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Comparative antioxidant activity of roots and fruits of *Piper sylvaticum* Roxb

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ABSTRACT

Objective: *Piper sylvaticum* Roxb is commonly known as Pahari pipul belongs to family: Piperaceae, traditionally used to treat liver disorder, gastrointestinal tract disorder and chronic kidney diseases. Therefore, it was chosen to evaluate its antioxidant activity.

Materials and Methods: In the present study, petroleum ether and chloroform extract of roots and fruits of *Piper sylvaticum* Roxb were subjected to phytochemical screening for the presence of phytoconstituents. Amongst all extracts chloroform extract of root and fruit part of *Piper sylvaticum* Roxb showed the presence of phenols and flavonoids. These extracts were then taken up for the comparative study of antioxidant activity by DPPH and superoxide anion radical method.

Results & Conclusion: The antioxidant activity of the *Piper sylvaticum* (fruit and root) was found to be in the following order: Chloroform extract (fruit) > Chloroform extract (root) > Petroleum ether extract (fruit) > Petroleum ether extract (root). The chloroform extract of fruits and roots of *Piper sylvaticum* Roxb was found to be most potent free radical scavenger. Antioxidant activity of these extracts may be attributed to the presence of phenolic compounds and flavonoids. These results suggest that the chloroform extract of *Piper sylvaticum* Roxb can be considered as a medicinal source for the treatment of many free radicals related diseases.

Keywords: *Piper sylvaticum* Roxb, Pahari pipul, Antioxidant, DPPH, Free radical.

INTRODUCTION

Free radicals and other reactive species, produced during aerobic metabolism, can cause oxidative degradation of amino acids, proteins, lipids and DNA in our body [1]. It has been reported that oxidative stress is one of the major cause of various chronic and degenerative diseases such as ageing, atherosclerosis, cancer, diabetes mellitus, immunosuppression, ischemic heart disease, hepato-toxicity and neurodegenerative disorders [2]. The most effective way to abolish free radicals is to use antioxidants. Antioxidants, both exogenous and endogenous, whether synthetic or natural, can be effective to scavenge free radicals and promoting their decomposition. Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), t-butyl hydroquinone (TBHQ) and 2-tert-butyl-4-

methylphenol (TBMP) are synthetic antioxidants used in food industry, out of which some have been reported to be dangerous for human health [3,4]. Recently, there has been growing interest in natural antioxidants of plant origin by virtue of their greater application in the food industry and better therapeutic efficacy. Some *in vitro* studies indicates plant extracts and their bioactive constituents (having antioxidant activity) are capable of exerting protective effects against oxidative damage responsible for various complications in biological systems. *Piper sylvaticum* Roxb belongs to family Piperaceae, commonly known as Pahari pipul, is effective against liver, spleen, gastrointestinal tract disorders etc. This plant was undertaken in the present study to evaluate their *in vitro* antioxidant activity and compared with antioxidant profile of the selected plant parts.

MATERIALS AND METHODS

Plant material

Piper sylvaticum Roxb (roots and fruits) was purchased from authorized dealer of our institute and taxonomically authenticated from Division of Taxonomy, National Botanical Research Institute (CSIR), Lucknow (Ref. No: NBRI/CIF/74/2009). Selected parts were washed with water and air dried under shade. Size reduction was carried out in grinder, passed through sieve no. 18, weighed and utilized for further studies.

Preparation of extracts

The powdered samples of plant roots and fruits were successively extracted with petroleum ether and chloroform using soxhlet apparatus. The extracts obtained were evaporated to dryness in rota evaporator (Laborota 4001 Efficient, Heidolph, Germany) and weighed. Dried extracts were used for evaluation of *in vitro* antioxidant activity.

Phytochemical screening

Phytochemical screening of the petroleum ether and chloroform extracts were carried out for flavonoids, polyphenols, alkaloids, steroids, terpenoids, amino acids, saponins and glycosides.

Determination of *in vitro* antioxidant activity

(a) 1, 1-Diphenyl, 2-picryl-hydrazyl (DPPH) free radical scavenging assay

DPPH was used to determine free radical scavenging activity (in triplicate) of the plant extracts using the method of Sánchez-Moreno et al., 1999 [5]. Different concentrations of the extracts (100, 200, 300, 400, 500 µg/ml) were prepared in methanol. 0.1 ml of test sample was added to 3.9 ml DPPH solution (0.025g/L) in methanol. Mixtures so obtained were incubated in dark for 30 minutes and absorbance was measured at 517 nm using UV-Visible spectrophotometer (Varian Cary-5000, Netherland). The percentage scavenging was calculated using calibration curve of DPPH and IC₅₀ was determined.

(b) Superoxide radical scavenging method

The superoxide anion radical (O₂^{•-}) scavenging capacity of the extracts (in triplicate) were determined by the method of Liu et al., 1997 and assayed by the reduction of nitro blue tetrazolium (NBT) [6]. Superoxide radicals were generated in 3 ml of Tris-HCl buffer (16 mM, pH 8.0) mixed with 1 ml of NBT (50 mM) solution, 1 ml NADH (78 mM) solution and 1 ml corresponding extract solution (100 mg/ml). 1 mL of phenazine methosulphate (PMS) solution (10 mM) was added to the mixture to initiate the reaction followed by incubation at 25 °C for 5 min. Absorbance was measured at 560 nm in a

spectrophotometer against blank samples. The superoxide radical scavenging was measured by using the formula:

$$\% \text{ scavenging activity} = [(A_c - A_s)/A_c] \times 100$$

Where A_c is the absorbance of the control and A_s is the absorbance of extract.

RESULTS

In this study, *Piper sylvaticum* Roxb were studied for their antioxidant activity against DPPH free radical scavenging assay and Superoxide radical scavenging method. Phytochemical constituents of the plants have been depicted in Table 1. Scavenging activity of different extracts on DPPH radical and Superoxide radical has been shown in Table 2 and 3 respectively. Data has been shown as mean ± standard error of mean.

DISCUSSION

Crude extracts of fruits, cereals, herbs, vegetables and other plant materials loaded with various phytoconstituents such as phenolics, flavonoids, triterpenoids etc. are increasingly of interest in the nutraceutical and pharmaceutical industry because of their antioxidant property [7-9]. Therefore, we have selected chloroform and petroleum ether extracts of *Piper sylvaticum* Roxb (roots and fruits) in order to evaluate their antioxidant activity. Two assays were used in the present study, i.e., ability to scavenge DPPH and superoxide radicals (NBT). DPPH assay of selected plant parts extracts expressed as a percentage scavenging of free radicals. Both extracts of selected parts of *Piper sylvaticum* Roxb showed a tendency to quench the free radicals as indicated by increase in percentage inhibition which indicates good antioxidant potential. Chloroform extract of fruit and root part of the plant has shown lower IC₅₀ value, which shows higher percentage of inhibition. NBT assay of selected plant parts extracts has been expressed as a percentage scavenging of superoxide anion radicals. Chloroform and petroleum ether extracts of *Piper sylvaticum* Roxb showed a tendency to scavenge superoxide anion radicals, as indicated by increase in percentage scavenging, indicates antioxidant effect of the plant. Chloroform extract of fruit and root part of selected plant has shown lower IC₅₀ value, which shows higher percentage of scavenging as compared to petroleum ether extracts. Antioxidant activity of plants has been partly ascribed to phenolic compounds and flavonoids. Most of the antioxidant potential of medicinal plants is due to the redox properties of phenolic compounds, which enable them to act as reducing agents, hydrogen donors and singlet oxygen scavengers [10]. Moreover, hydrogen-donating substituent's (hydroxyl groups) attached to the aromatic ring structures of flavonoids

Table 1: Preliminary phytochemical analysis of various extracts of *Piper sylvaticum* Roxb (roots and fruits)

Phytoconstituents	Test	Root (Pet. Ether extract)	Root (Chloroform extract)	Fruit (Pet. Ether extract)	Fruit (Chloroform extract)
Amino acids	Ninhydrin reagents	-	+	-	+
Alkaloids	Dragendroff's reagent	+	-	+	+
	Mayer's reagent	+	-	+	+
Flavonoids	Shinoda test	-	+	-	+
Glycosides	Borntrager test	-	-	-	-
Phenolics	5% alcoholic FeCl ₃ solution	-	+	-	+
Saponins	Foam test	-	-	-	-
Steroids	Liebermann-Burchard Test	+	-	+	-
Triterpenoids	Liebermann-Burchard Test	+	+	+	+

Absent -, Present +

Table 2: Percentage scavenging activity in DPPH free radical scavenging assay

Conc. (µg/ml)	Pet. Ether Extract (Root)	Chloroform Extract (Root)	Pet. Ether Extract (Fruit)	Chloroform Extract (Fruit)
100	50.04±0.49	53.50±0.52	54.12±0.448	55.32±0.46
200	52.01±0.51	56.75±0.54	57.24±0.56	56.22±0.51
300	58.04±0.54	56.95±0.55	62.26±0.58	58.25±0.48
400	61.07±0.59	67.28±0.59	63.15±0.61	65.21±0.59
500	66.04±0.62	77.61±0.68	74.02±0.69	75.25±0.63
IC₅₀	119.024	89.827	34.66	54.375

Values expressed as % scavenging activity (mean ± SEM); n=3

Table 3: Percentage scavenging activity in Superoxide radical scavenging assay

Conc. (µg/ml)	Pet. Ether Extract (Root)	Chloroform Extract (Root)	Pet. Ether Extract (Fruit)	Chloroform Extract (Fruit)
10	35.7±0.22	36.16±0.26	45.15±0.39	46.18±0.43
20	39.12±0.36	41.25±0.35	48.14±0.41	48.16±0.45
40	43.00±0.39	44.51±0.37	51.23±0.47	53.26±0.48
60	47.25±0.	52.00±0.46	55.21±0.48	57.85±0.56
100	55.18±0.54	58.12±0.53	60.49±0.58	64.52±0.63
IC₅₀	74.26	61.046	33.772	26.714

Values expressed as % scavenging activity (mean ± SEM); n=3

enable them to undergo redox reaction, which in turn, help them to scavenge free radicals [11]. These reports support our study, as chloroform extract of *Piper sylvaticum* Roxb (Roots and fruits) contained phenols and flavonoids.

CONCLUSION

The results of present research work revealed that all the extracts of *Piper sylvaticum* (Fruit and Root) were able to prevent the initiation of undesirable free radical mediated chain reaction. Among all the extracts, chloroform (Fruit) extract was found to be most potent free radical scavenger.

CONFLICT OF INTEREST

There is no conflict of interest.

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