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Research Article

Determination Phenolic Content and *In vitro* Antioxidant Activity of Leaves of Indian Lavender Plant *Bursera Penicillata* ENGL

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ABSTRACT

This study is intended to determine the antioxidant potential and total phenolic content of different solvent extracts of leaves of *Bursera penicillata* (Family: Burseraceae). The antioxidant potential of *B. penicillata* as revealed by free radical scavenging activities against DPPH, ABTS was carried out. In addition, reducing power assay and total phenol content have been determined. *B. penicillata* leaves showed high free radical scavenging activity as evidenced by the low IC_{50} values in DPPH (142.36±8.98 µg/mL), in ABTS (39.25±3.34 µg/mL) and higher reducing power and also the extract was found to have high levels of phenolic content (39.32±4.05 mg GAE/g). Based on our results, *B. penicillata* gains significance with regard to its antioxidant potential and its role in traditional medicine.

Keywords: *Bursera penicillata*, DPPH, ABTS, Total phenolic, Reducing power.

INTRODUCTION

Oxidative stress is induced by free radicals which can be any atom (or) molecule possessing unpaired electrons. The reactive oxygen species (ROS) are oxygen derived free radicals such as superoxide anion (O_2), hydroxyl (OH^-), hydro peroxyl (OOH^-), peroxyl (ROO^-) and alkoxyl (RO^-) radicals[1,2] and they can be formed in living organisms through endogenous means during the cellular metabolic activities and exogenously by radiation, pollutants, pesticides and organic solvents[3]. ROS cause oxidative damage to proteins, lipids and DNA leading to the degenerative processes related to ageing and diseases, like cancer, coronary arteriosclerosis, gastric ulcer, declination of the immune system, Alzheimer's disease and cataract[4-8].

In recent years, the research of natural antioxidants as alternative sources to synthesis antioxidants has emerged and the exploitation of the various secondary metabolites of the plants was highlighted[9]. Antioxidant compounds can prevent (or) repair the body's cells especially through free radical scavenging and quenching of singlet oxygen[10-12]. Natural antioxidants increase the antioxidant capacity of the plasma and reduce the risk of diseases and they occur in all higher plants and in all parts of the plants like leaves, fruits, roots, flowers, etc. These phytoconstituents such as phenolics, flavonoids and tannins are also known to have good antioxidant ability[13,14]. Several synthetic antioxidant agents are commercially available, however, they are reported to be toxic to animals including human beings and this has created an interest in many investigators to look for natural antioxidants[15]. The continued search for natural antioxidants has gained importance in recent years because of the increasing awareness of herbal remedies for many chronic diseases[16-19]. Scientific examination and validation of the traditional therapeutic use of the plant medicines may lead to the development of new and effective drugs as has occurred in the past[20].

The family Burseraceae includes more than 600 species in 20 genera distributed in the neotropical and temperate regions of the world and the genus *Bursera* is diversified in the tropical dry forests of Mexico[21]. *Bursera penicillata* is an aromatic essential oil plant introduced into India from Mexico at the beginning of the 20th century for the exploitation of its essential oils[22]. These plants are locally named as Indian lavender (or) Indian linaloe since they release a characteristic aroma from their aerial parts. The primary rich constituents of Burseras resin are terpenoid essential oils mainly linaloe and these resins are used topically in folk medicine in the treatment toothache, burns, headache, fever and stomachache[23-25]. Since there are no reports with regard to *B. penicillata's* antioxidant activity the present study has been undertaken to assess the DPPH and ABTS radical scavenging activity.

MATERIALS AND METHODS

Chemicals

DPPH (1,1-Diphenyl-2-picrylhydrazyl) and ABTS (2,2-azinobis(3-ethyl benzothiazoline-6-sulphonic acid diammonium salt) obtained from Sigma Aldrich (MO, USA) and sodium carbonate, sodium phosphate, potassium ferric cyanide, ascorbic acid, gallic acid, potassium persulphate and Folin-Ciocalteu reagent were purchased from Merck Pvt Ltd (Mumbai, India). All other chemicals and solvents were obtained from indigenous companies in India.

Collection and Preparation of Plant Material

Leaves of *B. penicillata* were collected from the botanical garden of Osmania University, Hyderabad, India and the

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Plant material was identified and authenticated by Prof. P. Ramachandra Reddy, Taxonomist, Department of Botany, Osmania University, Hyderabad. A voucher specimen (Bot/OU/0119/HYD) was deposited in the herbarium of the department of botany for future reference. Fresh leaves were collected and shade dried for 10 days then grounded into coarse powder. About 250g of powder macerated in 1500 mL of different solvents such as chloroform, acetonitrile, methanol and water in a dark room at room temperature for 3-5 days with intermittent shaking. The solvent extracts were filtrated and then concentrated at boiling temperature under reduced pressure by using a rotary evaporator to yield a semi solid mass. The crude extracts were measured and stored in refrigerator until further use.

Determination of total phenolic content (TPC)

The total phenolic content was estimated using Folin-Ciocalteu reagent method[26] and gallic acid has used as a standard for plotting calibration curve. A volume of 1 mL of the plant extract (1 mg/1 mL) was mixed with 2 mL of Folin-Ciocalteu reagent (diluted 1:10 with de-ionized water) and was neutralized with 2 mL of 20% sodium carbonate solution. The reaction mixture was incubated at room temperature for 30 min with intermittent shaking for color development. The absorbance of the resulting blue color was measured at 765nm using double beam UV-VIS spectrophotometer (Hitachi U-2910). The total phenolic contents of all the extracts were determined from the linear equation of a standard curve prepared with gallic acid and calculated as mean ± SD (n=3) and expressed as mg/g Gallic acid equivalent (GAE) of dry extract.

Antioxidant assays

Determination of DPPH radical scavenging activity

The free radical scavenging activity of the leaf extracts of *B. penicillata* was evaluated by using DPPH radical scavenging method as described[27]. The assay mixture contained 2 mL of 0.004% DPPH solution prepared in methanol and 0.2 mL of standard (or) sample solution of various concentrations (5-300 µg/mL) in ethanol. The absorbance of the resulting solution was measured after 30 min in dark at 517 nm using spectrophotometer (Hitachi U-2910) and ascorbic acid was used as a positive control. The percentage inhibition of activity was calculated by using the following formula:

Free radical scavenging activity (%) = $(A_{control}-A_{sample}) / A_{control} \times 100$

Where $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of samples.

Determination of ABTS radical scavenging activity

The total free radical scavenging activity of *B. pencillata* was measured by discoloration of $ABTS^+$ radical cation using the method[28]. ABTS radical cation was generated by oxidation of $ABTS^+$ (7 mmol/L) with potassium persulfate (2.4 mmol/L) which was dissolved in 5 mL of distilled water. After incubation for 12-16 h at room temperature in dark condition, blue/green $ABTS^+$ chromophore was produced. The resulting solution was then diluted with ethanol by mixing 1 mL of freshly prepared ABTS solution to obtain an absorbance of (0.700±0.001) at 734 nm using spectrophotometer. Fresh ABTS solution was prepared for each assay. The assay mixture contained 2 mL of ABTS solution and 0.2 mL of standard (or) sample solution of various concentrations (2-140 µg/mL) in ethanol. The absorbance of the resulting solution was measured after 30 min in dark at 734 nm. Lower absorbance of the reaction mixture indicates higher radical scavenging activity and the ABTS scavenging activity of the extracts was compared with that of ascorbic acid and percentage inhibition was calculated by using this formula:

ABTS radical scavenging activity = $(A_{control}-A_{sample}) / A_{control} \times 100$

where $A_{control}$ is the absorbance of ABTS radical with methanol and A_{sample} is the absorbance of ABTS radical with sample extract/standard.

Determination of reducing power

The reducing power of the extracts was quantified by the method[29]. Briefly, 2 mL of reaction mixture containing various concentrations of (10-100 µg/mL) standard (or) plant extracts in sodium phosphate buffer (0.2 M, pH 6.6), incubated with 1% potassium ferricyanide at 50 ^{o}C for 20 min. The reaction was terminated by adding 10% trichloro acetic acid solution and the mixture was centrifuged at 3000 rpm for 10 min. The supernatant (2 ml) was mixed with 2 mL of distilled water and 0.5 mL of 1% ferric chloride solution (Freshly prepared) and absorbance was read at 700nm. Increased absorbance of the reaction mixture indicates grater reducing power and the results were compared with that of the standard (L-ascorbic acid).

Statistical analysis

The determination of total phenolic content, ABTS and DPPH radical-scavenging activity and reducing power were carried out for three replicates and mean values and standard deviations (S.D.) were calculated. One way analysis of variance (ANOVA) was applied for comparison of the mean values and $P \leq 0.05$ was regarded as significant and the results were processed by using Origin and SPSS software.

RESULTS

Extraction yield

Different extraction yields of *B. penicillata* have been calculated and shown in Table 1 and among all fractions methanol extract has been found to be maximal (10.8%) followed by acetonitrile (7.6%).

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Total phenol content

The total phenolic content of the *B. penicillata* leaf extracts was expressed in terms of gallic acid and yield (%) (w/w) and shown in Table 1. The amount of the total phenolic content in different extracts was determined using the linear regression equation of the calibration curve ($y=0.0009x-0.0144$, $r^2=0.998$) and expressed as gallic acid equivalent. The amount of phenolic content was higher in acetonitrile extract in relation to the other extracts.

DPPH free radical scavenging

The free radical scavenging activity of *B. penicillata* leaf extracts was investigated with DPPH and it was compared with ascorbic acid (a known antioxidant with a IC_{50} value of 17.69±0.07 µg/mL) and the results have been expressed as % inhibition (Fig.1 A). Different extracts exhibited considerable free radical scavenging activity as indicated by their IC_{50} values (Table 1) and acetonitrile leaf extract has shown significant free radical scavenging activity compared to other extracts. However, scavenging ability of all the leaf extracts' has been found to be significant ($p\leq0.05$) in relation to that of ascorbic acid's.

ABTS radical scavenging activity

Fig.1B. Shows the free radical scavenging ability of different leaf extracts of *B. penicillata* and interestingly acetonitrile extract has shown a better and significant activity ($p\leq0.05$) than the other extracts (Table 1) and it comes closer to that ascorbic acid.

Reducing power activity

The reducing power activity was found to increase with the increase in concentration of the extract (Table 2) and the acetonitrile extract has been found to be significant when compared to the non-polar extracts such as chloroform and polar extracts such as methanol and aqueous (Fig.2). The reducing power activities of the extracts has been shown in descending order (ascorbic acid > acetonitrile extract > methanol extract > aqueous extract > chloroform extract) and ascorbic acid and chloroform registering highest and lowest activities respectively.

Table 1: Free radical scavenging activities, total phenolic content and % yield of the different extracts of *B. penicillata*

No	Extraction	% yield	IC_{50} values		Total phenolic content (mg/g GAE)
			ABTS	DPPH	
1	Chloroform	1.68	71.07±1.44[a]	194.66±15.8[a]	17.60±0.76*
2	Acetonitrile	7.6	39.25±3.34[a]	142.36±8.98[a]	39.32±4.05*
3	Methanol	10.8	45.33±3.41[a]	191.70±1.24[a]	29.96±2.33*
4	Aqueous	3.76	56.44±2.86[a]	201.47±15.42[a]	15.35±1.71*
5	Ascorbic acid	NA	5.06±0.09	17.69±0.07	NA

Values were represented as mean ± SD (*n=3*), Superscript [a] denotes in column are statistically significant (***p*≤0.05**) compared with standard (ascorbic acid). *Indicate in column are statistically significant (***p*≤0.05**). NA: Not analyzed, GAE: Gallic acid equivalent.

Table 2: The reducing power assay of the different extracts of *B. penicillata*

Concentration (µg/ml)	Chloroform extract	Acetonitrile extract	Methanol extracts	Aqueous extracts	Standard (Ascorbic acid)
20	0.034±0.00	0.091±0.06	0.050±0.01	0.029±0.00	0.472±0.01
40	0.081±0.00	0.193±0.13	0.203±0.01	0.085±0.01	0.973±0.06
60	0.152±0.01	0.521±0.13	0.304±0.02	0.176±0.02	1.602±0.02
80	0.244±0.02	0.745±0.02	0.427±0.03	0.256±0.19	2.219±0.05
100	0.308±0.01	1.001±0.04	0.596±0.02	0.448±0.01	2.592±0.03

Values were represented as mean ± SD (*n=3*)

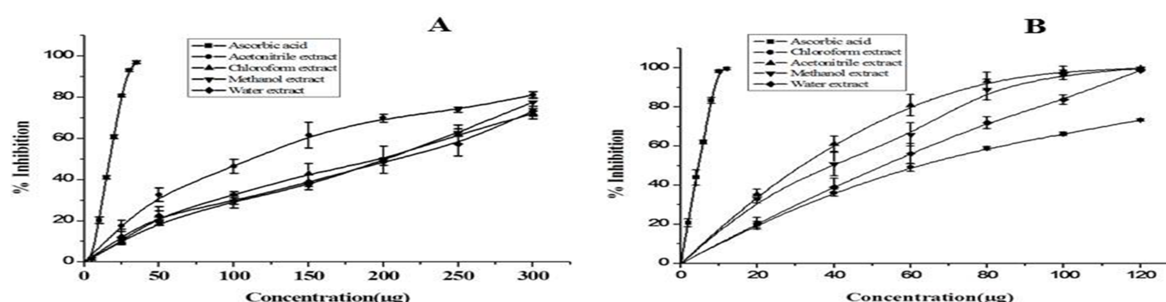

Figure 1: (A**)** DPPH radical scavenging activity and (B) ABTS radical scavenging activity of the various extracts of *B. penicillata* leaves and ascorbic acid (standard). Results represent means of triplicates of different concentrations analysed.

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DISCUSSION

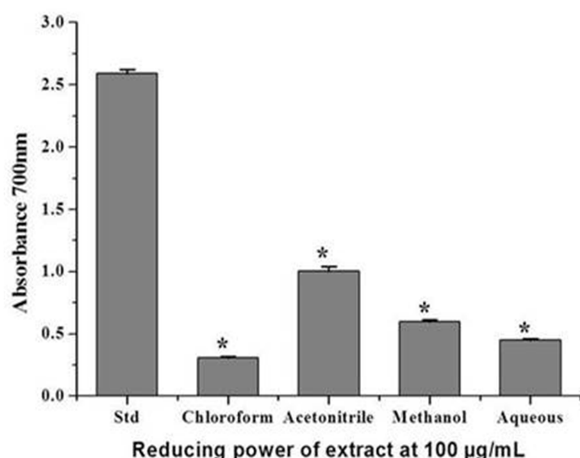

Figure 2: The assay of reducing power of different extracts of *B. penicillata* leaves and standard. The vertical bars represent ± SD (n=3). *denotes statistically significance compared with standard (ascorbic acid).

Medicinal plants since ancient times are known for their diverse pharmacological actions which are attributed to the presence of phytochemicals such as alkaloids, flavonoids, glycosides, tannins, and steroids, etc. Phenols and flavonoids have been identified as free radical scavengers and can prevent damage caused by oxidative stress[30,31]. And, in recent times they have caught the attention of researchers from the point of view of their protective abilities in oxidative damage in cellular systems. The phenolics of these natural sources have been identified as potential free radical scavengers that can very effectively prevent damage mediated by oxidative stress. Many reports have suggested that the active compounds/molecules are effective in reducing the risk and progression of many acute and chronic diseases like cancer, cardiovascular, neurodegenerative and diabetes by scavenging free radicals[32,33]. Among the four different extracts (chloroform, acetonitrile, methanol and water) of *B. penicillata*, methanol extract has been found to register a higher percentage of yield and this could perhaps be due to its high polarity[34]. Phenolic compounds are well known for their role in oxidative damage and acetonitrile extract of *B. pencillata* has registered a higher phenolic content compared to other leaf material[35].

The free radical scavenging method is commonly used to test in *in-vitro* antioxidant activity of plant extracts. DPPH is a stable, nitrogen centered free radical which produces thick purple color in methanol solution. The principle of this method is based on the reduction of purple colored methanolic DPPH solution in the presence of hydrogen donating antioxidants by the formation of yellow colored diphenyl-picryl hydrazine. DPPH reduction is directly proportional to the amount of antioxidant present in the extract. DPPH radical scavenging activity of leaf extracts of *B. penicillata* was compared to standard ascorbic acid and acetonitrile extract has been found to be a good scavenger of the free radicals with low IC_{50} value which can be attributed to the presence of phenolic hydroxyl group. The radical cation involved in ABTS assay is being measured on the basis of inhibition of absorbance by antioxidants and it has a characteristic wavelength at 734nm. The principle behind the technique involves the reaction between ABTS and potassium per sulfate to produce the ABTS radical cation which is a blue green chromogen. In the presence of antioxidant reductant, the coloured radical is converted back to colourless ABTS[36]. Chakraborty[37] reported that the decolourization of the $ABTS^+$ cation reflects the capacity of an antioxidant to donate electrons or hydrogen atoms to counter this radical species and acetonitrile extract was found to have a higher ABTS radical scavenging activity.

The protective nature of an extract is primarily due to its reducing power which serves as an indicator to measure its potential antioxidant activity that can terminate radical chain reaction by converting free radicals and reactive oxygen species to more stable product[38]. And, more the reductones the more will be antioxidant potential. The acetonitrile extract from *B. penicillata* had exhibited a better reducing activity which could be due to the presence of high amount of reductones that can react with free radicals in an attempt to counter them and thereby protecting cellular system. *B. penicillata* leaves showed a higher reducing power and free radical scavenging activity as evidenced by the low IC_{50} values of 142.36±8.98 µg/mL and 39.25±3.34 µg/mL in DPPH and ABTS respectively. Besides this, the extract was also found to have high levels of phenolic content. In summary, the results of the present investigation suggest that the leaves of *B. penicillata* are a rich natural source of phenolic content and merit for further investigation to exploit its antioxidant potential which could be of immense use in countering oxidative insult in biological systems.

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