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Research Article

EVALUATION OF ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF *PARIS POLYPHYLLA* SM.

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ABSTRACT

Objectives: *Paris polyphylla* Sm. is a very important anti-cancerous plant species found in the Himalayan region of India. The present study was carried out to determine the phenolic and flavonoid content, antioxidant and antimicrobial activity of its rhizome.

Methods: Antioxidant activity of rhizome extract was evaluated through 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. Antimicrobial activity was evaluated through disc diffusion assay against two bacterial and two fungal strains. The bacterial species used in the present study were *Escherichia coli* and *Staphylococcus aureus* and the fungal strains used were *Aspergillus niger* and *Trichoderma reesei.*

Results: The standard curve of Gallic acid revealed that the phenolic content of our sample is 43.01±0.17 mg Gallic acid equivalents (GAE)/g dry weight (DW). Similarly, flavonoids were obtained as 28±0.12 mg quercetin equivalent/g DW of the sample. It was observed that the methanolic rhizome extract showed higher antioxidant potential than water extract with the IC_{50} value of 1.09 mg/ml. Further, the rhizome extract of *P. polyphylla* species exhibited significant antimicrobial activity and it was observed that at concentration of 5 mg/ml of the sample, the percentage inhibition was 95-97% in *E. coli, S. aureus* and *A. niger* whereas in case of *T. reesei* it was 74%.

Conclusion: This study is first of its kind in the Indian subcontinent on this plant species and these findings indicate the tremendous and promising potential of this wonder herb. A lot of work has already been done in China on this species, therefore, further studies are required for the conservation and sustainable use in Indian subcontinent before this species is thrown into extinction as it has already been declared vulnerable.

Keywords: Antioxidant, Anticancer, Antimicrobial, Phenolics, Flavonoids.

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INTRODUCTION

Since time immemorial, use of plants has played significant role in the development of mankind and its culture [1,2]. Throughout human history, plants play basic role in medical treatments and such folk medicines are still widely practiced today [3]. Indigenous practice has major advantage over modern drugs as it is cost-effective in collection and plantations. Most of the world's population still depends on drugs derived from plants [4].

New drugs produced from medicinal plants are mostly due to the characteristic effects of secondary metabolites present in these plants [5]. Isolation, purification, identification, and structure of bioactive compounds and chemicals found in plants, known as phytochemistry, specifically describe its secondary metabolites [6]. It includes terpenoids, alkaloids, saponins, polysterols, amines, glucossinolates, flavonoids, cyanogenic glycosides, phenolics [7]. Indefinite number of the pharmaceuticals currently available such as aspirin, digoxin, quinine, and opium are derived from plants (viz: *Filipendula ulmaria, Digitalis purpurea, Cinchona officinalis,* and *Papaver somniferum*) that have millennia-long history drug information of use as folk herbalism [8].

Paris polyphylla Sm. (Singpan, Satuwa; Family Melanthiaceae) is a rhizomatous perennial herb native to China and the Indian subcontinent (Fig. 1). Diverse species of *Paris* are found extensively growing in Yunnan-Guizhou Plateau of China [9]. The species is growing in China, Bhutan, and Nepal. There are widely known subspecies and varieties of *P. polyphylla* distributed in Bhutan, Laos, Myanmar, Thailand, and Vietnam as well [10]. In India, the species have been recorded from Arunachal Pradesh, Himachal Pradesh, Jammu and Kashmir, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim, and Uttarakhand of Indian-Himalayan region [11].

P. polyphylla commonly known as "Rhizoma Paridis" in China was documented for the first time in the "Chinese pharmacopoeia" in 1985 [12]. The whole plant is primarily used to treat cancer and also it acts as a febrifuge. The dried rhizomes of *P. polyphylla* have been used as a natural remedy for the treatment of microbial infection, snake bite, convulsions, fractures, throat swelling, diarrhea, and liver cancer. The rhizomes of *P. polyphylla* possess rich bioactive compounds which can be utilized potentially as source of plant-derived drugs. Rajsekhar *et al.* reported large number of secondary metabolites from the rhizome of this plant including alkaloids, quinones, phenols, and tannins [13].

Recently, *P. polyphylla*, was in news in the state of Manipur, India because of its illegal export to China and other Southeast Asian countries through Myanmar. Some local newspapers of Manipur State, such as The Local Gazette, The Sangai Express and Poknapham, reported the massive illegal trading of rhizomes of *P. polyphlla* through Indo-Myanmar border by the local traders [14,15]. A research team working under Natural Resource Data Management System, Department of Science and Technology witnessed smuggling of the rhizomes in huge quantities in and around Senapati District of Manipur [16].

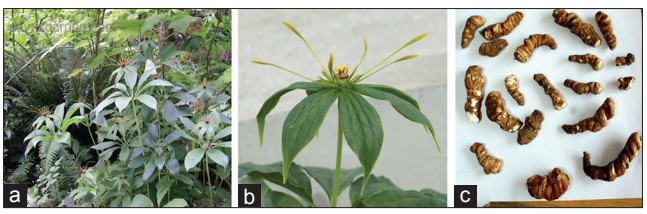

Fig. 1: *Paris polyphylla:* Habit (a), flower (b) and rhizome (c)

Due to its huge medicinal properties, this plant species is being illegally exported and unscientifically over-exploited in Northeastern states of India. At some places in Manipur, it is being uprooted. Besides above reasons and many others, this important plant species is facing the threat of extinction and recently has been declared as vulnerable. Therefore, present study was an attempt to elucidate the important parameters such as phenolics, flavonoids, antioxidant, and antimicrobial activities of this wonder herb. This work is the first of its kind in India as no work has been done on this plant species except a review on its distribution and properties [11].

METHODS

Plant material

P. polyphylla rhizomes were collected from the Ukhrul District, Manipur and identified by Dr. Arbeen Ahmad Bhat, Assistant Professor, School of Bioengineering and Biosciences, Lovely Professional University, Punjab, India. The rhizomes were washed and air-dried at room temperature for 2 weeks. Dried rhizomes were ground to fine powder and stored in air-tight containers.

Preparation of plant extracts

The rhizome powder (10 g) was extracted in Soxhlet apparatus with 300 ml solvent (methanol and distilled water) at 65°C and 100°C, respectively. Later, the extracts were evaporated using rotary evaporator. Both samples were collected and stored at 4°C for later use.

Determination of total phenolic contents

Folin-Ciocalteu colorimetric method was used for the determination of total phenolic content following the method of Ainsworth and Gillespie, 2007 [17] with little modifications. Methanolic extract of rhizome with concentration 20 mg/ml was prepared. Later, 20 µl of this extract was mixed with 1.58 ml distilled water, 100 µl Folin-Ciocalteu reagent, and 300 µl of 20% sodium carbonate followed by 30 minutes incubation in water bath at 40°C. The absorbance of the reaction mixture was recorded at 765 nm using distilled water as blank. The standard curve was prepared by taking different concentrations of Gallic acid in water, and then, the content of phenolics in extracts was expressed in terms of Gallic acid equivalent (mg of GA/g of extract).

Determination of flavonoid contents

The flavonoid content in the rhizomes was determined by aluminum chloride colorimetric method [18]. The reaction mixture contained 0.5 ml of rhizome extract in methanol, 1.5 ml methanol, 100 µl aluminum chloride, 100 µl potassium acetate, and 2.8 ml distilled water. Aluminum chloride replaced with distilled water was used as blank and absorbance was recorded at 415 nm after 30 minutes incubation at room temperature. The standard calibration curve was prepared by taking various concentrations of quercetin in methanol. The results were represented as mg QE/g dry weight (DW).

Evaluation of antioxidant activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was carried out following the method of Padmanabhan and Jangle [19] with little modifications. Different concentrations of sample were taken, ranging from 0.25 to 1.25 mg/ml. 2 ml of 0.1 mM DPPH methanol solution was added and absorbance recorded at 517 nm following 15 minutes of incubation in dark. DPPH radical scavenging activity was calculated using the following formula:

$$\text{Scavenging effect } (\%) = (1 - \frac{As}{Ac}) \times 100$$

Where, A_s=OD of sample with DPPH solution,
A_c=OD of DPPH methanol solution.

Antimicrobial activity

Microbial strains

The two bacterial strains (*Escherichia coli* and *Staphylococcus aureus*) and two fungal strains (*Aspergillus niger* and *Trichoderma reesei*) were identified and purchased from MTCC, IMTech, Chandigarh.

The bacterial inoculums were maintained in nutrient broth at 37°C and subculturing after every week. The fungal isolates were cultured in potato dextrose broth at 25°C and subculturing after 2 weeks.

Antibacterial activity

Disk diffusion technique was used to determine the antibacterial activity of the methanolic plant extract following Balouiri *et al.* [20]. Different concentrations of sample ranging from 1.25 to 5 mg/ml, were placed on the nutrient agar plates inoculated with test organisms. The sample extract replaced by streptomycin sulfate disc was used as positive control and with methanol only as negative control. After incubation at 37°C for 2 days, the diameters of the zones of inhibition were measured to the nearest millimeters.

Antifungal activity

Antifungal activity was evaluated again following the procedure of Balouiri *et al.* [20]. Again the procedure was same as in antibacterial activity except the media used here was Czapek Dox agar media and in positive control Fluconazole drug was used. After incubation at 27°C for 3 days, the diameters of the zones of inhibition were measured to the nearest millimeters.

Relative percentage inhibition

The relative percentage inhibition of the plant rhizome extract with respect to positive control was calculated using the following equation [21]:

$$\text{Relative percentage inhibition of the test extract} = \frac{(X - Y)}{(Z - Y)} \times 100$$

Where, X: Total area of inhibition of the plant extract,
Y: Total area of inhibition of the negative control,
Z: Total area of inhibition of the positive control.

The total area of the inhibition was calculated using area=πr^2, where, r=Radius of zone of inhibition.

RESULTS AND DISCUSSION

Yield of the extract

10 g of dried rhizome powder of *P. polyphylla* was extracted in methanol and water separately using a soxhlet apparatus. The yield of methanol extract (8.9%) was higher compare to aqueous extract (6.5%).

Total phenolic and flavonoid content

The methanolic rhizome extract was found to contain significant quantity of phenolic compounds and the results are shown in Table 1. From the calibration curve of Gallic acid (Fig. 2), it was calculated that our sample contains 43.01±0.17mg GAE/g DW of sample (R^2=0.99).

Flavonoids on the other hand also were quite significant in the sample and the calibration curve (R^2=0.99, Fig. 3) of quercetin revealed that our sample contains 28±0.12 mg quercetin equivalent/g DW of the sample.

DPPH antioxidant activity

DPPH radical is commonly used for the evaluation of the antioxidant activities of natural compounds. It is a stable free radical that can be reduced by the antioxidants. The transfer of either an electron or a hydrogen atom to DPPH causes the scavenging mechanism of natural compounds [22]. When the DPPH radical is scavenged, the color of the

Table 1: Total phenolic and flavonoid content of methanolic extract of *P. polyphylla* rhizomes

Total phenolics content[a]	*43.01±0.17
Total flavonoids content[b]	*28±0.12

[a]mg gallic acid equivalent (GAE)/g DW. [b]mg quercetin equivalent/g DW. Values are means of three replicates. *Each value is expressed as the mean±SD (n=3). *P. polyphylla: Paris polyphylla*, SD: Standard deviation

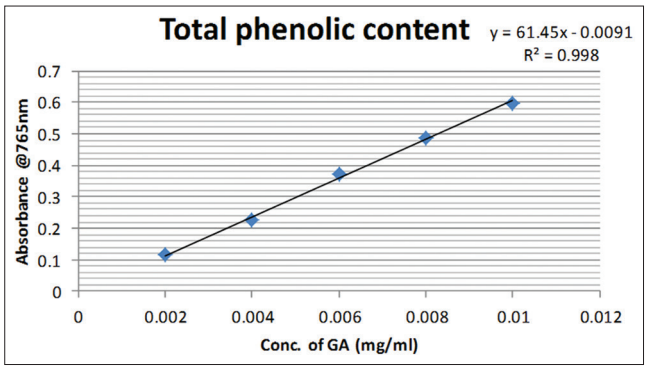

Fig. 2: Standard curve of gallic acid

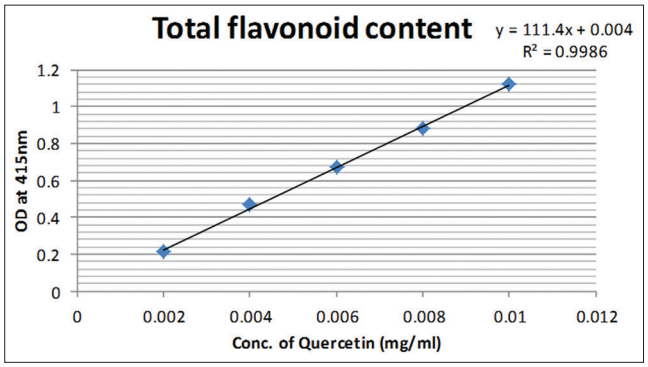

Fig. 3: Standard curve of quercetin

reaction mixture changes from purple to yellow with decreasing of absorbance at wavelength 517 nm.

It was observed that the methanol rhizome extract showed higher antioxidant activity than water extract with IC_{50} value of 1.09 mg/ml (Fig. 4). The results of IC_{50} value for rhizome extract were in contrast to the results obtained by Shen *et al.* who reported it as 0.25 mg/ml [23]. The percentage scavenging effect on DPPH radical was increased with the increase in the concentration of the sample (Fig. 4).

Antimicrobial activity

P. polyphylla is a potent antimicrobial agent. The main compounds responsible for its antimicrobial activity are the steroidal saponins, which are concentrated mostly in its rhizomes [24]. These compounds showed antimicrobial activity against a wide range of bacteria (*Agrobacterium tumefaciens, Bacillus subtilis, E. coli, S. aureus, Helicobacter pylori, Xanthomonas vesicatoria, Staphylococcus haemolyticus, Pseudomonas* spp. etc.) and fungi (*A. niger, Candida albicans, Fusarium graminearum, Phytophthora capsici, Botrytis cinerea, Magnaporthe oryzae*, etc) [25].

The present study revealed that the rhizome extract exhibited significant amount of antibacterial activity (Fig. 5). Highest activity of the extract was found against *E. coli* where the diameter of the zone of inhibition was found to be >31 mm (Table 2). Similarly, for *S. aureus,* the zone of inhibition was found to be as 30 mm. It is observed that the inhibition increases as we increase the concentration of the sample signifying the dose-dependent inhibiton. For *E. coli,* at the concentration of 1.25 mg/ml the zone of inhibition was 23 mm while at 5 mg/ml the zone of inhibition was found as 31 mm whereas for *S. aureus* at the concentration of 1.25 mg/ml the zone of inhibition was 26 mm and at 5 mg/ml it was found to be 30 mm. The results were found to be statistically significant at $p<0.05$.

Regarding antifungal nature the present plant species exhibited highest activity against *A. niger* with a diameter around 33 mm in zone of inhibition while in case of *T. reesei* the zone of inhibition was found as 31 mm at 5 mg/ml concentration of the sample.

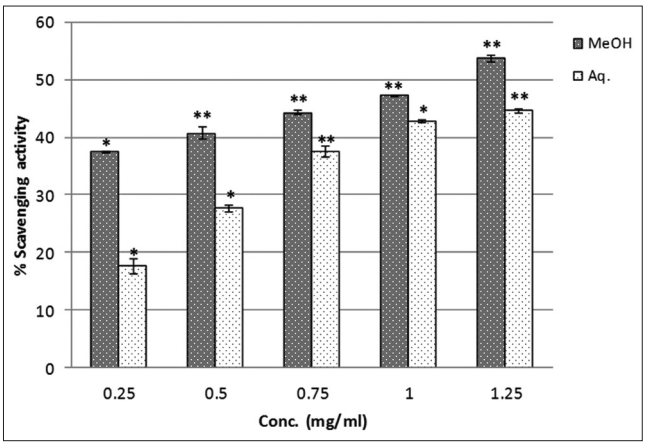

Fig. 4: 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity of *Paris polyphylla* rhizome (mean±SD, n=5). *Indicates statistically significant difference at p≤0.05 (Tukey's test) and **indicates statistically significant difference at p≤0.01 by Tukey's test

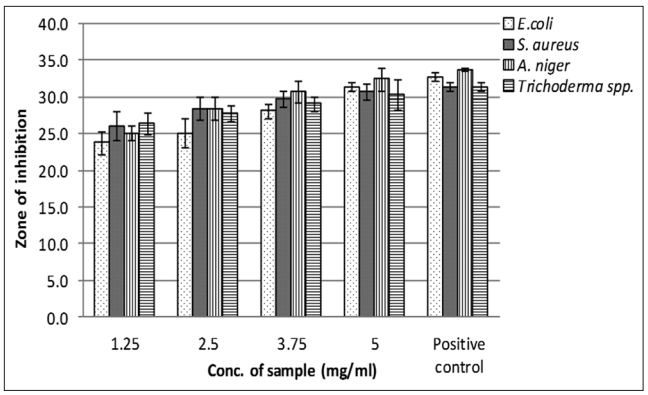

Fig. 5: Antimicrobial activity of test sample on different bacterial and fungal strains. At the concentration of 5 mg/ml of the extract, the inhibition effect of both positive control and sample is almost same (statistically significant at p<0.05 after Tukey's test; n=3)

Determination of relative percentage inhibition

The results for relative percentage inhibition are presented in Table 3. It was observed that the methanol extract of rhizome exhibited maximum relative percentage inhibition against *A. niger* (97.74%). *S. aureus* and *E. coli* exhibited same inhibition percentage, i.e., 95.58% while *T. reesei* showed 74.41% inhibition.

DISCUSSION

At present, there is an increasing interest worldwide in herbal medicines accompanied by increased laboratory investigation into the pharmacological properties of the bioactive ingredients and their ability to treat various diseases [26]. Free radicals have been considered as the major cause of various chronic and degenerative diseases in the living systems. The vast amounts of synthetic molecules are available for free radical scavenging antioxidants, but adverse side effects are associated with these compounds. An alternative solution for this problem is to consume the naturally available antioxidants from the medicinal plants because they are having lower side effects and comparatively safe [27].

The present work is the first of its kind on *P. polyphylla* rhizome extract in India and was designed to evaluate the antioxidant and antimicrobial activity of methanolic extract of the rhizome of *P. polyphylla* species. Secondary metabolites in plants (e.g., phenolics, flavonoids, carotenoids) are natural phytochemical antioxidants. Flavonoids are found to have medical importance as they possess anticancer, antidiabetic, and anti-inflammatory properties and help reduce coronary heart

Table 2: Antimicrobial activity of *P. polyphylla* rhizomes on different microorganisms

Concentration of sample (mg/ml)	Zone of inhibition (mm)			
	*E. coli**	*S. aureus**	*A. niger**	*T. reesei**
1.25	23.66±1.53	26±2.0	25±1.0	26.33±1.52
2.5	25±2.0	28.33±1.53	28.33±1.52	27.67±1.15
3.75	28±1.0	29.66±1.15	30.67±1.52	29±1.0
5	31.33±0.58	30.66±1.15	32.33±1.52	30.33±2.08
Positive control	32.66±0.57	31.33±0.58	33.67±0.22	31.33±0.66
Negative control	0	0	0	0

*Each value is expressed as the mean±standard deviation. *P. polyphylla: Paris polyphylla, E. coli: Escherichia coli, S. aureus: Staphylococcus aureus, A. niger: Aspergillus niger, T. reesei: Trichoderma reesei*

Table 3: Relative percentage inhibition of the test organisms

Concentration of sample (mg/ml)	Relative percentage inhibition (%)			
	E. coli	*S. aureus*	*A. niger*	*T. reesei*
1.25	50.55	58.78	59.29	51.21
2.5	65.77	65.77	84.58	61.53
3.75	91.33	89.18	95.43	69.42
5	95.58	95.58	97.74	74.41

E. coli: Escherichia coli, S. aureus: Staphylococcus aureus, A. niger: Aspergillus niger, T. reesei: Trichoderma reesei

diseases [28,29]. Natural phenolics from medicinal plants are novel anticarcinogens [30]. The rhizome extract of *P. polyphylla* species exhibited promising antioxidant activity owing to the presence of fair amount of phenolics and flavonoids in its rhizome. The DPPH scavenging activity of methanolic extract of *P. polyphylla* rhizome showed an IC_{50} value of 1.09 mg/ml. A dose-dependent increase in the scavenging activity of methanolic rhizome extract was observed in the present study. Similar results were obtained by Saiah *et al.* [31] in six algerian medicinal plants while evaluating their antioxidant and antimicrobial activities.

P. polyphylla has been found to be a potent antimicrobial agent. The main compounds responsible for its antimicrobial activity are the steroidal saponins, which are concentrated mostly in its rhizomes [24]. Besides large amount of phenolics and flavonoids have also been reported in the plant species which may be considered responsible for its antimicrobial activity. Further, it was observed that the zone of inhibitions at the concentrations of 5 mg/ml were almost equal to the zone of inhibitions of different standard drugs used in this study signifying the importance of this plant species against various infections and thus can be used as a substitute to chemically prepared harmful antibiotics.

CONCLUSION

The IUCN and CAMP listed *P. polyphylla* Sm. as vulnerable medicinal plant [32]. Random collection by uprooting the young or mature plants which sprouted either from seeds or fragmented rhizomes is one of the key factors for downsizing the population of *P. polyphylla.* With so much of work and researches done on the different aspects and potentials of *P. polyphylla* in China, not many works pertaining to the medicinal properties and other, of this plant has been reported from India. The need of the hour is for the scientific community to draw their attention to this wonder herb.

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