EFFECT OF PHENOLIC CONTENT ON ANTIOXIDANT ACTIVITY OF CASUARINA EQUISETIFOLIA

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Summary

The total amount of phenolic compounds in each extracts was determined as Tannic acid equivalents. The percentage of phenolic contents in Methanolic extracts of Wood, Bark, Fruit and Leaf were estimated by Folin-Ciocalteu method (22.97%, 14.00%, 2.63% and 1.76% w/w).

In vitro DPPH assay was carried out in the measurement of free radical scavenging ability of test extracts. Our findings showed the maximum activity of Wood extract as compared to Bark, Fruit and Leaf (IC_{50} 1.70>47.17>72.62>131.76). The wood extract showed remarkable antioxidant activity than Asocrbic acid (IC_{50} 14.10).The free radical scavenging abilities were stichometrically inhibited by all extracts. The results of this study substantiate to isolate the constituents responsible for Antioxidant activity.

Key words: Antioxidant activity, DPPH, *Casuarina equisetifolia*, Free radical scavenging activity, Folin-Ciocalteu method.

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Introduction

Casuarina equisetifolia (Casuarinaceae) is handsome tree with drooping branches, 10-50 m high[1]. It is found in dry hill sides and open forests of India, Sri Lanka and Australia.[2] The following phytoconstituents have been isolated from the plant so far; Kaempferol,quercetin.[3], Alicyclic acids (Shikimic and Quinic acid),amino acids[4].,taraxerol,lupenone, lupeol, gallic acid,β-sitosterol[5].,catechin and gallocatechin[6,7]. The plant is used as astringent[1],diarrhea[8], dysentery, cough, ulcers, toothache, lotion for swelling[2] and diabetes [9].Pharmacological investigations have shown that wood and bark possess significant anthelmintic and anticancer activities. [10,11] The biological activities, viz. anticancer, antibacterial[2], hypoglycemic, antifungal[12] of the leaf has been reported. The percent phenolic content in various parts was estimated spectrophotometrically by Folin-Ciocalteu method. The Folin's reagent is sensitive to reducing compounds, polyphenols there by producing blue colored complex. The quantative phenolic estimation was performed at 765 nm by change in intensity of Folin-phenolic compounds complex. The DPPH assay was used in the measurement of scavenging ability of isolated test compounds. The DPPH radical is reduced in the presence of an antioxidant molecule, the electron becomes paired off showing the color change from blue to uncoloured methanol solutions stichometrically; depending on the number of electron taken up.[13,14]

Materials and methods

Estimation of Phenolic contents[16]: -

Authentification of plant material: - The plant specimen was collected from Gangapur dam locality, Nashik (M.S.) identified as ***Casuarina equisetifolia*** Linn Family Casuarinaceae, Voucher no. ANA1, Ref. No. BSI /WC/ Tech./2005/867 dated 22.12.2005 by P. S. N. Rao, Joint Director, Botanical Survey of India, Pune (M.S.).

Preparation of Plant extracts: - Coarsely powdered materials of leaf, bark, wood and fruit (50 gm each) were subjected to reflux with 200 ml of methanol for 4 hrs coded as MEL, MEB, MEW and MEF followed by subsequent filtration and evaporation to yield extract (Table 2).

Preparation of Standard :- Tannic acid (Research Lab., Bombay) was purified by Dr.C.K.Kokate's method.

Folin-Ciocalteu reagent: - Qualigens Phenol reagent (Folin & Ciocateus) Prod.No. 35953,B.No.47066511-5 Glaxo –SmithKline Pharmaceuticals limited, Mumbai-30 (M.S.)

Sodium carbonate solution: - Sodium carbonate (200 gm) was dissolved at 70 ^{o} C - 80 ^{o} C in and volume was made with distilled water upto 1 lts. It was filtered through glass wool and allowed to stand overnight.

Calibration curve: - 1 ml of standard aliquot was taken in 25 ml volumetric flask, added with 10 ml of water,1.5 ml of Folin-Ciocalteu Reagent ,allowed to stand for 10 min. 4 ml of Sodium carbonate was added in each volumetric flask, volume was adjusted with distilled water. Readings were taken after 1 hr at 765 nm by U.V.Spectrophotometer 160 A (Schimatzu Japan) against reagent blank. The calibration curve (Fig 1) of absorbance Vs Concentration was plotted with Tannic acid solution in distilled water (Table 1).

Determination of Phenolic Content: - The stock solutions (1 mg/ml) of methanolic extracts according to plant parts stated in (Table 2) were prepared in distilled water. 1 ml of stock solution was transferred in 25 ml volumetric flask; similar procedure was adopted as above. With the help of calibration curve, the phenolic concentrations of extracts were expressed in terms of µg /ml (Table 1).

Table 1 Data for calibration curve for Tannic acid

Sr.No.	Concentration µg/ml	*Absorbance at 765nm
1	25	0.087
2	50	0.17
3	75	0.255
4	100	0.331
5	125	0.416
6	150	0.498
7	175	0.570
8	200	0.674

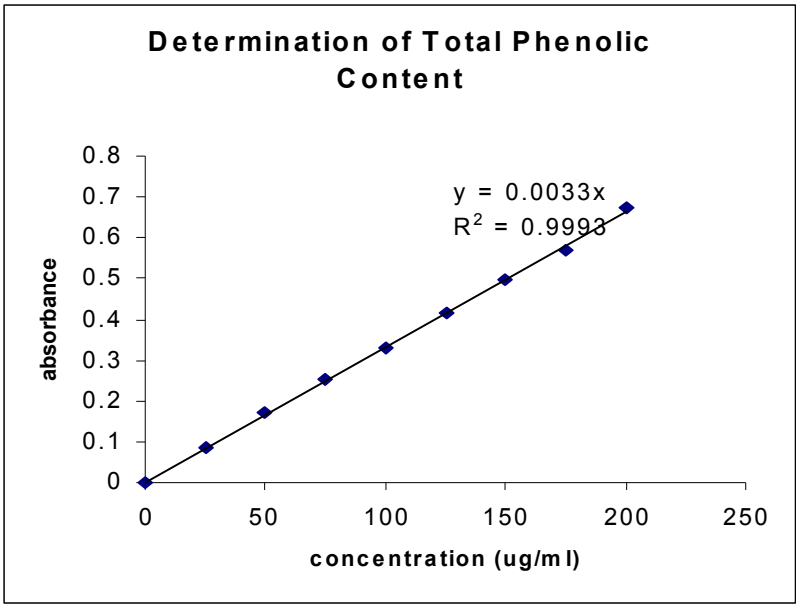
Averages of three readings

Table 2 Estimation of phenolic content in various plant parts

Sr.No.	Part used	% Extractive value	*Absorbance at 765 nm	Concentration of Phenols (µg /ml)
1	Leaf	17.90	0.058	17.576
2	Bark	12.54	0.462	140.000
3	Fruit	11.88	0.087	26.364
4	Wood	04.10	0.758	229.697

Averages of three readings

Figure 1: - Calibration curve for Tannic acid



DPPH radical scavenging activity[15,16] -

Methanolic solution of DPPH 100 µl (1.3 mg/ml) was added in 4 ml of methanol to give initial absorbance of 0.9 at 516 nm. To Methanolic solution of DPPH (100 µl), test extracts dissolved in methanol were added at different concentrations (25-100 µg /ml). The decrease in absorbance of test mixtures was read at 516 nm against test blank and the percentage inhibition (Table 3) was calculated by using the formula,

$$\% \text{ Inhibition} = \frac{\text{O.D. Blank} - \text{O.D. of Test}}{\text{O.D. Standard}} \times 100$$

Table 3:-Antiradical activity for total methanolic extracts for various plant parts observed with DPPH

Sr.No.	Extract	Percentage Scavenging (Mean ± SEM)				IC_{50}(µg/ ml)
		25µg/ml	50µg/ml	75µg/ml	100µg/ml	
1	MEW	57.37 ±1.84	70.54 ±5.80	81.82 ±3.79	86.63 ±4.14	1.70
2	MEB	38.43 ±6.62	55.08 ±3.74	61.86 ±5.32	68.42 ±3.29	47.13
3	MEL	5.61 ±4.51	14.09 ±6.15	24.97 ±4.72	37.44 ±4.67	131.76
4	MEF	28.12 ±3.52	40.74 ±5.70	50.54 ±1.63	62.38 ±7.48	72.62
5	Ascorbic acid	15.64 ±0.09 (5µg/ml)	34.51 ±0.11 (10µg/ml)	51.45 ±0.04 (15µg/ml)	73.87 ±0.01 (20µg/ml)	14.10

(Values are mean of three replicates)

Stastical analysis: - Results were expressed as mean ± standard deviation of three replicates and average value was considered. The IC_{50} value was calculated using linear regression analysis of the percent inhibition obtained using different concentrations. The regression equation was obtained and the concentration required to produce 50% inhibition (IC_{50}) was calculated.

Result and Discussion

The calibration curve for Tannic acid was found linear from 2.5 µg /ml /ml to 200 µg /ml /ml. The Correlation Coefficient (0.9996) (Table 4) indicates good linearity between concentration and absorbance. A phenolic content in Methanolic extracts of Wood was more compared with Bark, Fruit and Leaf estimated by Folin-Ciocalteu method (22.97%, 14.00%, 2.63% and 1.76% w/w).

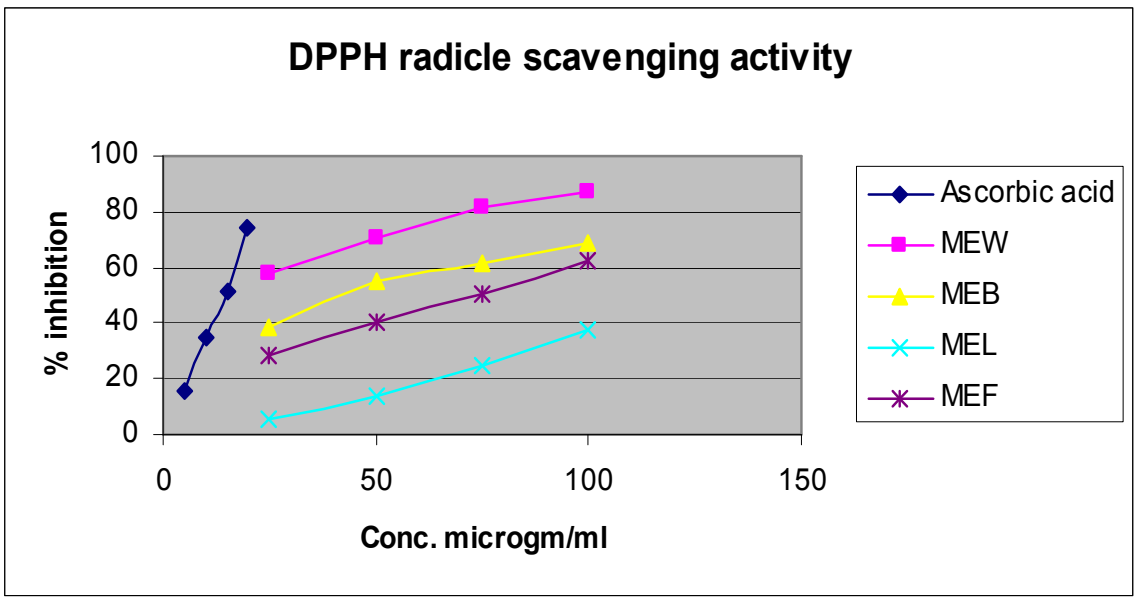
Table 4: - Statistical parameters for Tannic acid

Sr.No.	**Parameter**	Value
1	Absorption maxima	765 nm
2	Beer's law limit (µg/ml)	25 –200
3	Regression equation	y= 0.0252X + 0.0194
4	Intercept (a)	0.0194
5	Slope (b)	0.0252
6	Correlation Coefficient	0.9996

Radical scavenging activity:-

The methanloic extracts of wood exhibited a dose dependent inhibition of DPPH activity (Table 4), with 50% inhibition (IC_{50}) at the concentration of 1.70 compared with Bark, Fruit and Leaf (IC_{50} 47.17, 72.62, 131.76). Radical scavenging capacity of wood extract (Fig.2) was found to be even higher than reference antioxidant, Ascorbic acid (IC_{50} 14.10). Oxidation is involved in decomposition of pharmaceutical preparation containing steroids, vitamins and antibiotics. Reactive oxygen species (ROS) are produced continuously in the cells as accidental bye-products of metabolism which are cytotoxic and are important factors for several pathological conditions such as cardiovascular diseases, diabetes, inflammation, cancer etc.[17] Flavonoids and phenolic compounds widely distributed in plants which have been reported to exert multiple biological effect, including antioxidant, free radical scavenging abilities, anti-inflammatory, anticarcinogenic.etc.[18] DPPH is one of the free radicals, stable at room temperature generally used for testing preliminary radical scavenging activity of a compound or a plant extract. The decrease in the concentration of DPPH radical due to scavenging ability of compounds isolated from *Casuarina equisetifolia* showed better activity.

Fig. 2: - Free radical scavenging (DPPH model) of methanolioc extract of Casuarina equisetifolia



In conclusion, the results of the present study showed that extract of ***Casuarina equisetifolia*** wood which contains highest amount of pholyphenols exhibits greatest antioxidant activity through the scavenging of DPPH radical which traditionally used as anti cancer and for swelling.[2] They are endowed with potentially exploitable antioxidant activities. The potential of Antioxidant activity supported by Wood extract may be attributed to presence of Tannins and steroids as detected by Preliminary phytochemical studies.

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