INNOVARE JOURNAL OF SCIENCES

Vol 4, Issue 5, 2016

ISSN - 2321-5496

Research Article

COMPARISON OF PHYTOCHEMICAL SCREENING AND BIOLOGICAL ACTIVITIES OF AQUEOUS AND ALCOHOLIC FRACTIONS OF *PTEROSPERMUM ACERIFOLIUM* (LEAF PETIOLE)

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Received: 28 July 2016, Revised and Accepted: 08 August 2016

ABSTRACT

Objectives: The objective of the current work was to compare the two fractions (aqueous and alcoholic) of leaf petioles of *Pterospermum acerifolium*. The two fractions were compared for their phytochemistry and biological activities (antioxidant, antibacterial, and antifungal).

Methods: Phytochemistry was done using conventional natural products identification tests. Antioxidant activity was done using DPPH assay was used for free radicals scavenging. Similarly, antifungal and antibacterial assays were performed using agar tube dilution and agar well diffusion assays, respectively.

Results: Phytochemical screening using conventional natural products identification tests indicated the presence of different phytoconstituents. The phytoconstituents present in the methanolic extract were tannins, saponins, steroids, terpenoids, coumarins, and betacyanins while aqueous extract contains tannins, glycosides, reducing sugar, saponins, steroids, terpenoids, coumarins, and betacyanins. Both the extracts were analyzed for antioxidant activity. The aqueous extract showed a significant activity as compared to methanolic extract. The same two fractions were compared for their antibacterial and antifungal activities.

Conclusion: The methanolic and aqueous extracts obtained from *P. acerifolium* show strong activity against most of the tested fungal and bacterial strains; it is concluded that methanolic extract showed significant activity against fungal strains as compared to aqueous extract, whereas the antibacterial activity of aqueous extract is more active than methanolic extract. Efforts should be made to isolate and derive active compounds responsible for these activities.

Keywords: *Pterospermum acerifolium*, Phytochemistry, Antioxidant activity, Antibacterial activity, Antifungal activity.

INTRODUCTION

For fulfilling basic needs of health, about 80% of the world's population is dependent on medicinal plants. Medicinal plants are the significant source of natural drugs. *Pterospermum acerifolium* belongs to the family Sterculiaceae. Its common name is dinner plate tree. Matured leaves are too large, having a length and width of up to 35 cm. They can be used as actual dinner plates or as packaging and storage by wrapping materials inside. In India, they are shaped into regular dinner plates and soup bowls on molds, some even stitched together with twigs. In Burma, they are also used to dry tobacco upon. The plant is widely distributed in Indian sub-Himalayan tract, outer Himalayan valley, North America, and Pakistan [1]. *P. acerifolium* is known locally as Kanak Champa, Matsakanda Karnikara, and Muchukunda. The aqueous and alcoholic extracts of this plant possess numerous medicinal properties, for example, anti-inflammatory, analgesic, antipyretic, and antihyperglycemic activities [2]. The bark of the plant has been in use for the management of diabetes [3]. *P. acerifolium* ethanol and water extracts were found to be active against all bacterial strains under investigation [4]. The ethanol extract of flowers was reported to inhibit the growth of cancer cells [5]. Flowers and bark were known for treatment of smallpox [6].

The current work deals with the comparison of phytochemical screening tests and biological profile (antibacterial effectiveness, antifungal activity, and antioxidant activity) of methanolic and aqueous extracts of *P. acerifolium*.

METHODS

Plant material

Plant *P. acerifolium* was collected from the "Kuram Garhi" area of Bannu, KPK-Pakistan. The plant was identified in the Department of Botany, University of Peshawar. The voucher specimen was deposited at the herbarium of the department.

Extraction

Shade dried *P. acerifolium* plant leaves were earmarked in methanol and water each for 72 hrs for extraction of crude. After 4 days, the methanolic primary extract was concentrated by solvent removal in rotavap while aqueous crude extract was left for a couple of days at room temperature for evaporation of water. Methanolic extract was lyophilized to fine powder and stored at 4°C for further activities.

Phytochemical profiling

Standard methods of phytochemical screening were applied to investigate bioactive secondary metabolites [7-12].

Alkaloids

About 0.5 g of the crude with 2% H_2SO_4 was heated for 2 minutes. A few drops of Dragendroff's reagent were added to cooled mixture of the same. Signal of orange-red precipitous is the existence of alkaloids.

Tannins

About 1 g of rudimentary extract was intermixed with H_2O and warmed using a water bath. A few drops of ferric chloride were mixed in the mixture. Appearance of dark green solution shows the presence of Tannins.

Anthraquinones

About 0.5 g of rudimentary extract along with 10% HCl was boiled for few minutes in a water bath. The mixture was allowed to cool. $CHCl_3$ was added to the reaction mixture in a quantity of 2 ml. In addition,

a few drops of ammonia (10%) were added to mixture and heated. Anthraquinones are specified by the existence of rose pink look.

Glycosides
To hydrolyze the crude extract, HCl was used and it was then neutralized with NaOH solution. Fehling's solution A and B were added in a minute quantity to the mixture. Development of red precipitate implies the manifestation of glycosides.

Reducing sugars
For reducing sugar test, crude extract was supplemented with distilled water and shaked. The reaction mixture was allowed to boil with a small amount of Fehling's solution A and B for limited time. An orange-red precipitates indorse the presence of reducing sugars.

Saponins
For the detection of saponins, 3 ml of distilled H_2O was added to 0.5 g of extract and shaked. It was then heated till boiling. Frothing (appearance of creamy miss of small bubbles) ratifies the occurrence of saponins.

Flavonoids
The existence of flavonoids check was done by dissolving 0.2 g of crude extract in diluted NaOH. Few dribs of HCl were added. A yellow solution that turns colorless shows the presence of Flavonoids.

Phlobatannins
Crude extract in a quantity of 0.5 g was liquefied in distilled water. The mixture was boiled with 2% HCl solution. Red precipitous displays the positive results for the existence of phlobatannins.

Steroids
Acetic anhydride (2 ml) was added to the reaction mixtures of 0.5 g of crude extract and 2 ml H_2SO_4. The color turns from violet to blue or green express the steroids occurrence.

Terpenoids
About 0.2 g of each extract was mixed with 2 ml of chloroform and concentrated H_2SO_4 (3 ml) was prudently added to synthesize a layer. Formation of reddish brown coloration at the interface means the positive results for the presence of terpenoids.

Test for coumarin
Around 10% of NaOH (3 ml) was added to 2 ml of aqueous crude solution. The presence of coumarin can be confirmed by the appearance of yellow color.

Test for emodins
NH_4OH (2 ml) and benzene (3 ml) were added to crude extract. Appearance of red indicates the presence of emodins.

Test for anthocyanin and betacyanin
To 2 g of extract, 1 ml of NaOH (2N) was added and heated for 5 minutes at 100°C. Formation of yellow pigments designates the presence of betacyanins and creation of bluish green pigments shows the presence of anthocyanins.

Antioxidant activity using DPPH radical scavenging assay (RSA)
DPPH free radical rummaging test was used for determining antioxidant potential of the two extracts. Standard assay mentioned by Acharya and Patel, 2016, was used [13]. The oxidation capacities of the methanolic extracts of leaf petioles were determined from the blanching act of the DPPH solution in wood alcohol. For standard, 1 mM solution was prepared by dissolving DPPH in wood alcohol. 1 ml of this solution was assorted with 3 ml of fraction in methanol (containing 10-100 µg) and blank (without sample). The solution was kept in dark for 30 minutes, and then absorbance was taken at 517 nm. Decrease in the DPPH solution absorbance confirms an increase in the DPPH radical foraging activity. As percent radical scavenging activities (%RSA) using DPPH, the scavenging of free radicals was calculated as:

% DPPH = (OD blank/control – OD sample) × 100/OD blank

OD blank shows the absorbance of the blank solution, and OD sample shows the absorbance of standard and sample.

Antifungal activity
The method used for assessing antifungal activity was used as described by Martin *et al*., 2016 [14]. 22 mg from both extracts was taken in clean and sterile vials. These weighed samples were then liquefied in 1 ml sterilized dimethyl sulfoxide. They were then properly homogenized. SDA was used as growth media for the fungus. Now, 9.75 g of the powdered SDA was dissolved in 150 ml distilled water. It was then standardized. The media and micropipette tips were sterilized by autoclave. The tubes were cooled at 50°C, and test samples were encumbered into the non-solidified SDA in biological safety hood. Each sample was poured into test tubes for specified fungal cultures and negative control. Tubes were arranged with their corresponding samples in slanting positions at room temperature (for 24 hrs to form slants). After 24 hrs, slants were screened for their sterility and each sample tube was injected with 4 mm diameter of fungus taken from 7 days old cultures of fungus. The tubes were kept in fungal incubators at 27°C-29°C for 3-7 days for incubation. To calculate inhibition of fungal growth, the formula given below was used.

% inhibition = 100 – Linear growth in test sample (mm)/Linear growth in control (mm) ×100

Antibacterial activity
To assess antibacterial activity, standard protocol mentioned by MERLI M 2016 was used [15].

Klebsiella pneumonia, *Staphylococcus aureus, Bacillus subtilis,* and *Staphylococcus epidermidis* were used for the antibacterial activity. The bacteria were kept in Mueller-Hinton agar in the refrigerator at 4°C.

The cultures were taken in triplicates at cultivation temperature of 37°C for 1 to 3 days. The broth culture of the test organism was placed in a sterilized Petri dish to which 20 ml of the sterilized molten MHA was supplemented. Wells were bored into the medium using 0.2 ml of the extract. The standard antibacterial agent used was streptomycin (2 mg/ml). Inoculation was done for 1 hr to make possible the diffusion of the antibacterial agent into the medium. The inoculation plates were incubated at 37°C for 24 hr, and the diameter of the zone of inhibition of bacterial growth was measured in the plate in millimeters.

RESULTS AND DISCUSSION

Both the methanolic and aqueous extracts showed dynamic results. Phytochemical constituents present in both the extracts are illustrated in Table 1. The antioxidant behavior of both the extracts is demonstrated

Table 1: Phytochemical screening of methanolic and aqueous extracts of *P. acerifolium* leaf petioles

Test	Methanolic extract	Aqueous extract
Steroids	+	+
Terpenoids	+	+
Coumarins	+	+
Emodin	-	-
Anthocyanin	-	-
Betacyanin	+	+
Flavonoids	-	-
Alkaloids	-	-
Tannins	+	+
Anthraquinones	-	-
Glycosides	-	+
Reducing sugar	-	+
Saponins	+	+
Phlobatannins	-	-

P. acerifolium: Pterospermum acerifolium

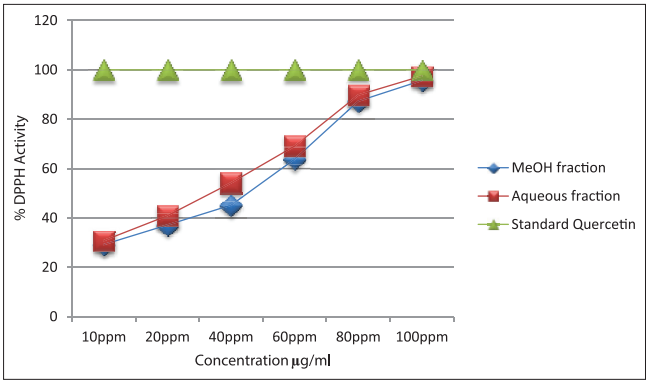

Fig. 1: Antioxidant activity of methanolic and aqueous extracts of *Pterospermum acerifolium* leaf petioles

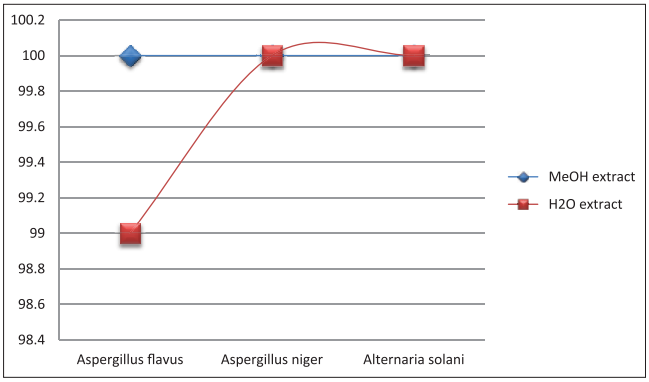

Fig. 2: Antifungal activity of methanolic and aqueous extracts of *Pterospermum acerifolium* leaf petioles

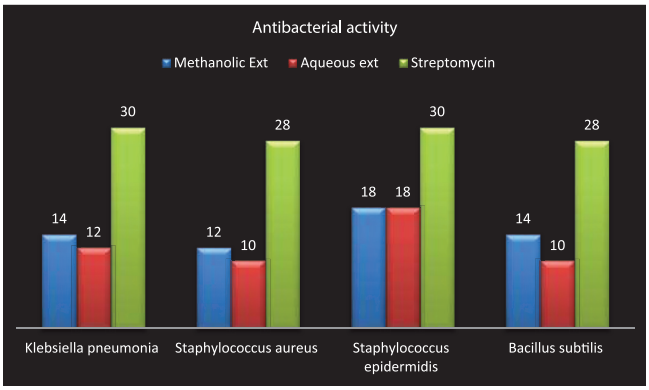

Fig. 3: Antibacterial activity of methanolic and aqueous extracts of *Pterospermum acerifolium* leaf petioles

in Fig. 1. Antimicrobial tests (antifungal and antibacterial) results are substantiated in Figs. 2 and 3, respectively. Phytochemical screening plays an important role in the isolation of new compounds and discovery of drugs. Natural products are produced by plants in most selective and precise way which is responsible for nitrating biological action. The methanolic and aqueous extracts of *P. acerifolium* were screened for phytochemical tests which showed that both extracts contained tannins, saponins, steroids, terpenoids, coumarins, and betacyanins but in aqueous extract glycosides and reducing sugar were also identified which showed that aqueous extract contains more phytochemical constituents as compared to methanolic extract (Table 1).

DPPH is a free radical compound and has been widely used to test the free radical scavenging ability of various samples. It is a stable free radical with a characteristic absorption at 517 nm and was used to study the radical scavenging effects of extracts. The decrease in absorbance of the DPPH radical owing to the scavenging capability of *P. acerifolium* extract is illustrated in Fig. 1. It is cleared from the figure that with the increase in concentration the scavenging power is also increased. Aqueous extract showed more scavenging power than methanolic extract (Fig. 1).

Antimicrobial properties of medicinal plants are being progressively reported from different parts of the world. The WHO guesstimates that plant extract or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population.

CONCLUSION

In the present work, the methanolic and aqueous extracts obtained from *P. acerifolium* shows strong activity against most of the tested fungal and bacterial strains (Figs. 2 and 3, respectively). It is clear from Fig. 2 that methanolic extract showed significant activity against fungal strains as compared to aqueous extract. Fig. 3 shows antibacterial activity which confirms that aqueous extract is more active than methanolic extract.

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