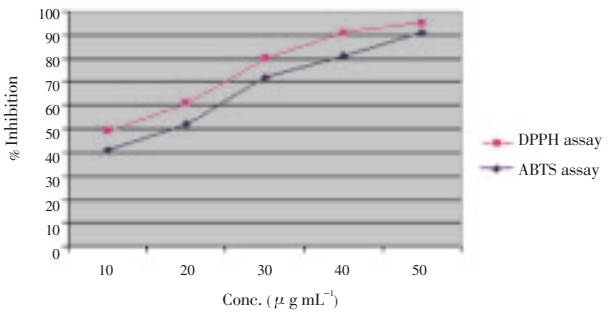

Fig. 3 Free radical-scavenging activity of extract (% Inhibition by DPPH and ABTS assay).

4. Discussion

Caesalpinia decapetala is one of the widely used drugs in traditional medicines. In the present study, preliminary phytochemical testing showed the presence of phenolics and tannins along with flavonoids. Isolated Gallic Acid showed significant in vitro free radical scavenging activity; at the concentration level of 20 μg mL^{-1}, 61.45±0.44 % and 52.94± 0.67 % inhibition of free radical was observed for ABTS and DPPH assay respectively. While 95.22±0.71 % and 91.99±0.59 % inhibition of free radical was observed for ABTS and DPPH assay respectively at the concentration of 50 μg mL-1 (Table 1).

As plants produce a lot of antioxidants to control the oxidative stress caused by sunbeams and oxygen, they can represent a source of new compounds with antioxidant activity. In this study Gallic Acid isolated from *Caesalpinia decapetala* extract was selected, since Gallic Acid possesses free radical scavenging effect. Scientific literature reveals that the carbonyl groups present in the flavonoids and phenolic compounds were responsible for the free radical scavenging activity. This investigation revealed that isolated Gallic Acid showed potent DPPH and ABTS scavenging activity; may be due to the presence of carbonyl groups which are considered to be responsible for the antioxidant activity. Thus research concluded that *Caesalpinia decapetala* extract can be used as potent antioxidant which can play vital role against the diseases like neurodegenerative disorders, inflammation, viral infections and gastric ulcer.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Maizura M., Aminah A., Wan Aida W. M., Total phenolic content

and antioxidant activity of kesum (Polygonum minus), ginger (Zingiber officinale) and turmeric (Curcuma longa) extract, *International Food Research Journal*, 2011; **18**: 529-534.

[2] The Wealth of India, Council of Scientific and Industrial Research, New Delhi, 1988, p.13

[3]Kirtikar K.R., Basu B.D., Ind Med Plants. 1984, p.850.

[4] Guha Bakshi D.N., Sensarma P., Pal D.C., A Lexicon of Medicinal Plants in India, Vol.I, Naya Prokash Publisher, Calcutta, 1999, p.336.

[5] Kiem P.V., Minh C.V., Huong H.T., Lee J.L., Kim Y.H., Asaldecan; cassane diterpenoid from the leaves of *Caesalpinia decapetala*. *Chem. Pharm. Bull.*, 2005; **3**: 428-430.

[6] Subhashini N., Thangathirupathi A., Lavanya N., Antioxidant activity of Trigonella foenum using various in vitro and ex vivo models International *Journal of Pharmacy and Pharmaceutical Sciences*, 2011; **3**(2): 111-120.

[7] Anna F., Dae-Ok K., Sang-Jin C., Sung I. K., Ock K. Chun, Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods, *Journal of Food Composition and Analysis*, 2011; **24** (7): 1043-1048.

[8] Rahmat A Khan, Muhammad R Khan, Sumaira S. and Mushtaq A., Evaluation of phenolic contents and antioxidant activity of various solvent extracts of Sonchus asper (L.), *Hill Chemistry Central Journal*, 2012; **6**:12.

[9] Alpinar K, Ozyurek M, Kolak U, Guclu K, Aras C, Altun M, Celik SE, Berker KI, Bektasoglu B, Apak R: Antioxidant capacities of some food plants wildly grown in ayvalik of Turkey, *Food Sci Tech Res*, 2009; **15**: 59-64.

[10]Kadry H., Abou Basha I., and A. Temraz, Antioxidant activity of aerial parts of Tribulus alatus in rats, Pak. *J. Pharm. Sci.*, 2010; **23** (1): 59-62.

[11]Nasir R., Komal R., Muhammad Z., Tahsin G., Iftikhar H. B., Mazhar A. G., Adeela A., Antioxidant activity of various extracts and organic fractions of Ziziphus jujube, *International Journal of Phytomedicine* 2011; **3** (3): 147.

[12]Harbone J.B.Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Chapman and Hall Ltd., New York, 1998; p.42-43.

[13]Hanane E. H., Nadya L., Katim A., Yahya C., Abdellah F., Abdesslam E., Brahim E. B. and Mohammed L., Antioxidant properties and total phenolic content of three varieties of Carob tree leaves from Morocco, *Rec. Nat. Prod.*, 2010; **4** (4): 193-204.

[14]Sawant L, Prabhakar B, Pandita N, Quantitative HPLC analysis of Ascorbic Acid and Gallic Acid in Phyllanthus Emblica. *J. Anal. Bioanal Techniques*, 2010; **1**: 111.

[15]Hafsa D., Pradnya J. P., Development of RP-HPLC method for qualitative analysis of active ingredient (Gallic acid) from Stem Bark of Dendrophthoe falcate Linn., *International Journal of Pharmaceutical Sciences and Drug Research*, 2011; **3**(2): 146-149.

[16]Ramshankar YV, Vinay P, Vijayan P. Antioxidant, antimicrobial and cytotixicity properties of the methanolic extract from Grewia tiliaefolia Valh. *Pharmog Mag*. 2008; **4**: 330-4.

[17]Krzysztof P., Wieslawa B., Beata J., Irena M., Maria S., Henryk M., And Grazyna B. B., Antioxidant activity of flavonoids of different polarity, assayed by modified ABTS Cation Radical Decolorization and EPR Technique, *Acta Biologica Cracoviensia Series Botanica;* 2010; **52** (1): 97-104.