Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

QUALILATIVE SCREENING AND EVALUATION OF ANTIOXIDANT ACTIVITY OF *ARTOCARPUS HETEROPHYLLUS* BARK

Neelu Yadav*[1], Jyoti Saxena[2], Mamta Maheshwari[1]

[1]*Department of Chemistry, Sarojini Naidu Govt. Girls (PG Autonomous) College, Shivaji Nagar Bhopal, Madhya Pradesh, India*

[2]*Institute for Excellence in Higher Education (IEHE) Bhopal, Madhya Pradesh, India*

**Corresponding author: neeelu113yadav@gmail.com*

ABSTRACT

The aim of the present investigation was to evaluate the phytochemical constituents, free radical scavenging activities and antioxidant properties of the chloroform, ethyl acetate and methanol extract of bark of *Artocarpus heterophyllus*. The phytochemicals in the chloroform, ethyl acetate and methanol extract of bark of *A. heterophyllus* were determined qualitatively and quantitatively using standard methods. The antioxidant activities were carried out by DPPH free radical scavenging assay and reducing power assay. The methanol extract of *A. heterophyllus* showed good radical scavenging activities and reducing power activities which were found to increase with the increasing concentration of the extract. The study indicated that the presence of the major phytochemicals *viz*. flavonoids and phenols in the methanol extract of *A. heterophyllus* were 86.75 mg/g and 524.86 mg/g, respectively. The present study revealed that the methanol extract of leaves of *A. heterophyllus* showed significant antioxidant activities as well as phenolic and flavonoidal content.

Keywords: *Artocarpus heterophyllus*, Phytochemicals, Phenol, Flavonoids, Antioxidant, DPPH, $OH^{\bullet}$, $NO^{\bullet}$, reducing power.

1. INTRODUCTION

Use of plants as medicinal substances is as old as human civilization and mankind continues to rely on them for healthcare [1]. At present, around 80% population residing in the developing or underdeveloped countries still use plant-based medicines to combat their ailments [2]. Naturally-derived compounds have significantly contributed in the discovery of new chemical entities. The process of drug discovery from nature involves multi-disciplinary approach and is interconnected with many disciplines like ethnobotany, phytochemistry, biology and various chemical separation processes along with combinatorial synthetic techniques. It is currently estimated that around 87% of drugs are derived directly or indirectly from nature. Approximately, 420 000 plant species occur in nature [3]. Oxidative stress is considered as the principal cause of human ailments. Oxidation of lipids, proteins, and DNA is related to several life-threatening diseases like cancer [4], atherosclerosis [5], heart disease [6], diabetes [7], preeclampsia [8], and neurodegenerative diseases like Huntington's disease, amyotrophic lateral sclerosis, Alzheimer's disease, celiac disease [9-12] and Parkinson's disease [13]. Several free radicals are produced throughout metabolic process; however, the body balances oxidation and antioxidation using its multiple defense mechanisms [14-16]. Aging process is directly linked to systemic oxidative stress. Declined nutritional antioxidants availability and accumulation of oxidation products have been recognized as main contributors in human aging [17]. According to the Denham Hartman's free radical theory of aging, it is believed that consequences of building-up of biomolecules, spoiled through free radicals leads to aging [18, 19]. Antioxidants are substances that are accountable for the prevention of reactive oxygen species formation or scavenge them [20]. Most of the dietary antioxidants are derived from plants. Moreover, antioxidants, obtained from medicinal plants, have attracted the researchers' attention due to the risks, associated with several available synthetic antioxidants including butylated hydroxyanisole and/or butylated hydroxytoluene [21]. *Artocarpus heterophyllus* Lam. belongs to the family Moraceae. *Artocarpus heterophyllus* is a monoecious evergreen tree grown in several tropical and subtropical regions. Different parts of this

tree have been used for ailments; leaves for stimulating lactation in women and animals, seeds for relieving sick, constipation and diarrhoea and the roots for alleviating asthma and fever [1]. Wood chips yield a dye, which gives orange-red colour to the robes of Buddhist priests. The leaves are also useful to treat fever, ulcers, boil wounds, skin diseases and are antidiarrhoeal, analgesic and immune modulator. The ripe fruits are sweet, cooling, laxative, aphrodisiac and tonic. The plant is known to produce prenyl flavonoids, stilbenes, triterpenes, and sterols. Some of these compounds have exhibited interesting biological activities such as cytotoxicity, antioxidant activity, anti-inflammatory activity and antimalarial activity, inhibition of tyrosinase and melanin biosynthesis and inhibition of 5α-reductase [2]. Hence, in the present investigation, we evaluated the phytochemical constituents, free radical scavenging activities and antioxidant properties of the methanol extract of leaves of *Artocarpus heterophyllus*.

2. MATERIAL AND METHODS

2.1. Extraction of plant materials

In present study, plant material was extracted by continuous hot percolation method using Soxhlet apparatus. Powdered material of plant was placed in thimble of soxhlet apparatus. Soxhlation was performed at 60°C using petroleum ether as non-polar solvent. Exhausted plant material (marc) was dried and afterward re-extracted with chloroform, ethyl acetate and methanol solvent. For each solvent, soxhlation was continued till no visual colour change was observed in siphon tube and completion of extraction was confirmed by absence of any residual solvent, when evaporated. Obtained extracts was evaporated using rotary vacuum evaporator (Buchitype) at 40°C. Dried extract was weighed and percentage yield for each extract was determined using formula:

% Yield= (Weight of extract/Weight of Plant material used) x 100

Prepared extracts were observed for organoleptic characters (percentage yield, colour and odour) and were packed in air tight container and labelled till further use [5].

2.2. Qualitative Phytochemical Estimation of Extracts

Detailed phytochemical testing was performed to identify presence or absence of different phyto-constituents in *Artocarpus heterophyllus* extracts by using standard procedures [6]. The extracts prepared in Petroleum ether, Ethyl acetate and Methanol were subjected to following *In vitro* antioxidant assay

2.3. DPPH scavenging assay

The DPPH free radical scavenging ability of the extracts was assessed. 0.1mM DPPH solution (4mg/100ml) was prepared in methanol. Extract Samples were prepared to get concentration of 1mg/ml in methanol, various concentrations of sample solution were further diluted with methanol to 2ml then added 1ml of DPPH solution incubated at room temperature for 10 min and absorbance was measured at 517 nm against blank [6]. The free radical scavenging activity was expressed as the percentage inhibition which was calculated by using the following formula:

% Inhibition = {(Absorbance $_{Blank}$ - Sample Absorbance) /Absorbance $_{Blank}$ }X 100

The inhibition concentration (IC_{50}) value was determined from extrapolating the graph of % Inhibition versus the concentration of extract (using linear regression analysis), which is defined as the amount of antioxidant necessary to reduce the initial radical concentration by 50 %. Lower the IC_{50} value, higher the antioxidant effects.

2.4. Reducing Power assay

This method is based on the principle of increase in the absorbance of the reaction mixture. Increase in the absorbance indicates increase in the antioxidant activity. Substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanides (Fe^{2+}), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm. Increase in absorbance of the reaction mixture indicates the reducing power of the samples.

1 mL of various concentrations of extract was mixed with 2.5 ml phosphate buffer solution (pH a6.6) and 2.5 ml of 1% potassium ferricyanide. The solution was properly mixed and placed in incubator for 20 min at 50ºC. After incubation, the resulting solution was cooled and 2.5 ml of 10% tri chloro acetic acid was added to reaction mixture, followed by centrifugation at 3000 rpm for 10 min. After centrifugation, 2.5 ml of supernatant was mixed with equal volume of distilled water and finally 0.5 ml of 0.1% ferric chloride was added. The reaction mixture was shaken and kept at room temperature for 10 min. The absorbance was measured at 700 nm.

3. RESULTS AND DISCUSSION

3.1. Percentage Yield

In phytochemical extraction, the percentage yield is very crucial in order to determine the standard efficiency of extraction for a specific plant, various sections of the same plant or different solvents used. The yield of extracts received from the *Artocarpus heterophyllus* are mentioned in table 1.

3.2. Preliminary observations

The crude extracts were prepared in three solvents. All the extracts were semi solid in nature. Qualitative phytochemical testing of extracts was done to study the presence or absence of various phytochemical constituents using standard tests. Phytochemical screening revealed the presence of bioactive components such as flavonoids, glycosides, phenolics in the extract.

Table 1: Percentage Yield of crude extracts of *Artocarpus heterophyllus* extract

Extract	*Artocarpus heterophyllus*
Pet ether	0.112
Chloroform	0.134
Ethyl acetate	1.786
Methanol	4.588

Table 2: Phytochemical analysis of *Artocarpus heterophyllus*

S. No.	Experiment	Observations			
		Pet Ether Extract	Chloroform Extract	Ethyl acetate Extract	Methanolic Extract
Test for Carbohydrates					
1.	Molisch's Test	-ve	-ve	+ve	+ve
2.	Fehling's Test	-ve	-ve	+ve	+ve
3.	Benedict's Test	-ve	-ve	+ve	+ve
4.	Barfoed's Test	-ve	-ve	+ve	+ve
Test for Alkaloids					
1.	Dragendorff's Test	-ve	-ve	+ve	+ve
2.	Wagner's Test	-ve	-ve	+ve	+ve
3.	Mayer's Test	-ve	-ve	-ve	-ve
4.	Hager's Test	-ve	-ve	+ve	+ve
Test for Triterpenoids and Steroids					
1.	Libermann-Burchard Test	-ve	+ve	+ve	+ve
2.	Salkowski Test:	+ve	+ve	+ve	+ve
Test for Saponins					
1.	Froth Test	-ve	-ve	+ve	+ve
Test for Tannin and Phenolic Compounds					
1.	Ferric Chloride Test	-ve	-ve	+ve	+ve
2.	Gelatin Test	-ve	-ve	+ve	+ve
3.	Lead Acetate Test	-ve	-ve	+ve	+ve
Test for Flavonoids					
1.	Shinoda's Test	-ve	+ve	+ve	+ve
2.	Alkaline reagent test	-ve	+ve	+ve	+ve
Test for Glycosides					
1.	Borntragers Test	-ve	+ve	+ve	+ve
2.	Keller Killiani Test	-ve	+ve	+ve	+ve
	Test for Protein				
1.	Biuret Test	-ve	-ve	-ve	-ve
2.	Ninhydrin Test	-ve	-ve	-ve	-ve
3.	Million Test	-ve	-ve	-ve	-ve
	Test for Oil	+ve	-ve	-ve	-ve

3.3. Total phenolic contents

The total phenolic contents of *Artocarpus heterophyllus* extracts were calculated with a regression equation based on a standard curve using Gallic acid (20-100μg/ml) as standard. The methanolic extract of *Artocarpus heterophyllus* had the phenolic content, 174.73 mg GAE/g extract and ethyl acetate extract of *Artocarpus heterophyllus* contain 191.60 mg GAE/g extract.

3.4. Total Flavonoids content (TFC)

The total flavonoid content of bark of *Artocarpus heterophyllus* extracts was measured with the aluminium chloride colorimetric assay using quercetin as standard. The absorbance was measured 510 nm using water as blank by UV Spectrophotometer. Rutin (20-100 μg/ml) was used as standard. The total flavonoid contents of bark of *Artocarpus heterophyllus* extracts were calculated with a standard curve. Flavonoids contribute to majority of plant secondary metabolites.

Table 3: Total Phenolic Content of extract *Artocarpus heterophyllus* bark

Extracts	Total Phenolic content (mg/gm equivalent of Gallic acid)
Ethyl acetate	191.60±1.058
Methanol	174.73±1.006

Table 4: Total Flavonoid Content of extract *Artocarpus heterophyllus*

Extracts	Total Flavonoid content (mg/gm equivalent of rutin)
Ethyl acetate	115.33
Methanol	92.33

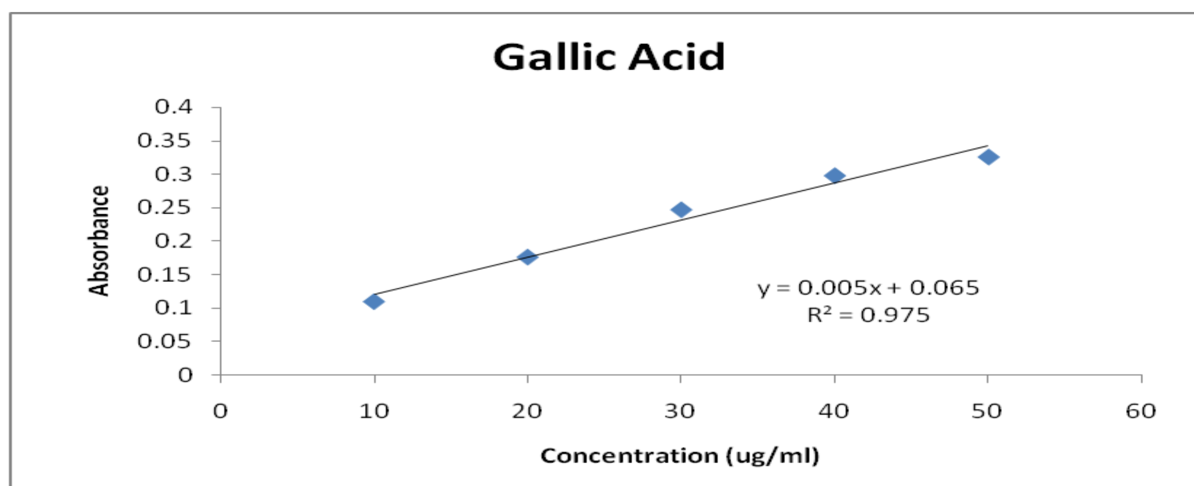

Fig. 1: Standard Calibration curve of Gallic acid for Total Phenolic Content determination

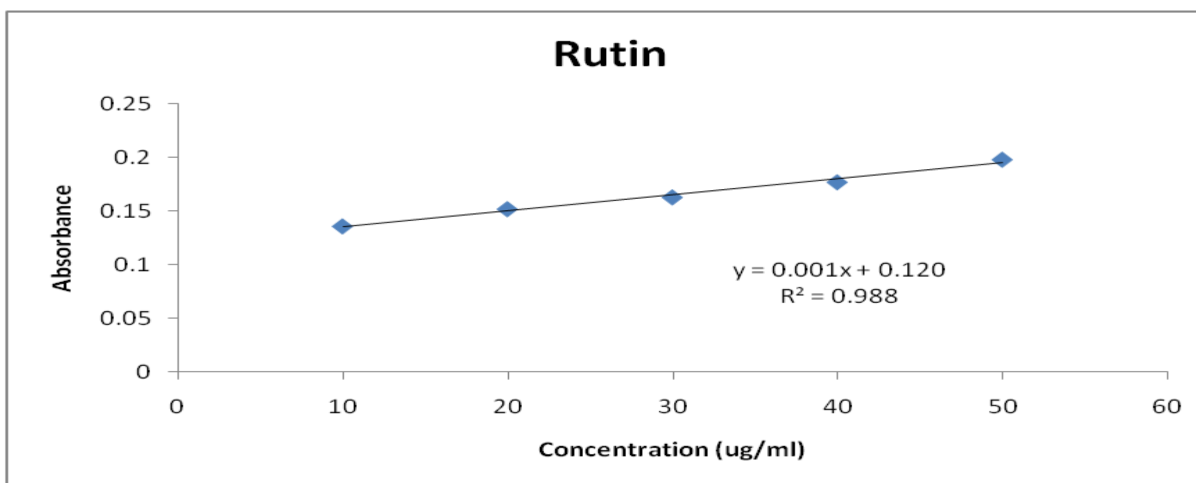

Fig. 2: Standard Calibration curve of Rutin for Total Flavonoid Content determination

3.5. DPPH 1, 1- diphenyl-2-picryl hydrazyl Assay

DPPH is a stable free radical, purple in color. It decolorizes on accepting an electron in presence of antioxidant molecule. This is measured by spectro-photometer from the changes in absorbance at 517 nm. The reduction ability of DPPH radical was determined by the decrease in absorbance induced by plant antioxidants [6]. The scavenging activity of extracts and standard on the DPPH radical expressed as IC_{50}values. AA was used as standard.

3.6. Reducing Power Assay

The reducing power of extracts is shown graphically by depicting absorbance as a function of concentration. The reducing power of all the extracts increased with increase in concentration. Reducing power of methanol extract is highest which is comparable to standard compound ascorbic acid.

Table 5: DPPH radical scavenging activity of Std. Ascorbic acid

Concentration	% Inhibition
20 μg/ml	50.59
40 μg/ml	57.05
60 μg/ml	63.16
80 μg/ml	70.80
100 μg/ml	75.55
IC_{50}	17.86

Table 6: DPPH radical scavenging activity of plant extract

Concentration	% Inhibition (Chloroform extract)	% Inhibition (EA Extract)	% Inhibition (Methanolic Extract)
20 μg/ml	28.69	39.90	36.84
40 μg/ml	31.58	42.95	44.82
60 μg/ml	35.31	51.95	49.92
80 μg/ml	41.94	56.03	54.84
100 μg/ml	46.18	61.97	59.93
IC_{50}	118.84	58.07	62.59

Table 7: Reducing power assay of plant extracts

Concentration	Absorbance of Ascorbic acid	Absorbance of Chloroform extract	Absorbance of Ethyl acetate extract	Absorbance of Methanolic extract
20 μg/ml	0.087	0.041	0.071	0.052
40 μg/ml	0.114	0.049	0.082	0.06
60 μg/ml	0.129	0.072	0.11	0.086
80 μg/ml	0.146	0.094	0.131	0.107
100 μg/ml	0.162	0.119	0.149	0.132

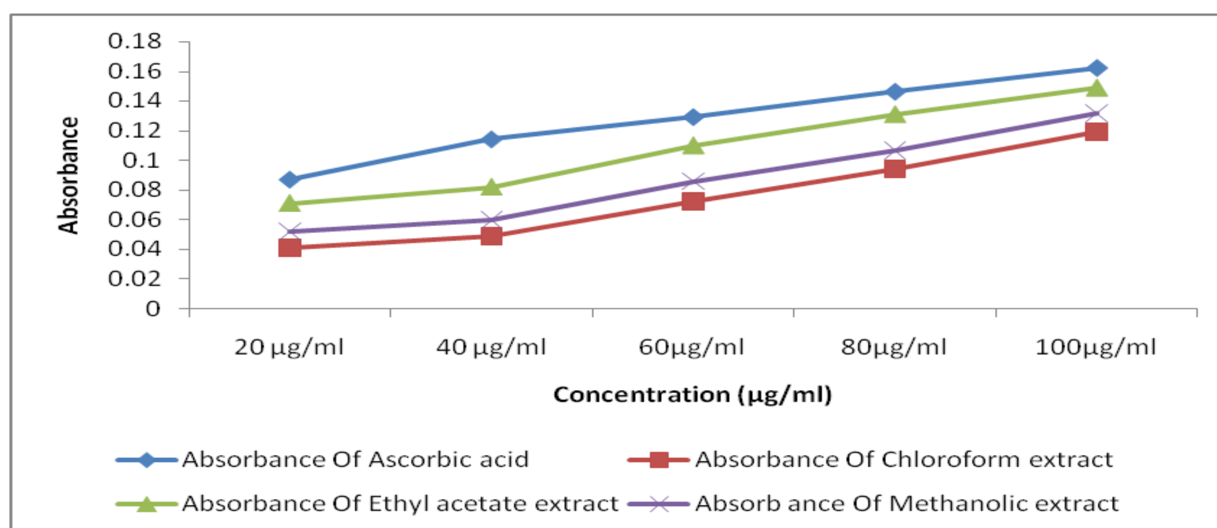

Fig. 3: Reducing capacity of plant extracts of *Artocarpus heterophyllus*

4. CONCLUSION

The results of the present work indicated that the chloroform ethyl acetate and methanol extract of bark of *A. heterophyllus* is a potential source of natural antioxidants and could dose-dependently and significantly inhibit free radicals. The difference in the antioxidant activity may be ascribed to their different group of phenolic and flavonoids compounds. The methanol extract of *A. heterophyllus which* showed higher phenolic content contributes to the higher antioxidant activity. Based on the results obtained, it can be concluded that the plant contains essential phyto-chemical constituents and possesses active antioxidant property. Further investigations on the isolation of the active component of the extract will throw more information on the mechanism of action.

Conflict of interests

None declared

5. REFERENCES

1. Khan M, Omoloso A, Kihara M. *Fitoterapia,* 2003; **74:**501-505.
2. Shimizu K, Fukuda M, Kondo R, Sakai K. *Planta Med.,* 2000; **66:**16-19.
3. Little EL, Wadsworth FH. Common Trees of Puerto Rico and the Virgin Islands. Agriculture Handbook No. 249. Washington, D. C:U. S. Department of Agriculture, Forest Service;1964.
4. Raaman N. Phytochemical techniques. New Delhi: New India Publishing Agency; 2006. p.306.
5. Trease GE, Evans WC. Textbook of Pharma-cognosy, 12th Edn. London: Balliese Tindall and Company Publisher; 1983. p. 343-83.
6. Harbome JB. Phytochemical Methods. Chapman and Hall Ltd: London; 1973. p. 49-188.
7. Singleton VL, Orthofer R, Lamuela-Raventos RM. *Methods Enzymol.,* 1999; **299:**152-178.
8. Chang C, Yang M, Wen H, Chern J. *J Food Drug Analysis* 2002; **10:**178-182.
9. Blois MS. *Nature,* 1958; **29:**1199-2000.
10. Ulyana A, Daniel E, Michel H, Edward J, Kennelly S. *Phytotherapy Res.,* 2002; **16:**63-65.
11. Oyaizu M. *Jpn J Nutr.,* 1986; **07:**307-315.
12. Prieto P, Pineda M, Anguilar M. *Anal Biochem.*, 1999; **269:**337-341.
13. Scott BC, Butler J, Halliwell B, Aruoma OI. *Free Rad Res Commun.,* 1993; **19:**241-253.
14. Kunchandy E, Rao MNA. *Int J Pharmacol.,* 1990; **58:**237-240.
15. Hochestein P, Atallah AS. *Mutat Res.,* 1988; **202:**363-375.
16. Kappus H. Lipid peroxidations Mechanism and biological relevance. In:Aruoma O I, Halliwell B, Eds. Free Radicals and Food Additives. Taylor and Francis:London, UK 1991. p.59-75.
17. Moncada A, Palmer RMJ, Higgs EA. *Pharmacol Rev.,* 1991; **43:**109-142.
18. Gordon MF. The mechanism of antioxidant action *in vitro*. In:B. J. F. Hudson (Ed.). Food antioxidants. London:Elsevier Science;1990.p.1-18.
19. Xing R, Liu S, Guo Z, Yu H, Wang P, Li C, Li Z, Li P. *Bioorg Med Chem.,* 2005; **13:**1573-1577.
20. Dorman HJD, Peltoketo A, Hiltunen R, Tikkanen MJ. *Food Chem.,* 2003; **83:**255-262.
21. Aderogba MA, Okoh EK, Idowu TO. *Hochst Biol Sci.,* 2005; **5:**239-242.