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ANTIDERMATOPHYTE AND ANTIOXIDANT POTENTIAL OF *REMISATIA VIVIPARA* (ROXB.) SCHOOT (ARACEAE)

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ABSTRACT

The present study describes the antidermatophyte and antioxidant activity of methanolic extract of an edible plant Remisatia vivipara (Roxb.) Schoot (Araceae). Human dermatophytes namely Trichophytum rubrum and Chrysosporium indicum were tested for their sensitivity to methanolic extract by Agar well diffusion method. Different concentrations of methanolic extract was subjected to antioxidant activity by DPPH free radical scavenging and Fe^{+3} reducing power assays. A marked dose dependent antioxidant activity of extract was observed. Among fungi, Trichophytum rubrum was found to be more susceptible to solvent extract than Chrysosporium indicum. The results of the study indicate the antioxidant and antifungal potential of extract. Further studies on isolation of constituents and biological activities of isolated compounds in vivo are to be carried out.

Key words: *Remisatia vivipara (Roxb.) Schoot; DPPH; reducing power assay; Agar well diffusion; Zone of inhibition*

Introduction

The superficial fungal infections in humans (dermatomycoses) are caused by dermatophytes, a group of filamentous fungi that invade and draw nutrients from the keratinized tissues such as skin, hair and nails. Among the dermatophyte genera, *Trichophyton*, *Microsporum* and *Epidermophyton* are most important. As the dermatophytes have developed resistance to antimycotic drugs, there is an urgent need for nontoxic, safe and cost effective antifungal agents [1, 2]. Free radicals are chemical species containing one or more unpaired electrons that makes them highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability [3]. Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS [4, 5]. In recent years much attention has been devoted to natural antioxidant and their association with health benefits [3]. Biologically active compounds present in the medicinal plants have always been of great interest to scientists working in this field. In recent years this interest to evaluate plants possessing activity for various diseases is growing [6]. Plants readily synthesize substances for defense against attack by insects, herbivores and microorganisms. *Remisatia vivipara* (Roxb.) Schoot belonging to the family Araceae is a tuberous herb emitting long leafless bulbiferous shoots form the

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crown of the tuber. Leaf is solitary, entire, peltate, orbicular, membranous, ovate or cordate. Flowers monoecious and spathe coriaceous, spadix very short. Male and female inflorescences are distinct. Ovary closely packed one celled with parietal plancentation. Fruits are small clustered berries and the seeds are small. It is commonly known as Maragesu in Kannada and in Sanskrit it is known as Lakshmana. It is flowering and fruiting in April to May and commonly appears in trunksite of tree during the rainy season in the evergreen forests of Western Ghats. The juice of the plant mixed with cow urine for an alexipharmic. Roots compound with turmeric into an ointment for itch. Leaves are edible; locally they are cooked with rice floor to prepare a popular food item called Pathrode [7]. The present study describes the antidermatophyte and antioxidant activity of methanolic extract of an edible plant *Remisatia vivipara* (Roxb.) Schoot (Araceae).

Materials and Methods

Extraction and phytochemical analysis

Leaf material was shade dried and powdered mechanically. About 250g of powdered material was subjected to soxhlet extraction and exhaustively extracted with methanol for about 48 hours. The extract was filtered and concentrated in vacuum under reduced pressure using rotary flash evaporator and dried in the dessicator. The methanolic extract was subjected to preliminary analysis for screening the presence of various phytoconstituents namely alkaloids, steroids, flavonoids, saponins, tannins, steroids and glycosides by chemical tests [8].

Antioxidant activity of solvent extracts by DPPH free radical scavenging assay

Different concentrations of solvent extracts and ascorbic acid (standard) namely 25, 50, 100, 200 and 400 mcg/ml were prepared in methanol. DPPH (0.002% in methanol) was used as free radical. Equal volume of different concentrations of solvent extracts and DPPH were mixed in clean and labeled test tubes separately and the tubes were incubated at room temperature in dark for 30 minutes. The optical density was measured at 517nm using UV-Vis Spectrophotometer. The degree of stable DPPH* decolorization to DPPHH (reduced form of DPPH) yellow indicated the scavenging efficiency of the extract. The scavenging activity of the extract against the stable DPPH* was calculated using the following equation.

Scavenging activity (%) = A – B / A x 100

Where A is absorbance of DPPH and B is absorbance of DPPH and extract combination [9,10].

Reducing power assay

Different concentrations of solvent extracts and tannic acid (25, 50, 100, 200 and 400 mcg/ml) in 1ml of methanol were mixed with 2.5ml of phosphate buffer (200mM, pH 6.6) and 2.5ml of 1% potassium ferricyanide separately. The mixtures were placed in a water bath for 20 min at 50^{o}C, cooled rapidly, mixed with 2.5ml of 10% trichloroacetic acid and 0.5ml of 0.1% Ferric chloride. The intensity of iron (II) - ferricyanide complex was determined by measuring the formation of Perl's Prussian blue at 700nm after 10min. The higher absorbance of the reaction mixture indicates increased reducing power [11].

Antifungal activity of methanol and aqueous extracts

Fungi namely *Trichophytum rubrum* (MTCC3272) and *Chrysosporium indicum* (MTCC4965) were tested for their susceptibility to the solvent extracts by Agar well diffusion method [12]. The fungal cultures were obtained from IMTECH, Chandigarh, India. The fungal inocula

were aseptically swabbed on sterile and solidified Sabouraud dextrose agar plates. Then, aseptically wells of 6mm diameter were bored in the inoculated plates with the help of gel puncher and the extracts (10mg/ml of 10% DMSO), Standard (Amphotericin B, 1mg/ml) and Control (10% DMSO) were added into the respectively labeled wells. The plates were incubated at 28^{o}C for 72 hours in upright position and the zone of inhibition formed around the well was recorded. The experiment was carried in triplicates to get average reading.

Results and Discussion

Qualitative Phytochemical analysis

The preliminary phytochemical analysis of methanolic extract showed the presence of phytoconstituents namely saponins, alkaloids, steroids, glycosides and tannins in the methanolic extract (Table-1). Symbol (+) indicates the presence of different phytochemicals and (-) indicates the absence of phytochemicals.

Table1: Phytoconstituents detected in methanolic extract

Phytoconstituents	Methanolic extract
Alkaloids	+
Steroids	+
Saponins	+
Glycosides	+
Terpenoids	-
Flavonoids	-
Tannins	+

Antifungal activity

Antifungal activity of methanolic extract against human dermatophytic fungi is shown in Table-2. *T. rubrum* was found to be more susceptible when compared to *C. indicum.* The inhibitory activity of standard was greater than the methanolic extract. Control (10% DMSO) did not reveal any inhibition of test fungi.

Table 2: Antifungal activity of methanolic extract

Test fungi	Zone of inhibition in mm		
	Methanolic extract	Standard	Control
C. indicum	11	28	-
T. rubrum	14	23	-

DPPH free radical scavenging activity

The result of antioxidant activity of different concentrations of methanolic extract and standard (ascorbic acid) is shown in Figure 1. The extract exhibited marked antioxidant activity by scavenging DPPH* (free radical) and converting into DPPHH. A dose dependent radical scavenging activity was observed. The scavenging activity of ascorbic acid was greater than that of solvent extracts. There are several methods available to assess antioxidant activity of compounds. An easy, rapid and sensitive method for the antioxidant screening of plant extracts is free radical scavenging assay using 1,1, diphenyl-2-picryl hydrazyl (DPPH) stable radical spectrophotometrically. In presence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases [13]. In this study, the scavenging activity of methanol extract was found to be dose dependent i.e., higher the concentration, more was the scavenging activity. Though the DPPH radical scavenging abilities of the extracts were less than that of ascorbic acid, the study showed that the extracts have the proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants.

Reducing Power Assay

The result of reducing power of different concentrations of solvent extracts and tannic acid is represented in Figure 2. In this study, the absorbance was found to increase with the dose of extracts and standard which is suggestive of reducing power. In the Fe^{+3} reducing assay, the reducing power of crude solvent extracts was found to increase with the dose. The presence of reductants such as antioxidant substances in the antioxidant samples causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Therefore, Fe^{2+} can be monitored by measuring the

formation of Perl's Prussian blue at 700 nm [14]. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity [15]. However, the antioxidant activity of putative antioxidants have been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, and radical scavenging [16].

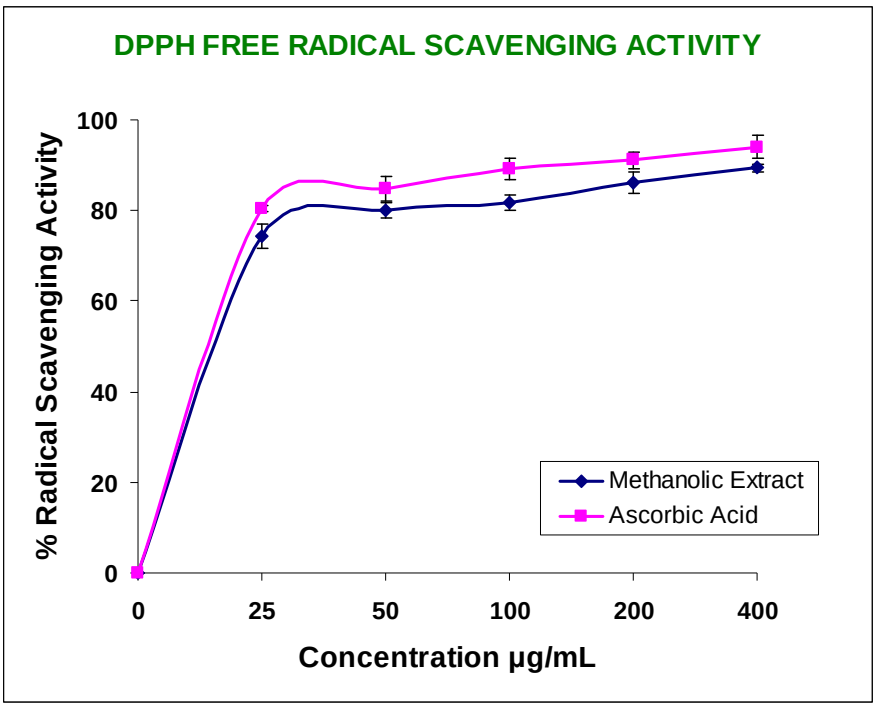

Figure 1: DPPH free radical scavenging activity of different concentrations of methanolic extract. Ascorbic acid was used as standard.

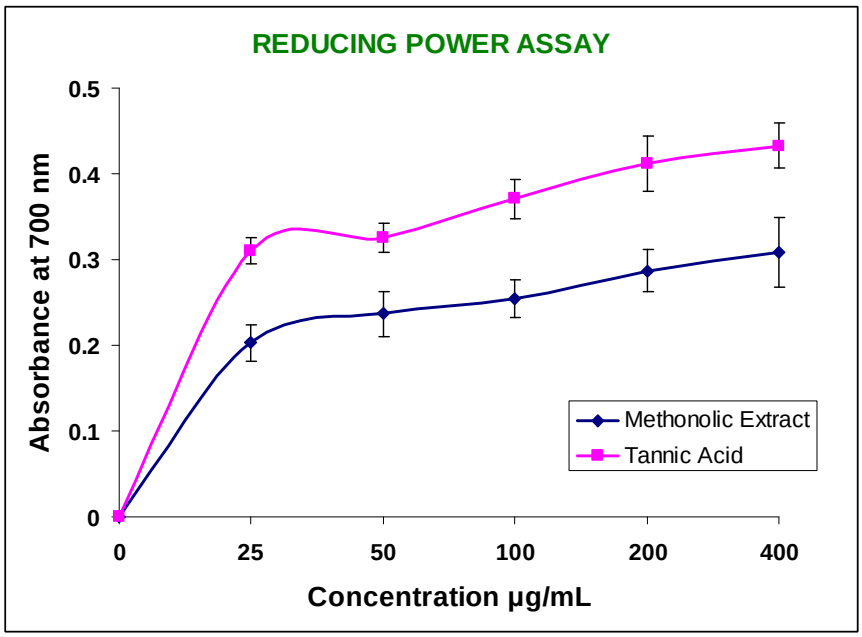

Figure 2: Fe^{+3} reducing activity of different concentrations of methanolic extract and Tannic acid was used as standard

The use of plant extracts to treat infections is an age-old practice in a large part of the world, especially in developing countries, where there is dependence of traditional medicine for a variety of diseases [17]. In developing countries like India where poverty and malnutrition is rampant, knowledge of plant derived metabolites could reduce the cost of health care. India has a rich history of using various herbs and herbal components for treating various diseases [18]. Infectious diseases caused by bacteria, fungi, viruses, and parasites remain a major threat to public health, despite tremendous progress in human medicine. Their impact is particularly great in developing countries because of the relative unavailability of medicines and the emergence of widespread drug resistance [19]. Interest in plants with antimicrobial properties has revived as a result of current problems associated with the use of antibiotics [20].

Conclusion

The traditional medicines hold a great promise as a source of easily available effective antifungal agents to the people, particularly in developing countries, including India. Indigenous system of medicine reports a number of plants for their antifungal efficacy. However, their scientific evaluation as compared to commercial agents is limited. The extract of the plant used in this study was found to be effective against the human dermatophytes tested. Due to negative effects of synthetic antioxidants, nowadays, much attention has been placed on phytoconstituents. Many of the phytoconstituents are beneficial and many of them are acting as natural antioxidants. The results of the present investigation were suggestive of the potential of solvent extracts in scavenging free radical. According to our study, the presence of phenolic constituents in the solvent extract may be responsible for the high radical scavenging activity. Further studies on isolation of phytoconstituents and *in vivo* biological activities will be carried.

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