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Antioxidants and Polyphenolic Compounds Studies from *Spondias mangifera* Willd. extracts

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Abstract

Spondias mangifera Willd. is an economical and medicinal plant. This plant is used for the treatment of various diseases. This study aims to estimate antioxidant activity and polyphenolic compounds from various extracts of *in vitro* leaf and callus. Callus and *in vitro* leaf extract were used for the estimation of antioxidant activity was evaluated through DPPH assay and beta-carotene/linoleic acid bleaching assay. Total phenolic content, total flavonoid content and reducing potential were estimated through standard method. Highest antioxidant and polyphenolic compound was found in methanolic extract of callus and *in vitro* leaf. Maximum reducing potential was observed in ethanolic extract of callus and methanolic extract of *in vitro* leaf. From the obtained results, it is concluded that *in vitro* leaf and callus are rich source of different phytochemicals and natural antioxidants.

Keywords: *Spondias mangifera; Phytochemical Analysis; Antioxidant Activity; Reducing Potential; In Vitro Cultures*

Introduction

Free radicals are very unstable due to presence of a free electron and react quickly with other compounds and try to capture the needed electron to gain stability. Free radicals have important roles in many biological processes like energy production, phagocytosis, cell growth regulation and synthesis of biologically important compound but its excessive amount also causes harmful effect [1]. These are consistently formed in the human body of as by-products of aerobic metabolism generally as reactive oxidant species (ROS) such as superoxide, hydroxyl radical (OH), peroxyl radical (RO_2), nitric oxide (NO) and peroxynitrite ($ONOO^-$) [2]. Lipids, proteins and DNA are the main targets of free radicals. Excess free radicals result in oxidative stress that causes many degenerative diseases like atherosclerosis, aging, coronary heart disease and cancer [3].

Antioxidant compounds can scavenge free radicals and activate anti-oxidative enzyme such as catalase, superoxide dismutase, glutathione and so forth [4]. Antioxidants are also used as food additives as these antioxidants protect food from oxidative degradation [5,6]. Synthetic antioxidants includes butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have suspected for cause of liver damage and carcinogenesis [7]. Therefore, natural antioxidants should be preferred over the synthetic one because natural antioxidants are side effect free due to natural origin [8,9]. A number of phytochemical have also been reported for their various medicinal properties such as antioxidant, antimicrobial, anticancer etc. [8,10,11].

Spondias mangifera Willd. (family - Anacardiaceae) is a glabrous tree with characteristic pleasurable smell of wood distributed on throughout the tropical and subtropical regions [12]. This plant is also known as wild mango tree, Amara and in ayurveda it is known by Amrarta. The fruit of this plant is edible and used for making the pickles. Different parts of this plant are known for their various pharmacological properties. The bark is used as tonic, and in treatment of articular and muscular rheumatism. Bark is also used for the treatment of diarrhea and dysentery [13]. Root of this plant also possesses antipyretic, anti-spasmodic and antihistamine and hypoglycemic activity

[14,15]. The fruit is astringent and anti-scorbutic and also useful in bilious dyspepsia. The plant is reported to contain antitubercular properties [16]. Daucosterol, β-sitosterol, stigmast-4-en-3-one, cycloartanone 24-methylene and lignoceric acid have been reported in the aerial parts. Other constituents like β-amyrin and oleanolic acid were also reported from the fruits.

Objective of the Study

The objective of the present investigation was to evaluate and compare polyphenolic compounds free radical scavenging activity and reducing potential from leaf derived callus and *in vitro* leaf extracts of *Spondias mangifera*. This is first report of phytochemicals study using callus and *in vitro* leaf extract of this plant.

Materials and Methods

Chemicals

Ascorbic acid, 1,1-diphenyl,2-picryl hydrazyl (DPPH), gallic acid, thiobarbituric acid (TBA), rutin trihydrate, sodium dodecyl sulphate (SDS), trichloro acetic acid (TCA), potassium ferricyanide ($K_3[Fe(CN)_6]$), ferrous sulphate ($FeSO_4$) and ferric chloride ($FeCl_3$), sodium carbonate (Na_2CO_3), sodium nitrite ($NaNO_2$), aluminum chloride ($AlCl_3$), sodium hydroxide (NaOH), Folin-Ciocalteu phenol reagent. All chemicals were purchased from Hi-Media Ltd.

Callus induction and extracts preparation

In vitro green leaves (1g) isolated from micropropagated plants [17]. Callus was induced using *in vitro* leaf explants on MS media supplemented with 5 mg l^{-1} of 2, 4-D. Four-week-old callus (1g) and *in vitro* leaf (1g) were ground using chilled mortar and pestle in 5 ml different solvents (aqueous, ethanol or methanol) and filtered using muslin cloth. Final volume was maintained 10 ml using respective solvents, stored till further use.

Antioxidant assay through DPPH

The free radical scavenging activity of different extracts was determined according to the method of with slight modification [18]. Plant extract with various concentrations (200 and 400 µg/mL) of volume 1mL were mixed with 3 mL of methanolic solution of DPPH (0.004%) followed by vigorous shaking and incubated for 15 minutes at room temperature. Absorbance of different samples was recorded at 517 nm by using spectrophotometer (Thermo Scientific, UV1). The results were expressed as the percent inhibition of free radicals by different samples and calculated by the formula:

DPPH free radical scavenging activity (%) = $\frac{(A-B)}{A} \times 100$

(A= absorbance of control, B= absorbance of sample)

Antioxidant activity through β-carotene and linoleic acid assay

This free radical scavenging assay was carried out by the method (Miller (1971) with some modification [19]. This modification was done as discussed by Singh., *et al* [20].

Measurement of total phenolic content

Total phenolic content (TPC) was measured by Folin-ciocalteu assay [21]. In short, 5 ml of folin reagent (1:10 diluted with DDW) was mixed with 0.5 mL (200 µg/mL) of extract and then 4 mL of aqueous sodium carbonate was added in this reaction mixture. Incubation of this reaction mixture was done at room temperature for 15 minutes and absorbance was recorded at 765 nm. The standard used was gallic acid prepared in methanol and DDW (1:1). Total phenolic content was expressed in terms of gallic acid equivalent (GAE, mg/g of dry mass).

Estimation of total flavonoid content

Total flavonoid content (TFC) was measured by using method of Zhu., *et al.* with few modifications [22]. In Plant extract (2 mL, 200 µg/mL) 0.6mL sodium nitrite (5%, w/v), 0.5 mL aluminium chloride (10%, w/v), 3 mL sodium hydroxide (4.3%, w/v) was added respectively

and final volume of approximately 10 mL was maintained by DDW. After every addition of solution, reaction mixture was shaken vigorously for 6 min in order to complete the reaction and incubated at room temperature for 15 minutes. The absorbance was recorded at 500 nm. Reaction mixture without plant extract was treated as control. Rutin was used as standard. TFC were expressed as rutin equivalent (RE, mg/g).

Estimation of reducing power capacity

Reducing potential of the extract was evaluated by the method of Nabavi., *et al.* with minor modification as carried out by Singh and Kumari [8,23].

Results

Free radical scavenging activity

Plant extracts of *Spondias mangifera* showed high antioxidant potential. Among all extracts, maximum antioxidant potential was observed in methanolic extracts of both callus (51.11%) and *in vitro* leaves (57.62%) (Table 1). Significant free radical scavenging activity was observed in all extracts of callus and *in vitro* leaf through Beta-carotene bleaching assay (Figure 1). Through this assay maximum free radical scavenging activity was observed in the methanolic extract of *in vitro* leaf.

Conc. of sample (µl ml^{-1})	Callus extract			In vitro leaf extract		
	Percentage inhibition (%)					
	Ethanolic	Methanolic	Aqueous	Ethanolic	Methanolic	Aqueous
200	20.64	23.58	19.41	27.03	29.24	22.11
400	47.91	51.11	45.45	52.71	57.62	50.62
IC_{50}	-----	391.93	------	378.89	346.30	395.57

Table 1: *Antioxidant activity of in vitro leaf and callus extracts of Spondias mangifera.*

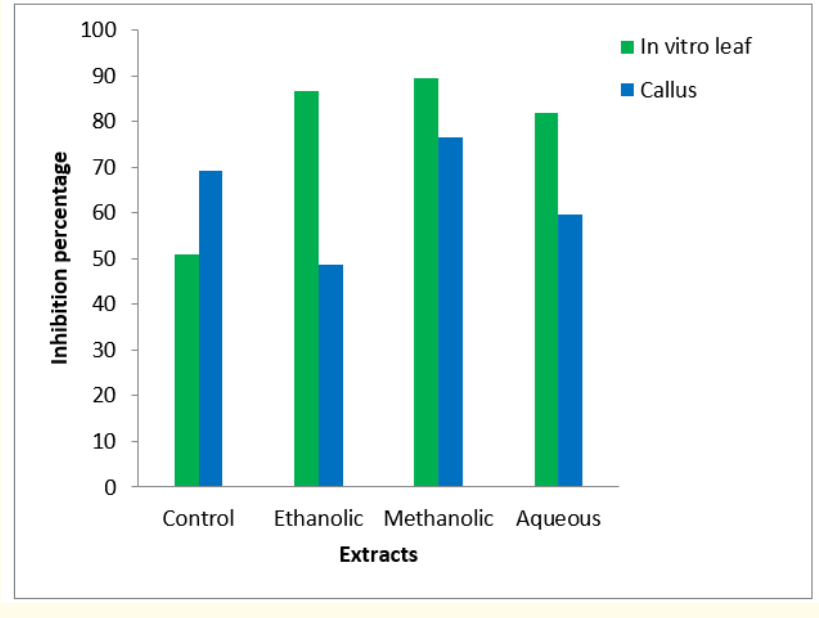


Figure 1: *Beta-carotene bleaching assay of callus and in vitro leaf extracts.*

Total phenolic content and total flavanoid content

Callus and *in vitro* leaf extracts have shown high amount of TPC and TFC (Table 2). Methanolic extract of callus contained highest amount of TPC (58.02 mg g^{-1} of GAE and TFC (250.22 mg g^{-1} of RE). Among the of *in vitro* leaf methanolic extract contained highest amount of TPC (67.68 mg g^{-1} of GAE and TFC (272.31 mg g^{-1} of RE).

	Callus extract			In vitro leaf extract		
	Ethanolic	**Methanolic**	**Aqueous**	**Ethanolic**	**Methanolic**	**Aqueous**
TPC	45.34	58.02	7.16	51.02	67.68	12.15
TFC	212.20	250.22	175.16	229.14	272.31	195.25

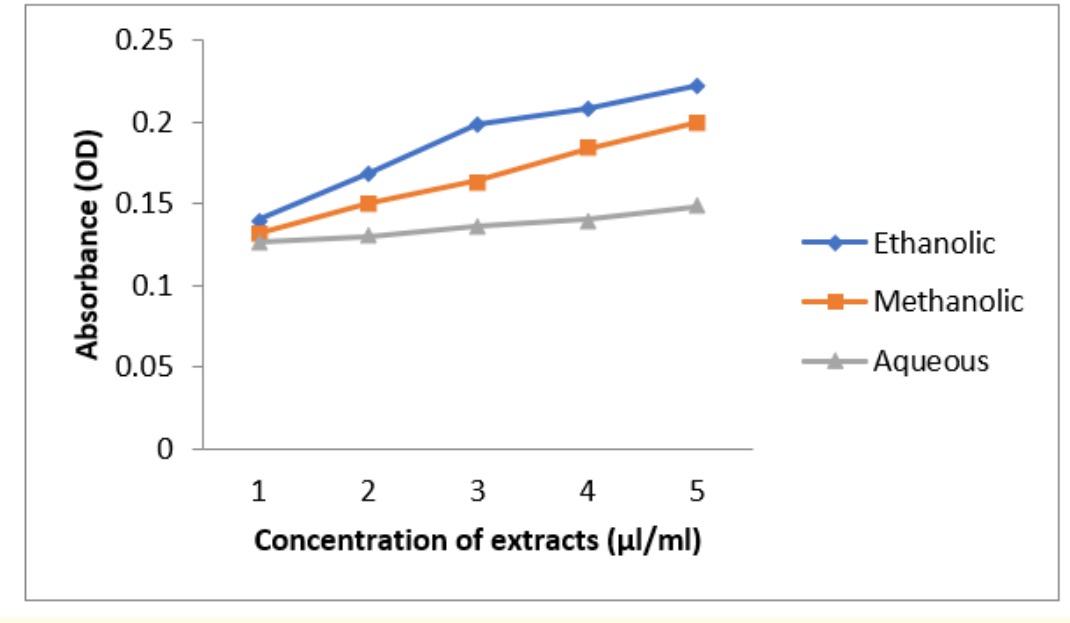
Table 2: *Total Phenolic content (TPC) and Total Flavonoid content (TFC) of in vitro leaf and callus extracts of Spondias mangifera.*

Reducing capacity

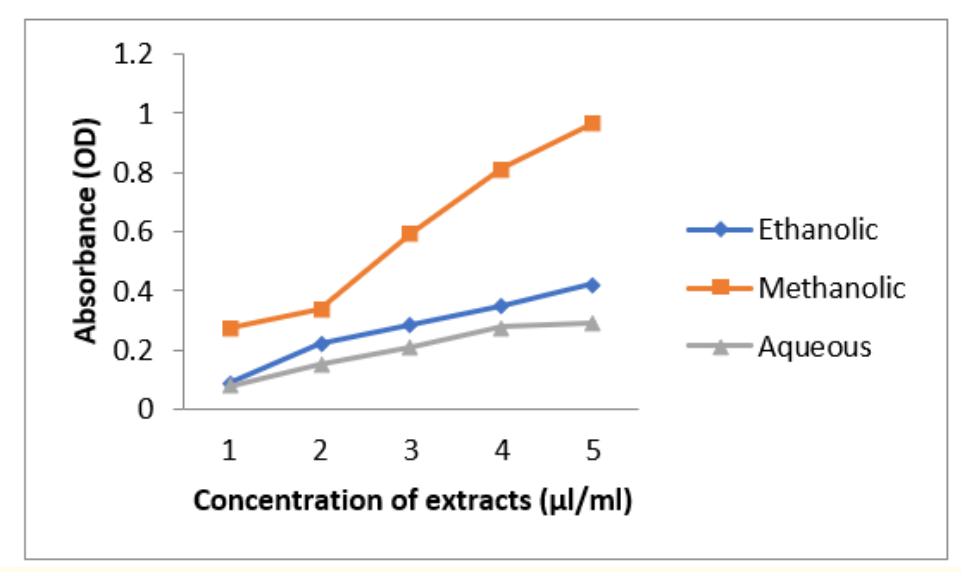
All the extracts of callus and *in vitro* leaf have shown the reducing potential (Figure 2). Reducing potential increased in the concentration dependent manner of extracts. In callus, maximum reducing potential was observed in ethanolic extract (Figure 2A). Among the all extract of *in vitro* leaf, methanolic extract have shown the highest reducing potential (Figure 2B).

Discussion

Spondias mangifera is an economical and medicinal plant. The *in vitro* leaf and callus extracts have shown the significant antioxidant activity. Several researchers also accessed the antioxidant activity using *in vitro* plant materials. DPPH assay and β-carotene/linoleic acid bleaching assay is easy and rapid method for the estimation of antioxidant activity from the plant sample. These methods are frequently used in several other plants [20,24]. Total TPC and TFC is a most significant natural compounds and pharmaceutically important. TPC and



(A) Callus
(Note: 1=100, 2==200, 3=300, 4=400, 5= 500)



(B) In vitro leaf
(Note: 1=100, 2==200, 3=300, 4=400, 5= 500)

Figure 2: *Reducing potential of extracts of callus and in vitro leaf (A) Callus (B) In vitro leaf.*

TFC have also been compared in *Passiflora alata* from *in vivo* and *in vitro* materials [25]. Similar pattern of reducing potential from *in vitro* leaf and callus extracts have also been reported in the other plants [26].

Conclusion

It is concluded form the obtained result that callus and *in vitro* leaf is rich in total phenolic content and total flavonoid content. Presence of high antioxidant activity and reducing potential also showed the importance of *Spondias mangifera* Willd. as medicinal plant. *In vitro* leaf and callus of *S. mangifera* may be used as raw material for the production of pharmaceutically important phytochemicals and for the development of new pharmacophores.

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