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In vitro antioxidant activity of *Petrospermum acerifolium* barks.

Manna A. K.*, Jena J., Manna S., Behera A K., Bag S., Karmakar S., Maity S., Panda.B.R., Hati M.R., Kar S.***

Seemanta Institute of pharmaceutical sciences, Jharpokharia, Mayurbhanj, Orissa , 757086.
***** Dept. of Pharmaceutical Technology. ,Division of pharmacology , Jadavpur University Kolkata 32***

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ABSTRACT

Antioxidant activity of ethanolic extract of *Petrospermum acerifolium* barks was studied for it free radical scavenging property in different in vitro models as 1, 1- Diphenyl – 2 picryl hydrazyl, nitric oxide, superoxide and hydroxyl radical model. The extract shows significant dose dependent free radical scavenging property in all model. The extract showed the presence of high phenolic content suggesting the plant to exhibit antioxidant activity.

Keywords: Antioxidant, phenolic content, *P. acerifolium.*

INTRODUCTION

Pterospermum acerifolium wild (Sterculiaceae) commonly known as 'Kanak champa' is a shrubs distributed in tropical Asia. It has been traditionally used for blood troubles, inflammation, ulcer, tumors, leprosy and for small pox eruptions (wealth of India).In an earlier study in our laboratory, the ethanolic extract of P. acerifolium were found to possess antiulcer activity. The work was aimed at the scientific validation of the ethnopharmacological calim about anti inflammatory and analgesic properties of the bark extract.

Materials and methods:-

Plants materials *P. acerifolium* bark were collected from East Midnapur (West Bengal, India) in July 2007 and authenticated by comparison with a voucher. Specimen in Botanical survey of India, Kolkata.

One kg. of the air dried barks were blended to a fine powder and extracted with Pet. Ether, chloroform and ethanol for 6 days (144hours). The extract was concentrated using a rotavapor. The extract was dissolved in normal saline before experimentation

Phytochemical screening:-

The extract and its fraction were tested by the lierman Burchard, Ferric chlorides, Magnesium tracings and Vanillin sulphuric acid tested to determine the presence of sterols phenolic compound, flavonoids and saponins respectively.

Chemicals :

All the chemicals used were in analytical grade 1, 1 – diphenyl – 2 picryl hydrazyl (DPPH) was obtained from sigma chemicals USA and rest of chemical were obtained from SISCO Research Laboratories Pvt. Ltd., Mumbai.

DPPH (Free radical) scavenging activity:-

DPPH free radical scavenging activity of *P. acerifolium* extract (10, 25, 50 and 100mg/ml) was estimated according to the method of Luseinskas et al. Methanolic solution of DPPH (0.3mM) produced a violet coloured which is stable at room temperature. 1ml of this solution was added to 2.5ml of sample solution containing different concentration of the test drugs. After 30min of reaction time, the absorbance was measured at 518nm against appropriate blank and control. Finally DPPH scavenging activity was considered to be proportional to the decrease in optical density (cotelle et al 1996) quercetin (25 and 50mg/ml) and Vit-C (25 and 50mg/ml) was used as standard antioxidant.

Nitricoxide (NO) scavenging activity:-

Nitric Oxide generated from sodium nitropruside (in aqueous solution at physiological PH on interaction with oxygen produced nitrite ions which was measured by Griss reaction. The reaction mixture (3ml) containing sodium nitropruside (10mm) in phosphate buffer salaine and the P. acerifolium extract (10, 25, 50 and 100mg/ml) was incubated at 25^{0}C for 150min. after incubation 1.5ml of the reaction mixture was removed and 1.5ml of the Griss reagent (1% sulphanilamide, 2% orthophosphoric acid and 0.1% Napthylethyline diamine hydrochloride) was added. The absorbance of the chromophore formed was read at 546nm. A decrease in absorbance indicated higher number scavenging activity (Green et al., 1994). Querectin (25 and 50mg/ml) and Vit. – C (25 and 50mg/ml) was used as standard antioxidant.

Superoxide anion (O_2^-) scavenging activity:-

Superoxide anion scavenging activity of P. acerifolium extract (10, 25, 50 and 100mg/ml) was performed according to the method of Shorwaikar et al., 2004) with few modification. About 1ml of nitroble tetrazolium (NBT) solution (156mm NBT in 100ml of phosphate buffer PH 7.4. 1ml of NADH solution (468mm in 100ml phosphate buffer PH 7.4) and 0.1ml of sample solution of *P. acerifolium* extract were mixed thoroughly. The reaction was initiated by adding 100mm of phenazine methosulphate (PMS) solution (60mm PMS in 100mm phosphate buffer, PH 7.4) to the mixture. The reaction mixture was incubated at 25^{0}C for 5min and the absorbance at 560nm was measured against appropriate blank. Decreased absorbance indicated increased superoxide anion scavenging activity of the reaction mixture. Quercetin (50 and 100µg/ml) was used as standard free radical scavenger.

Hydroxyl radical (OH˙) scavenging activity: -

Hydroxyl radical were generated by phenyl hydrazine in solu-

***Corresponding author.**
*Tel.: + 91-*9474188582
Telefax: +91-
E-mail: amanna73@ymail.com

Table No.1 : Effect of *P. acerifolium* extract on DPPH free radical scavenging activity (Results are expressed as mean ± S.E; n=6)

Drug	Dose mg/ml	DPPH free radical scavenging (measured at 518nm)	% of Inhibition
Control	-	0.621 ± 0.0014	-
P.acerifolium extract	10	0.070 ± 0.0033*	88.7
	25	0.058 ± 0.00033*	90.48
	50	0.054 ± 0.0033*	91.29
	100	0.052 ± 0.00033*	91.45
Quercetin	25	0.086 ± 0.00033*	86
	50	0.085 ± 0.00033*	86.29
Vitamin – C	25	0.071 ± 0.00033*	88.5
	50	0.067 ± 0.00033*	89.19

P value vs. control (by students 't' test.) * P <0.001

Table No. 2 : Effect of alcoholic extract of *P. acerifolium* bark on nitric oxide scavenging activity. (Results are expressed as mean ± S.E; n=6)

Drug	Dose µg/ml	Nitric oxide scavenging activity	% of Inhibition
Control	-	0.048 ± 0.0044	-
*P.acerifolium*bark extract	10	0.132 ± 0.0043**	63.6
	25	0.301 ± 0.0098*	84.05
	50	0.532 ± 0.0017*	90.97
	100	0.864 ± 0.03*	94.4
Quercetin	25	0.707 ± 0.0043*	93.2
	50	0.935 ± 0.016*	94.8
Vitamin – C	25	1.264 ± 0.042*	96.2
	50	1.656 ± 0.018*	97.1

P value vs. control (by students 't' test.) *P <0.001 **P <0.05

Table No. 3 : Effect of alcoholic extract of *P. acerifolium* bark on Superoxide scavenging activity.(Results are expressed as mean ± S.E; n=6)

Drug	Dose mg/ml	Superoxide radical scavenging activity	% of Inhibition
Control	-	0.0682 + 0.039	-
P. acerifolium bark extract	10	0.632 ± 0.00033*	7.3
	25	0.570 ± 0.0026*	16.4
	50	0.418 ± 0.010*	38.7
	100	0.309 ± 0.0069*	54.7
Quercetin	25	0.581 ± 0.017*	14.8
	50	0.573 ± 0.0078*	15.98
Vitamin – C	25	0.543 ± 0.0043*	20.38
	50	0.428 ± 0.0046*	37.2

P value vs. control (by students 't' test.) *P <0.01

Table No. 4 : Effect of Ethanolic extract of *P. acerifolium* bark on Hydroxyl radical scavenging activity. (Results are expressed as mean ± S.E; n=6)

Drug	Dose µg/ml	Hydroxyl radical scavenging activity	% of Inhibition
Control	-	0.390 ± 0.0035	-
P.acerifolium bark extract	10	0.171 ± 0.0049*	56.2
	25	0.153 ± 0.00058*	60.77
	50	0.149 ± 0.0058*	61.79
	100	0.147 ± 0.0018*	62.31
Quercetin	10	0.114 ± 0.0029*	70.77
	25	0.101 ± 0.0026*	74.10
Vitamin-c	50	0.071 ± 0.026*	81.8
	100	0.056 ± 0.00058*	85.6

P value vs. control (by students 't' test.) *P <0.01

Table: 5 In vitro anti oxidant activity *P. acerifolium* bark extract [IC_{50} ,(µg/ml)]

Test material	[IC_{50} ,(µg/ml)] DPPH radical scavenging activity	Nitric oxide scavenging activity	Superoxide scavenging activity	Hydroxyl radical scavenging activity
P.acerifolium bark extract	97.8	90.01	31.64	65.13
Vitamin-c	118.35	127.9	38.3	111.58
Quercetin	114.77	125.25	20.4	96.56

IC_{50} value was calculated by using formulae

$$IC_{50} = a+b(50)$$

$$a = y_{1\_} b x_1 \quad ; \quad b = H xy / Hx^2$$

where , b = regression co-efficient of x on y; a = intercept of the line ; x = concentration in µg/ml ;y = % of scavenging ; x_1 = mean of the concentration ; $y_{1=}$ mean of the % scavenging.

tion which was measured by appearance of pink colour (TBA) – MDA chromgen (due to OH^- mediated decomposition of 2-Deoxyribose) (Haliwell and Gutteridge, 1981). The reaction was performed in incubation mixture containing 50mm phosphate buffer (PH 7.4), 1mm deoxyribose, 0.2mm phnylhydrazine hydrochloride and P. acerifolium extract (10, 25, 50 and 100µg/ml) or (10 and 20) mm manitol. The final reaction value was made upto 2ml. incubation was terminated after 1hr or 4 hrs. with 2.8% TCA (1ml). Thiobarbituric acid (1%w/v) was than added to the reaction mixture followed by fitting for 10min on a boiling water bath. The tube were than cooled briefly and absorbance was taken at 532nm. A decreased in absorbance indicated hydroxyl radical scavenging activity.

Determination of total phenolic compounds:-

Total soluble phenolic compound of *P. acerifolium* extract were determined with Polin-ciocalteu reagent (according to the method of Slinkad and singleton, 1977) using Catechin as standard polyphenol. The P. acerifolium extract and Polin-ciocalteu reagent (1ml was mixed thoroughly in a volumetric flask (50 ml). After 3min 3ml of sodium carbonate (5% w/v) was added and the mixture was allowed to stand for 2hr in dark with intermittent shaking. The absorbance was measured at 760nm. The concentration of total phenolic compound in the P. acerifolium extract was determined with the help of standard curve for catechin. Finally, the total phenolic content was expressed as µg of catechin equivalent.

The equation in used as:

absorbance = 0.001 x Pyrocatechol (µg) + 0.0033.

Results:

Alcoholic extract of *P. acerifolium* was analyse for total soluble phenolic content (expressed as µg of catechin per ml) and it was found to 23%. However, in all the different experimental model the total phenol content was maintained at 18%.

In DPPH scavenging activity model it was observed that*P. acerifolium* extract (in dose of 10, 25, 50 and 100µg/ml) significantly scavenged (88.7%, 90.48%, 91.29% and 91.45%) respectively DPPH free radical in a concentrated dependent manner. However, quercetin (25 and 50µg/ml) and vitamin – C (25 and 50µg/ml) showed even more effective free radical scavenging (86%, 86.29%, 88.5% and 89.19%) respectively. (**Table No. 1.)**

P. acerifolium extract (in the dose of 10, 25, 50 and 100 µg/ml) significant scavenged (63.6%, 84.05% and 94.4%) the nirticoxide radical. Although the standard antioxidant like quercetin (25 and 50µg/ml) and vitamin – C (25 and 50µg/ml) were found to produce a more potent number radical scavenging activity (96.2%, 97.1%).(**Table No. 2.)** In Superoxide scavenging activity model the extract in the concentration of (10, 25, 50 and 100µg/ml) singnificantly scavenged (7.3%, 16.4%, 38.7%, 54.7%) respectively has significant inhibition of activity with respect to control value similarly quercetin found to be demonstrate potent (14.8%, 15.98%) scavenging activity(**Table No. 3.)**

In hydroxyl radical scavenging activity (10, 25, 50 & 100 µg/ml) and quercetin and vitamine – c are used as standard (70%, 74%, 81.8% and 85.6%) OH^- radical scavenged significantly inhibited OH^- radical mediated degradation of 2-Deoxyribose after 1 hr. **Table No. 4**.

Discussion

The extract showed significant antioxidant activity in vitro by free radical scavenging model. Free radicals are chemical entities that can exist separately with one or more unpaired electrons. The propagation of free radical can brings about many adverse reaction leading to extensive tissue damaged. Lipid proteins are all susceptible to attack by free radical (Cotran 1999, Yu et al 1992). DPPH is a relatively stable free radical and the assay to determine the ability of P. acerifolium extract to reduce DPPH radical to the corresponding hydrazine by converting the unpaired electrons to pairs one, which in fact is the action of antioxidant. Various reactive free radicals, nitric oxide (NO) arouses interest due to reports of its multiple physiological roles (Nakagawa et al, 2002). Furthermore, literature survey also reveals the usefulness of synthetic free radicals, this free radical is chemically stable and it accepts an electrons or hydrogen to produce a stable diamagnetic molecules (Soares et al 1997). The effectiveness of a compound showing scavenging activity against DPPH free radical is indicated by a decrease in absorbance. Similar decrease in optical density is used for evaluation of NO scavenging activity. In the present study P. acerifolium extract showed significant scavenging of NO. The extract also significantly scavenged DPPH radical in a dose of dependent manner.

There are many popular theories to explain oxygen mediated toxicities and among them superoxide (O_2^-) theory is by far one the most important one. The propose that oxygen toxicity is due to enormous production of (O_2^-) which may take place due to unwanted oxidation of biologoically important molecules. Accordingly, superoxide radical scavenging activity was estimated following PMS / NADH – NBT system and P. acerifolium extract exhibited significant reduction in the appearance of this radical indicating its superior scavenging property.

According to Mc Cord (1974), OH^- radical (generated secondarily by the reaction of superoxide and hydrogen peroxide) actually brings about depolymerisation of hyaluromic acid as a OH^- scavenger can return the viscosity of hyaluromic acid solution. Hydroxyl radical (OH^-) is closely associated with inflammatory disorder like arthritis where a progressive loss of hyaluromic acid in joint important feature of disease. In our study P. acerifolium extract produce a dose dependent scavenging of OH^- radical. The activity of extract was most effective in the first hour of the study. The observed dose dependent scavenging effect can be explained by the understanding the nature and generation of radicals as well as studying the different in physical and chemical properties of the natural occurring antioxidant (Khanamm et al 2004, Schwart et al 2001). The stable radicals like DPPH react stoichometrically with antioxidant which are hydrogen donors (Blois et al 1958, Schwart et al 2001, Gardner et al 1998). But antioxidant which are effective chelators of transition metal ions may contribute differently to the antioxidant response in hydroxyl radical inhibition assay. A substance may act as an antioxidant to its ability to reduce reactive oxygen species by donating hydrogen atom (Jayprkash et al 2001.).

The reducing property of P. acerifolium extract indicates that it is capable of donating hydrogen atoms in dose dependent manner. The high content of phenolic compound in the extract may be a associating factor towards antioxidant activity, because the phenolic compounds are known to have direct antioxidant property due to presence of hydroxyl group (Arnason et al 1981, Dreosti et al 2000, Duh et al 1999).

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