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Evaluation of total phenolic content, flavonoid content and antioxidant activity of stem bark of *Bridelia retusa* (Linn.) spreng

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ABSTRACT

In this study we have determined the total phenolic, flavonoid content and antioxidant activity of aqueous and alcoholic extract of the stem bark of *Bridelia retusa* Linn. (Euphorbiaceae). Folin-ciocalteu method was used to determine the total phenolic content and flavonoid content of these extract were assessed by aluminium chloride method. The antioxidant activities were determined by DPPH method. The aqueous as well as alcoholic extract showed antioxidant activity, alcoholic extract contained more phenolic and flavonoids and hence possessed greater antioxidant activity as compared to aqueous extract. The results suggest that the plant is good source of antioxidants and support their use in various diseases.

KEYWORDS

Bridelia retusa;
Total phenol;
Flavonoids;
Antioxidant;
DPPH.

INTRODUCTION

Bridelia retusa Linn (Euphorbiaceae) is a deciduous shrub or a tree up to 18 m in height, found throughout India up to an altitude of 1000 m except in the very dry regions[1]. The bark is traditionally used for rheumatism[2], astringent[3], arthritis and antifertility[4]. It is well known that free radicals play a fundamental role in several diseases. Free radicals contribute to more than hundred disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer, diabetes mellitus and AIDS[5,6]. Recently there has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing such free radical induced tissue injury. Besides well known and traditionally used natural antioxidants from tea, wine, fruits, vegetables and spices, some natural antioxidant (e.g. rosemary and sage) are already exploited commercially either as antioxidant additives or a nutritional supplements. Many other plant species have also been investigated in the search for novel antioxidants, but generally there is a continuous demand to find more information concerning the antioxidant potential of plant species occurring naturally. Phenolic compounds including flavonoids, tannins and phenolic acids are currently of growing interest due to their biological effects in human health. It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. Phenolic compounds are a class of antioxidant agents which act as free radical terminators[7].

Our study was aimed in determining the total phenolic and flavonoid content and evaluating the antioxidant activity of alcoholic and aqueous extracts of stem bark of *Bridelia retusa* linn. (Euphorbiaceae). An easy,

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rapid and sensitive method for the antioxidant screening of plant extracts is free radical scavenging assay using 1,1-diphenyl-2-picryl hydrazyl (DPPH) stable radical by spectrophotometrically.

MATERIALS & METHODS

The stem bark of the *Bridelia retusa* linn. was collected from Dajipur jungle (Radhanagri wild life sanctuary), Kolhapur, Maharashtra. The said plant was authenticated in the botany department of Willigdon College, Sangli and also by Dr. S. S. Sathe. The voucher specimen has been preserved in our laboratory for future reference.

DPPH and quercetin were purchased from Hi-media laboratories Pvt. Ltd. Mumbai, Folin-ciocalteu was purchased from loba chemie Pvt. Ltd Mumbai, then gallic acid, ascorbic acid and aluminium chloride were purchased from Research Labs Mumbai. All the chemicals used were of AR grade. Double beam UV-Vis Spectrophotometer (Jasco V-550) was used in the study.

Preparation of extract

The dried barks were subjected to size reduction to obtain a coarse powder. Alcoholic extract of the bark powder was obtained by soxhlet extraction while for aqueous extract the powder was macerated for seven days using distilled water. Both the extracts were concentrated with the help of rotary vacuum evaporator (make- medica, model – roteva).

Determination of total phenol content

Total phenolic content was determined by Folin ciocalteu reagent[8]. The dilute extracts (0.5ml of 1mg/ml) were mixed with Folin ciocalteu reagent (5ml, 1:10 diluted with distilled water) and aqueous sodium carbonate (4 ml, 1 M). The mixtures were allowed to stand for 60 min and total phenols were determined using double beam UV-Vis spectrophotometer at 765nm. Total phenolic values were expressed as gallic acid equivalent in g/100g of extract which is a common reference compound. The concentration of polyphenols in samples were derived from a standard curve of gallic acid ranging from 10 to 50 µg/ml.

Total flavonoids determination

Aluminum chloride colorimetric method was used for flavonoids determination[9]. Alcoholic and aqueous extracts of the bark (2ml) were mixed with 0.1ml of 10% w/v aluminium chloride, 0.1ml of 1M potassium acetate and 2.8ml of distilled water. It was kept at room temperature for 30 minutes and the absorbance of the reaction mixture was measured at 415nm using double beam UV-Vis spectrophotometer. The calibration curve was plotted using quercetin solutions at concentrations 12.5 to 100 µg/ml in methanol.

Free radical scavenging activity determinations

The free radical scavenging capacity of the extracts was determined using DPPH method[10]. Plant extracts and ascorbic acid were weighed and dissolved in methanol to obtain six different concentration (1, 5, 10, 50, 100 and 500 µg/ml). Aliquots were prepared suitably by diluting with methanol.

DPPH was weighed and dissolved in methanol to make 0.004% w/v solution. 3ml of 0.004% DPPH solution was added to each test tube with the help of calibrated pipette to obtain the desired concentrations. The prepared mixtures were incubated at 37°C for 30 min. The absorbance value of each test tube was determined using UV-Visible spectrophotometer at 517nm. The percentage inhibition values were calculated using equation.

$$\text{DPPH scavenged (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of text sample}}{\text{Absorbance of Control}} \times 100$$

IC_{50} was determined from % inhibition vs concentration graph. IC_{50} expressed the antioxidant activity defined as the concentration in ml that inhibits the formation of DPPH radicals by 50 %.

RESULTS AND DISCUSSION

Total phenol and flavonoid contents of the extracts

The results of total phenolic contents was obtained from regression equation of calibration curve ($y = 0.0037x + 0.017$, $r^2 = 0.9614$). The Values are expressed in gallic acid equivalents (GAE) for phenols (TABLE 1).

The flavonoids contents expressed in quercetin

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equivalents (QE)/100g of extract, were determined from regression equation of calibration curve (y = 0.0165x - 0.0235, r^2=0.9973). Values were expressed in quercetin equivalents (QE) (TABLE 1)

TABLE 1 : Phenol and flavonoids content

Extracts	Phenols* (GAE g/100gms)	Flavonoids* (QE g/100gms)
Alcoholic	6.37 ± 0.89	2.01 ± 0.22
Aqueous	4.00 ± 0.43	1.68 ± 0.10

*** Each value is average ± SD (n = 3)**

According to our study, the alcoholic extract of *B.retusa* showed high contents of phenol & flavonoids as compared to aqueous extract.

Antioxidant activity

DPPH is the best, easiest and widely used method for testing preliminary free radical scavenging activity of a compound or a plant extract[11]. In the present study $IC_{50.}$ values of aqueous and alcoholic extracts were calculated and compared with IC_{50} value of acorbic acid as a standard (IC_{50}= 12.42 µg/ml).

This investigation was based on the measurement of the relative inhibitory effect of extract tested at different concentrations. TABLE 2 represents the % inhibition of both aqueous as well as alcoholic extracts. Figure 1 is graphical representation of % inhibitory activity Vs Concentration.

TABLE 2 : % Inhibition of aqueous and alcoholic extracts

conc. (µg/ml)	% Inhibition	
	Aq.	Alcoholic
0	0	0
1	1.85±1.26	8.05±0.89
10	9.57±5.76	24.32±2.21
50	44.96±2.81	70.39±3.33
100	73.96±1.37	74.05±2.11
500	77.27±3.32	78.06±1.53

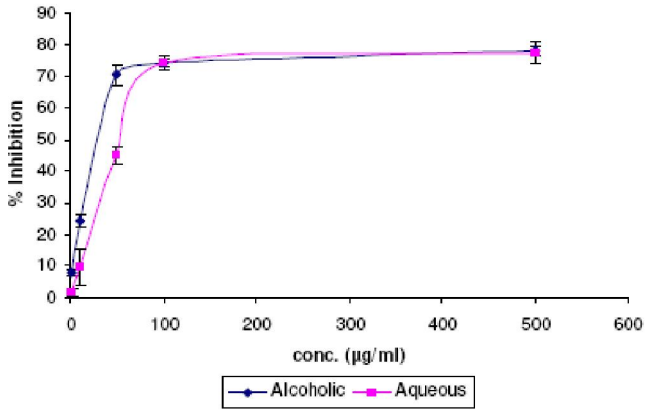

Figure 1 : Anti oxidant effect of stem bark of *Bridelia retusa*

TABLE 3 shows capacity of both extract to scavenge the DPPH radical. Both the extracts showed antioxidant activity.

TABLE 3 : Free radical scavenging activity

Extract	Alcoholic extract	Aqueous extract
IC_{50} (µg/ml) for DPPH scavenging activity	25 µg/ml	60 ug/ml

The result of the present study showed that the alcoholic extract of *B. retusa,* which contained high amount of phenolic and flavonoids content exhibited the greater antioxidant activity as compared to aqueous extract. Polyphenols, tannins and flavonoids are very valuable plant constituents in the scavenging action due to their several phenolic hydroxyl groups[12]. Hence it is clear that there is direct relationship between phenolic, flavonoids content of *B. retusa* and its antioxidant activity.

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