International Journal of Pharmaceutical Chemistry
ISSN: 2249-734X (Online)
Journal DOI: 10.7439/ijpc
CODEN: IJPCH3 (American Chemical Society)
Research Article

Phytochemical analysis and antioxidant activity of *Pterospermum acerifolium* (Sterculiaceae)

Mehreen Jabeen*, Fatima Arshad and Bashir Ahmad Chaudhary

Faculty of Pharmacy, Bahauddin Zakariya University, Multan, Pakistan, South Punjab

Abstract

The objective of present research work was to determine the antioxidant activity and presence of secondary metabolites in bark of *Pterospermum acerifolium* belonging to family Sterculiaceae. The phytochemical analysis of bark of *Pterospermum acerifolium* showed the presence of anthraquinones, cardiac glycosides, flavonoids, saponins and tannins while alkaloids were absent. Oxidative compounds are responsible for prognosis of many diseases like Alzheimer's disease, ischemic heart disease, Parkinson disease and atherosclerosis. Antioxidants are the compounds responsible for curing such ailments that are produced by oxidative stress to the cells. Antioxidant activity of bark of *Pterospermum acerifolium* was determined by 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH). Extracts of plant material were prepared in dichloromethane and methanol solvents. Gallic acid was used as standard drug. Methanol extract exhibited excellent radical scavenging activity with IC^{50} 14.506± 0.07.

Keywords: *Pterospermum acerifolium*, phytochemical, antioxidant activity, free radicals.

1. Introduction

Oxygen is essential for life. Human body consumes oxygen for energy production by metabolism of biomolecules. The metabolic pathways also produce reactive oxygen species (ROS) like superoxide (O_3^-), hydrogen peroxide (H_20_2) and hydroxyl radical (OH^o). These free radicals and ROS produced in adequate amount in the body. An excessive production of these ROS leads to oxidative stress and causes cellular and sub cellular damage by peroxidation of lipid membranes, denaturing of proteins, opening of DNA strands and disrupting of cellular functions [1]. Endogenous antioxidants such as glutathione peroxidase, α- tochopherol, superoxide dismutase and melatonin are synthesized within human body to protect the cells from oxidative stress induced by ROS and free radicals [2, 3]. In pathological conditions endogenous antioxidants are insufficient to fight with ROS. This lead to the way that disease progression and oxidative damage to cells may be retarded by supplementing endogenous antioxidant by exogenous antioxidants obtained from plants. In plants, antioxidants are phenolic compounds that vary from very simple phenolic acids to highly polymerized compounds like tannins. These compounds help in survival of plants and also promote health in humans [4]. Endogenous and exogenous antioxidants function as free radical scavengers and improve human health. Antioxidant substances inhibit the production of free radicals involved in pathogenesis of many harmful diseases like Parkinson's disease, Alzheimer's disease, atherosclerosis, ischemic heart disease and aging process [5]. Aging and age related diseases may occur due to long term oxidative damage to the cells and tissues of the body that arises primarily as a result of aerobic metabolism [6]. Many medicinal plants contains large number of antioxidants that play a vital role in absorbing and neutralizing free radicals, quenching super oxides or decomposing peroxides. Plants extracts, in addition to have minerals and primary metabolites also contains diverse groups of secondary metabolites with antioxidant potential. *Pterospermum acerifolium* processes anticancer activity [7], antibacterial and antifungal activity [8], anti-inflammatory and anti-nociceptive activity [9, 10], antipyretic activity [11], anti-ulcer [12], immunomodulatory activity [13], antihelmenthic activity [14], and hyperlipidemic activity [15]. By considering the above mentioned information the

* Correspondence Info
Mehreen Jabeen
Faculty of Pharmacy,
Bahauddin Zakariya University, Multan,
Pakistan, South Punjab
E mail: mehreenjabeen@gmail.com

main objective of the present study was to evaluate the bark of *Pterospermum acerifolium* for antioxidant property by radical scavenging mechanism.

2. Material and method

2.1 Plant material

Plant material was collected from Bahauddin Zakariya University, Multan in the month of September 2014 and was identified as *Pterospermum acerifolium* by Prof. Dr. Zafarullah Zafar, Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan. All chemicals and reagents are of analytical grade.

2.2 Preparation of extract

Shade dried bark of *Pterospermum acerifolium* was grounded and plant extracts were prepared according to standard protocols. The plant material was soaked in a measured volume of dichloromethane in an extraction bottle. After 24 hours filtration was carried out. The process of extraction was repeated three times with dichloromethane. The marc was extracted with methanol by same procedure. The extracts were concentrated under reduced pressure using rotary evaporator at 40ºC.

2.3 Antioxidant activity

2.3.1 DPPH radical scavenging assay

DPPH solution (95µl, 300µM) in Ethanol is mixed with test solution (5µl, 500µM). The reaction is allowed to progress for 30 min at 37ºC and absorbance is monitored by multiplate reader, SpectraMax340 at 517 nm. Upon reduction, the color of the solution fades (Violet to pale yellow). Percent Radical Scavenging Activity (%RSA) is determined by comparison with a DMSO containing control. The concentration that causes a decrease in the initial DPPH concentration by 50% is defined as IC_{50} value. The IC_{50} values of compounds were calculated by using the EZ-Fit Enzyme kinetics software program (Perrella Scientific Inc. Amherst, MA, USA). The decrease in absorbance indicates increased radical scavenging activity which was determined by the following formula [16, 17].

Percent scavenging activity = [100- (absorbance of test compound/ absorbance of control) x 100

2.4 Phytochemical analysis

2.4.1 Tests for alkaloids

In a test tube 0.5- 1g of finely powdered drug was boiled with 10 ml of dilute hydrochloric acid solution for one minute and filters it. The filtrate was made alkaline to litmus paper with dilute ammonia solution and extracted with 5 ml chloroform. Two layers were formed. Separate the chloroform layer and extracted with 10 ml of dilute acetic acid. The extract was treated Dragendroff's reagent, orange red color precipitates indicates the presence of alkaloids [18].

2.4.2 Keller Kiliani test for cardiac glycosides

1 g of powdered drug was boiled with 10 ml 70% alcohol. Filtered the extract and diluted with double volume of water. 1ml strong lead acetate solution was added to filtrate for precipitation of chlorophyll. Extract the filtrate with chloroform. Separate chloroform layer and evaporated it to the dryness in china dish over water bath. Add 3 ml of 3.5% ferric chloride in glacial acetic acid to the crucible containing residue and allowed to stand for 1 minute. Transferred the solution in test tube and add 1.5 ml strong sulphuric acid solution along the wall of the test tube. Brown color of deoxy sugars at the interface confirmed the presence of cardiac glycosides [18].

2.4.3 Test for tannins

10 % w/v aqueous extract of powdered plant material was prepared by boiling it with distilled water for 10-20 minutes. Add 2 ml ferric chloride solution to 1-2 ml of filtrate, a blue black precipitates showed the presence of tannins [19].

2.4.4 Tests for anthraquinone

Borntrager's test: 0.1g of powdered drug was extracted with 10 ml hot water and filtered. Aqueous extract was partitioned with 10 ml carbon tetrachloride. The carbon tetrachloride layer was separated and washed with 5 ml of water and shaken with 5 ml of dilute ammonia solution. Pink to cherry red color in upper layer showed the presence of free anthraquinone.

Modified Borntrager's test: 0.1 g of powdered drug was boiled with mixture of 10 ml Ferric chloride solution and 5 ml hydrochloric acid solution. Filtered while hot and filtrate was extracted with 10 ml carbon tetrachloride. The carbon tetrachloride layer was separated and washed with 5 ml of water and shaken with 5 ml dilute ammonia

solution. More intense pink color in the upper layer indicates the presence of anthraquinone glycosides in the drug [18].

2.4.5 Test for saponin glycosides

0.5 g of powdered drug and water was taken in a test tube and shake well, persistent forth indicate the presence of saponin glycosides [18].

2.4.6 Tests for flavonoids

2g of dried powdered plant material was boiled with 20 ml of distilled water and filtered. The filtrate was acidified with few drops of dilute hydrochloric acid. 5 ml of filtrate was made alkaline up to pH 10 with NaOH test solution; a yellow color indicated the presence of flavonoid compounds [20].

3. Results

Phytochemical analysis of bark of *Pterospermum acerifolium* revealed the presence of many constituents like cardiac glycosides, tannins, flavonoids, terpenoids etc. that are listed below in Table 1. Dichloromethane and methanol extracts of crude plant material were made by maceration process. The percentage yields of extracts are given in Table 2. Both extracts of selected plant were evaluated for antioxidant activity using DPPH method. Gallic acid was used as reference standard drug. Dichloromethane extract was found to be inactive while methanol extract showed excellent antioxidant activity with IC^{50}. The results are shown in Table 3.

Table 1: Phytochemical analysis of bark of *Pterospermum acerifolium*

Chemical constituents	*Pterospermum acerifolium*
Alkaloids	-
Flavonoids	+
Tannins	+
Cardiac glycosides	+
Saponin glycosides	+
Terpenoids	+
anthraquinones	+

Table 2: Weights and percentage yield of crude extracts of bark of *Pterospermum acerifolium*

Pterospermum acerifolium (100g)	Weight (g)	% yield
Dichloromethane extract	3.6	3.6
Methanol extract	15	15

Table 3: Antioxidant activity of DCM and methanol extracts of bark of *Pterospermum acerifolium*

Pterospermum acerifolium	Conc. (mg/ml)	IC^{50}±SEM	% RSA	St. drug	Conc. of St. drug (mg/ml)	IC^{50}±SEM of St. drug (mg/ml)	%RSA of St. drug
PTBD	0.5	-	42.74	Gallic acid	0.094	4.3±0.43	93.13
PTBM	0.0625	14.506±0.07	88.23				

PTBD = dichloromethane extract of *Pterospermum acerifolium;* PTBM = methanol extract of *Pterospermum acerifolium;* - = inactive; St. Drug= standard drug

4. Discussion

Plants contain a wide variety of vital components that are involved in development of new therapeutic agents. Plant tissues are rich source of many phenolic compounds like phenolic acids flavonoids and tannins. These phenolic compounds are radical scavengers and possess antioxidant activity due to their redox properties, hydrogen donor and singlet oxygen quenchers [21]. Phenolic compounds are also responsible for many other biological activities such as anticancer, hepatoprotective, anti-inflammatory and anti-atherosclerotic activities. These activities are might be related to their free radical scavenging activity [22]. Flavonoids are the compounds that have ability to reduce the formation of free radicals and scavenging of free radicals. Natural antioxidants present in plants are responsible of inhibiting the oxidative stress. In the present research work we have evaluated the crude methanol extract of bark of *Pterospermum acerifolium* for free radical scavenging activity by DPPH method. Phytochemical analysis of selected plant revealed the presence of many important chemical constituents such as glycosides, flavonoids, tannins and terpenes. Thus the phenolic compounds such as flavonoids that are abundantly present in the bark of *Pterospermum acerifolium* are might be responsible for antioxidant potential of this plant.

References

[1] Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. Clarendon press oxford; 1986.

[2] Mojzisova G, Kuchta M. Minireview: Dietary flavonoids and risk of coronary heart disease, *Physiol Res* 2001; 50:529-536.

[3] Oktay M, Glucin, Kufrevioglu OI. Determination of in vitro antioxidant activity of fennel (*Foeniculum vulgare)* seed extracts. *Lebensmittel-wissenschaft und-technology* 2003; 36:268-271.

[4] Lampart-Szcapa E, Korczakb J, Nogala-Kalukaa M, Zawirska-Wojtasiak R. Antioxidant properties of lupin products. *Food chem* 2001; 88: 279-185.

[5] Aruoma OL. Methodological consideration for characterizing potential antioxidant actions of bioactive components of food plants. *Mut Res* 2006; 523:9-20.

[6] Balaban RS, Nemoto FT. Motochndria, oxidants and aging cell 2005; 120:483-495.

[7] Saboo S, Deore SL, Khadabadi SS, Deokate UA. Evaluation of antimitotic and anticancer activity of the crude extracts of *Pterospermum acerifolium* willd leaves (Sterculiaceae). *Nigerian J Nat Prod Med* 2007; 11:76-79.

[8] Khond M, Bhosale JD, Arif T, Mandal TK, Padhi MM, Dabur R. Screening of some selected medicinal plants extracts for *in vivo* antimicrobial activity. *Middle East J Sci Res* 2009; 4:271-278.

[9] Mazumder PM, Pattnayak SP, Priyadarshini B, Sasmal D. Anti-inflammatory and antinociceotive activity of the flowers of *Pterospermum acerifolium*. *Pharmacol Online* 2008; 2:420-439.

[10] Bhalke DR, Pal SC. Anti-inflammatory and ant nociceptive activity of *Pterospermum acerifolium* leaves. *Asian J Pharm Clinical Res* 2012; 5:23-26.

[11] Datta R, Bera B, Das GA, Guha RS. Anti-inflammatory, analgesic and antipyretic activity of the leaves of *Pterospermum acerifolium*. *J Pharm Sci Tech* 2011; 1:35-40.

[12] Manna AK, Jena J, Manna S, Behera AK, Bag S, Karmarkar S, *et al. In-vitro* antioxidant activity of *Pterospermum acerifolium* barks. *J Pharmacy Res* 2009; 2.

[13] Soni VK, Dixit P, Bano N, Kumar P, Jayendra, Kumar Y, *et al.* Relative immune modifier activities of *Pterospermum acerifolium*, *Murraya koenigh* and Withania coagculans in Balb/ c mouse. *Int J Pharmacy Pharm Sci* 2011; 3:360-365.

[14] Parida S, Patro VJ, Mishra UK, Mohapatra L, Sannigrahi S. Anthelmentic potential of crude extracts and its various fractions of different parts of *Pterospermum acerifolium* linn. *Int J Pharm Sci Review Res* 2010; 1:107-111.

[15] Senapa AK, Giri RK, Swain SR, Satyanarayana S. Protective effect of lupeol isolated from *Pterospermum acerifolium* in experimental hypercholesterolemic rats. *Int J Res Pharmacol Pharmacotherap* 2011; 49-54.

[16] Thadhani VM, Choudhary MI, Ali S, Omar I, Siddique H, Karunaratne V. Antioxidant activity of some lichen metabolites. *Nat Prod Res* 2011; 25:1827-1837.

[17] Lee SK, Mbwambo ZH, Chung H, Luyengi L, Gamez EJ, Mehta RG, *et al.* Evaluation of the antioxidant potential of natural products. *Comb Chem High Throughput Screen* 1998; 1:35-46.

[18] Brain KR and Turner TD. The practical evaluation of phytopharmaceuticals. Published by *wright scientechnica*, *Bristol.* 1975.

[19] Trease and Evans. Text book of Pharmacognosy. 15th edition, W.B Saunders, 1996, 1-578.

[20] Olemy EL, Muhtadi MM, Afifi AF. Experimental phytochemistry- a laboratory manual. *King Saud University*. 1994.

[21] Banerjee SK, Bonde CG. Total phenolic content and antioxidant activity of extracts of *Bridelia retusa* Spring Bark: Impact of dielectric constant and geographical location. *J Med Plants Res* 2011; 5:817-822.

[22] Chung KT, Wong TY, Huang Y. Tannins and human health: Chung KT, A review. *Crit Rev Food Sci Nutr* 1998; 38:421-464.