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Research Article

ANTIOXIDATIVE AND ANTIMICROBIAL STUDY OF *SPONDIAS MANGIFERA* WILLD ROOT

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ABSTRACT

The present investigation evaluates the antioxidative and antimicrobial activities of the methanol and aqueous extracts of *Spondias mangifera* (Family: Anacardiaceae) root. Three principal bioactive compounds such as saponins, flavonoids and tannins are positive for both the extracts, alkaloids are detected only in methanol extract and are absent in aqueous extract. Both methanol and aqueous extracts have shown promising antibacterial activity against gram positive bacteria viz. *B. subtilis* and *S. aureus*. The extracts were screened for their in vitro antioxidant potential. Inhibition of oxygen-derived free radicals, viz., assays for free radical scavenging by DPPH, reducing power ability and nitric oxide scavenging were performed. All the antioxidant activities were compared with standard antioxidant such as ascorbic acid. Both the extracts of this plant showed effective free radical scavenging activity, reducing power and nitric oxide scavenging activity. All these antioxidant properties were concentration dependent. The highest antioxidant activity was observed with methanol extracts that could be attributed due to the presence of flavonoids and saponins.

Keywords: *Spondias mangifera*, Phytochemical, Antioxidative, Antimicrobial.

INTRODUCTION

Recently extracts of plants have provoked interest as sources of natural products. They have been screened for their potential uses as alternatives medicines for the treatment of many infectious diseases and also in preservation of food from the toxic effects of oxidants. In modern days the antioxidants and antimicrobial activities of plant extract have formed the basis of many applications in pharmaceuticals, alternative medicines and natural therapy[1]. Because of the possible toxication of synthetic antioxidants like Butylated hydroxy anisole (BHA) and Butylated hydroxyl toluene (BHT), an increased attention has been directed towards natural antioxidants. The trend to use extracts of plants may act as natural antimicrobial and antioxidants influence the health[2]. Some of the active principles of bioactive compounds are preferred for their therapeutic purposes either singly or in combination to inhibit the life processes of microbes[3, 4].

Spondias mangifera Willd. (Family- Anacardiaceae) is a glabrous tree upto 10.5 m high with straight trunk and smooth ash coloured bark having characteristic pleasant smell of wood [5]. In India it is cultivated in Punjab, Maharashtra, Orissa, West Bengal and Assam for the edible fruits [6]. Ethnomedicinally, the bark is used as tonic, refrigerant and for the treatment of articular and muscular rheumatism and in diarrhoea and dysentery[9]. The leaves are aromatic, acidic and astringent used for flavouring while its juice is applied in ear ache[8,10]. The root bark powders have been recommended for regulation of menstruation[11-15] and, antitumor [16], antipyretic, antispasmodic and antihistamine activities were also reported [17]. The aerial parts are reported to contain daucosterol, β-sitosterol, stigmast-4-en-3-one, cycloartanone 24-methylene and lignoceric acid. Other constituents like β-amyrin and oleanolic acid were also reported from the fruits [18].

In the present study anti microbial and antioxidative activities of methanol and aqueous extracts of *S. mangifera* are investigated. The antimicrobial activities were determined by using disk diffusion assay and Minimal Inhibitory Concentration (MIC) values. The antioxidant activities are determined by scavenging of DPPH radical, reducing power determination and scavenging of Nitric oxide method.

MATERIALS AND METHODS

Plant material

The plant material (roots) was collected from the forests of Ganjam district of Orissa during June 2007 and identified by the taxonomists of the Botanical Survey of India, Shibpur, and Howrah. A voucher specimen [Sp. No: CNH/ I-I / (17)/2009/Tech.II/28] has been kept in our research laboratory for further reference.

Preparation of extract

The powdered root (500 g) after defatting with petroleum ether (60-80^0 C) for 48 h was successively extracted with methanol and aqueous for 48 h in a soxhlet extractor. Following extraction, the liquid extracts were concentrated under vacuum to yield dry extracts. Standard methods [19-20] were used for preliminary phytochemical screening of the methanol and aqueous extracts and then crude extract was used for further investigation for antimicrobial and antioxidant properties.

Anti microbial activity

Methanol and aqueous extracts were tested against a panel of micro organisms including *Bacillus subtilis* UC 564, *Staphylococcus aureus* NCTC 8530, *Pseudomonas aerugenosa* 25619, *E. coli* ATCC 2457T obtained from stock cultures of Indian institute of cholera and Enteric diseases, Kolkata, India. Stock cultures were maintained on nutrient agar medium at 40^0 C, then subcultures in nutrient Broth at 37^0 C, prior to each anti microbial test.

Determination of zone of inhibition

The zone of inhibition of the test samples was performed by disc-diffusion method as suggested by *Awoyinka et al*., 2007 [21]. The dried plant extracts were dissolved in 5 per cent dimethylsulphoxide (DMSO; Merck, Germany) and then in sterile water, to reach a final concentration of 30 mg/ml and sterilized by filtration by 0.22 µm Millipore filters. The media used were Mueller Hinton Agar (HiMedia) for the bacteria. The discs (6 mm in diameter) were impregnated with 10 µl of the extracts (300 µg/disc) at a concentration of 20 mg/ml and placed on the inoculated agar (10^6 Cfu/ml). Tetracyclin (30 µg/ml) were served as positive reference standards to determine the sensitivity of the tested microbial strains. Control tests with the solvent DMSO (5%) employed to dissolve the plant extracts were performed for all assays and showed no inhibition of microbial growth. The inoculated plates were incubated at 37°C for 24 h for bacterial strains. Antimicrobial activity was evaluated by measuring the zone of inhibition against the tested organisms. All inhibition assays and controls were made in triplicate.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentrations (MIC) of methanol and aqueous extracts of *S. mangifera* with respect to different test microorganisms were determined by broth dilution method[22].

Mueller Hinton broth (HiMedia) was used for the antibacterial study. The extracts dissolved in 1 per cent of DMSO were first diluted to highest concentration (200 mg/ml) to be tested, and then serial two-fold dilution were made in a concentration range from 0.39 to 200 mg/ml in sterile water. For broth dilution, 0.1 ml of standardized suspension of a strain (10^6 CFU/ml) separately was added to each tube containing various extracts at concentrations of 0 (control), 0.39, 0.78, 1.56, 3.125, 6.25, 12.5, 25, 50, 100 and 200mg/ml in the broth medium. The tubes were incubated at 37° C for 24 h for bacterial strains and looked for visible growth after vortexing the tubes gently. The lowest concentration of test extract in a tube that failed to show any visible macroscopic growth was considered as its MIC. Inhibition of proliferation was assessed by optical density measurements (625 nm). The MIC determination was performed in triplicate for each organism.

Antioxidative activity

Scavenging of DPPH radical

This assay[23] based on the measurement of the scavenging ability of antioxidant test extracts towards the stable radical. The free radical scavenging activity of methanol and aqueous extracts of root of *S. mangifera* were examined *in-vitro* using DPPH [1, 1-Diphenyl, 2-picryl-hydrazyl] radical. The test extracts were treated with different concentrations from a maximum of 250 µg/ml to minimum of 4µg/ml. The reaction mixture consisted of 1ml of 0.1mM DPPH in methanol, 0.95 ml of 0.05 M Tris-HCL buffer (pH-7.4), 1ml of methanol and 0.05 ml of herbal extract. The absorbance of the mixture was measured at 517 nm exactly 30 sec after adding extracts. The experiment was performed in triplicate and percentage of scavenging activity was calculated using the formula 100-[100/blank absorbance X sample absorbance]. The blank was also carried out in similar manner, using distilled water in the place of extracts. The activity was compared with ascorbic acid, which was used as a standard antioxidant.

Reducing power determination

Different amounts of the extracts in methanol and in aqueous solutions [24] were mixed with 2.5 ml of (pH-6.6) 0.2 M phosphate buffer and 2.5 ml of 1%potassium ferricyanide [$K_3Fe(CN)_6$]. The mixture was incubated at 50^0 C for 20 minutes. 2.5 ml of 10% trichloroacetic acid was added to the mixture, which was then centrifuged for 10 minutes at 1000 rpm. 2.5 ml upper layer of solution was mixed with 2.5ml of distilled water and 0.5 ml of 0.1% ferric chloride. The absorbance was measured at 700 nm. The blank was also carried out in similar manner, using distilled water in the place of extracts. Increase in the absorbance of the reaction mixture indicated the increase in the reducing power. The activity was compared with ascorbic acid, which was used as a standard antioxidant.

Scavenging of nitric oxide

Sodium nitroprusside (5 µM) in standard phosphate buffer solution [25] was incubated with different concentration of the methanol and aqueous extracts dissolved in standard 0.025 M phosphate buffer (pH-7.4) and the tubes were incubated at 25^0 C for 5 hours. After 5 hours, 0.5 ml of incubation solution was removed and diluted with 0.5 ml of Griess reagent (prepared by mixing equal volume of 1% sulphanilamide in 2% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride in water). The absorbance of chromophore formed was read at 546 nm.

RESULTS

The result of phytochemical screening reveals those three active compounds: saponins, flavonoids and tannins are positive for both the extracts. Alkaloids are detected only in methanol extract and are absent in aqueous extract (Table 1).

The results of antimicrobial susceptibility (Table 2) reveals methanol and aqueous extracts have promising antibacterial activity against gram positive bacteria (*B. subtilis* and *S. aureus*) as compare to gram negative bacteria (*E. coli* and *P. aerugenosa*).

DPPH scavenging

The methanol and aqueous extracts of root of *S. mangifera* showed promising free radical scavenging effect of DPPH in a concentration dependant manner up to a concentration of 250 µg/ml. Methanol extract showed more scavenging activity than the aqueous extract. The reference standard ascorbic acid also showed a significant radical scavenging potential in the concentration of 1 µg/ml. The DPPH radical inhibition of aqueous, methanol and ascorbic acid was 50.13%, 52.12% and 78.12% respectively (Table 3).

Reducing power determination

As the concentration of the extracts increased, the absorbance was also increased correspondingly, indicating that both the methanol and aqueous extracts have potent anti-oxidant activity by reducing power ability. Methanol extracts showed more reducing power ability than aqueous extract, when compared with that of the standard anti-oxidant ascorbic acid (Table-4).

Nitric oxide scavenging

Methanol extract of root of *S. mangifera*, showed significant free radical scavenging action against nitric oxide (NO) induced released of free radicals at the concentration 250 µg/ml, the percentage of scavenging was found to be 47.92% than aqueous extract as 22.22% of NO inhibition, respectively. Ascorbic acid was used as the reference standard and its percentage of inhibition was 72.23% (Table-5).

Table 1: Phytochemicals screening of methanol and aqueous extracts of root of *S. mangifera*

Phytochemical compound	Methanol extract	Aqueous extract
Flavonoids	+	+
Saponins	+	+
Tannins and phenolic compounds	+	+
Gums and mucilages	-	+
Alkaloids	+	-
Carbohydrates	-	+

(+): Present; (-): Absent.

Table 2: Antibacterial activity of methanol and aqueous extracts of *S. mangifera* against bacterial strain

Microorganism	Diameter of inhibition zone (mm)			MIC (µg/ml)	
	Methanol extract	Aqueous extract	Tetracyclin	Methanol extract	Aqueous extract
E. coli	-	-	32.0 ± 0.7	-	-
P. aerugenosa	13.2 ± 0.5	-	34.3 ± 0.8	200	-
B. subtilis	28.8 ± 0.2	12.2 ± 0.6	35.0 ± 0.3	6.25	50
S. aureus	30.6 ± 0.9	13.0 ± 0.45	34.6 ± 0.2	12.5	25

Note: The control disc used for solvent had no zone of inhibition, so there data was omitted from the above data. Inhibition zones including the diameter of the paper disc (6 mm). Results are expressed as the mean ± SEM of triplicate measurements

Table 3: In vitro free radical scavenging effect of *S. mangifera* by DPPH method

	Percentage scavenging (Mean ±SEM) of triplicates							
	4 µg/ml	**8µg/ml**	**15µg/ml**	**30µg/ml**	**60µg/ml**	**125µg/ml**	**250µg/ml**	**IC_{50}µg/ml**
Methanol extract	25.02±0.002	25.86±0.002	27.85 ±0.001	31.3±0.001	42.44 ±0.001	44.03±0.002	52.01±0.002	203
Aqueous extract	21.35±0.002	22.54±0.001	23.34±0.001	30.77±0.001	37.40±0.001	45.22±0.002	50.13±0.002	213
	0.1µg/ml	**0.2µg/ml**	**0.4µg/ml**	**0.6µg/ml**	**0.8µg/ml**	**1µg/ml**		
Ascorbic acid	5.90±0.002	13.36±0.001	31.51±0.001	46.18±0.003	62.15±0.001	78.12±0.001		0.65

Table 4: In vitro free radical scavenging effect of *S. mangifera* by reducing power determination

	Absorbance (Mean ±SEM) of triplicates							
	4µgml	**8µg/ml**	**15µg/ml**	**30µg/ml**	**60µg/ml**	**125µg/ml**	**250µg/ml**	**500µg/ml**
Methanol extract	0.088±0.002	0.162±0.002	0.272±0.001	0.388±0.001	0.434±0.001	0.596±0.002	0.705±0.002	0.898±0.002
Aqueous extract	0.054±0.002	0.117±0.001	0.156±0.001	0.232±0.001	0.300±0.001	0.372±0.002	0.425±0.002	0.511±0.002
	0.1µg/ml	**0.2µg/ml**	**0.4µg/ml**	**0.6µg/ml**	**0.8µg/ml**	**1µg/ml**		
Ascorbic acid	0.056±0.002	0.180±0.001	0.242±0.001	0.492±0.003	0.805±0.001	0.968±0.001		

Table 5: In vitro free radical scavenging effect of *S. mangifera* by nitric oxide scavenging method

	Percentage scavenging (Mean ±SEM) of triplicates							
	4µgml	**8µg/ml**	**15µg/ml**	**30µg/ml**	**60µg/ml**	**125µg/ml**	**250µg/ml**	**IC_{50}µg/ml**
Methanol extract	42.6±0.002	42.71±0.002	42.90±0.001	43.19±0.001	43.77±0.001	46.28±0.002	47.92±0.002	325
Aqueous extract	1.13±0.002	6.02±0.001	6.49±0.003	7.53±0.003	9.03±0.004	14.68±0.001	22.22±0.002	614
	0.1µg/ml	**0.2µg/ml**	**0.4µg/ml**	**0.6µg/ml**	**0.8µg/ml**	**1µg/ml**		
Ascorbic acid	3.14±0.001	13.54±0.002	29.28±0.001	40.33±0.001	61.47±0.004	75.23±0.001		0.67

DISCUSSION

The free radical scavenging activity of the extracts was evaluated based on the ability to scavenge the synthetic DPPH. This assay provided useful information on the reactivity of the compounds with stable free radicals, because of the odd number of electrons. DPPH shows a strong absorption band at 517 nm in visible spectrum (deep violet color). As the electron became paired of in the presence of free radical scavenging, the absorption vanishes on the resulting discoloration stoichiometrically coincides with respect to the number of electrons taken up. The bleaching of DPPH absorption is representative of the capacity of the methanol and aqueous extracts to scavenge free radicals independently.

The reducing properties are generally associated with the presence of reductones, which has been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom. Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. Increase in the absorbance of the extracts indicated the antioxidant activity.

Nitric oxide is an essential gas required for several normal physiological processes like neural signal transmission, immune response and control of blood pressure. However the elevation of nitric oxide level was found in several pathological conditions including cardio vascular disease, diabetes etc Sodium nitroprusside serves as a chief source of free radicals. The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with naphthylethylene diamine is used as a marker for nitric oxide scavenging activity [26]. The chromophore formation was not complete in the presence of extracts (methanol and aqueous), which scavenges the NO thus formed from the sodium nitroprusside and hence the absorbance decreases as the concentration of the extract increases in a dose dependent manner.

These values revealed that the antioxidant activity of methanol extract is better than aqueous extracts that could be attributed due to the presence of high content of crude flavonoids and saponins [2].

The results obtained from phytochemical analysis, antioxidant and antimicrobial activity of *S. mangifera* could consider this plant as a natural herbal source that can be used in pharmaceutical industry. Further investigation may lead to the development of phytochemicals drugs.

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