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Research Article

SCURRULA PARASITICA L.: A MEDICINAL PLANT WITH HIGH ANTIOXIDANT ACTIVITY

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ABSTRACT

Scurrula parasitica L. is a medicinal plant reported to have anticancer effect on human cancer cell lines. Generally, anticancer activity of medicinal plants is believed to be due to their antioxidant properties. In this study, the antioxidant capacity of aqueous and methanolic extracts of matured and tender leaves of *S. parasitica* L. were estimated by four different *in vitro* methods *viz*. DPPH free radical scavenging assay, FRAP, total phenolic content assay and Ascorbate-Iron (III) induced lipid peroxidation inhibition assay. The antioxidant activity was expressed as mg Trolox equivalent (TE) per gram of dry leaves for DPPH and FRAP assay and as mg Gallic acid equivalent (GAE) per gram of dry leaves for total phenolic content. Methanolic extract of the matured leaves showed highest value of 190.51 and 134.92 mg TE/gm of dry leaves in DPPH and FRAP assay respectively and similarly it showed highest antioxidant activity in all the assays. Again the ability of the extracts to inhibit lipid peroxidation in bovine brain extract has been correlated with the other three assays. Data analysis showed that there was a positive correlation between lipid peroxidation inhibition and other antioxidant activity of the extracts (DPPH, R^2 = 0.8508; FRAP, R^2 = 0.5324; Lipid peroxidation inhibition, R^2 = 0.467). The findings of the present study indicates that this medicinal plant with high antioxidant property might prove to be a natural source of a very potent anticancer agent and should further be investigated for novel drug discovery.

Keywords: *Scurrula parasitica* L., Antioxidant activity, DPPH, FRAP, Total phenol content, Ascorbate-Iron(III) induced Lipid peroxidation inhibition

INTRODUCTION

Medicinal plants have become one of the important topics in the recent researches related to human health because of their therapeutic potentials as antioxidants. These compounds play an important role, preventing free radical induced diseases such as cancer and atherosclerosis[1]. Antioxidants can scavenge free radicals produced due to environmental pollutants, radiations, chemicals, toxins, deep fried and spicy foods as well as physical stress, thereby preventing harmful effects on DNA, intracellular proteins, membrane lipids, and change in gene expression and can raise the level of endogenous antioxidant defense. There is dynamic balance between the amount of free radicals generated in the body and antioxidants against their deleterious effects[2]. However, the amounts of these protective antioxidant principles present under the normal physiological conditions are sufficient only to cope with the physiological rate of free radical generation. It is therefore obvious that any additional burden of free radicals, either from environment or produced within the body can tip the free radical (pro-oxidant) and anti free radical (antioxidant) balance leading to oxidative stress; which may result in tissue injury and subsequent diseases[3]. In order to maintain the level of antioxidant in the body, for healthy living, external supplementation is necessary. Supplementation of natural antioxidants through a balanced diet could be more effective and also more economical than the supplementation of an individual antioxidant, such as vitamin C or vitamin E, in protecting the body against oxidative damage under various conditions[4]. So the interest on medicinal plants focused not only on the discovery of new biologically active molecules by the pharmaceutical industry, but also on the adoption of the crude extract of the plants, such as infusions, for self medication by general public[5].

Scurrula parasitica L. is a medicinal plant grown as parasite on a wide range of hosts, including species of Apocynaceae, Euphorbiaceae, Fabaceae, Fagaceae, Lythraceae, Moraceae, Punicaceae, Rosaceae, Rutaceae, Sapindaceae, Theaceae, and Ulmaceae. Polysaccharides isolated from the leaves of *S. parasitica* L. was found to inhibit cancer cell proliferation and promote cancer cell apoptosis by down-regulating the expression of Ki-67, CyclinD1 and Bcl-2 protein, and up-regulate the expression of Bax protein[6] (Xiao *et al*., 2010). Flavonoid extracts of *S. parasitica* L. parasitizing on *N. indicum* are reported to exhibit anticancer activity on human leukemia cell line HL-60[7] (Xiao *et al*., 2008). Many animal studies indicate that antioxidants may slow or possibly prevent the development of cancer. Keeping in view of the above points, this study has been undertaken to find out the antioxidant activities of different extracts of *S. parasitica* L. leaves by DPPH (1,1-diphenyl -2-picrylhydrazyl) free radical scavenging assay, FRAP (Ferric Reducing Antioxidant Potential) assay, total phenol content (TPC) estimation and to correlate with their ability to inhibit Ascorbate-Iron(III) induced lipid peroxidation.

MATERIALS AND METHODS

Plant extracts

S. parasitica L. (both matured and tender) leaves were collected from forests of Mizoram and were air dried in shade and powdered separately. One gram each of the powdered materials was then soaked separately in 40 ml deionised water and methanol and kept for 48 hr with intermittent shaking. The extracts were filtered and the crude extracts thus obtained were diluted 10 times and used for estimation of antioxidant activities and total phenolic content.

Chemicals and reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), 6-hydroxy-2,5,7,8–tetramethyl chromane-2-carboxylic acid (Trolox), Gallic acid, Bovine brain extract (Sigma, B-3635) and Thiobarbituric acid (TBA) were purchased from Sigma Chemicals Co. (St. Louis, USA); Methanol, Ethanol, Sodium acetate trihydrate, ferric chloride hexahydrate ($FeCl_3$. $6H_2O$), Folin-Ciocalteu Phenolic reagent, Sodium carbonate were obtained from Merck (Darmstall, Germany). Trichloroacetic acid (TCA) was obtained from Sisco Research Laboratories (SRL), Mumbai. All the chemicals used were of analytical grade.

DPPH free radical scavenging assay

The free radical scavenging activity was measured by the 1,1-Diphenyl-2-picrylhydrazyl (DPPH) method proposed by Leong and Shui[8]. DPPH solution of 0.1 mM was prepared in methanol and the initial absorbance was measured at 517 nm. 40 µl of extract was added to 3 ml of DPPH solution and the decrease in absorbance was measured at different time intervals until the absorbance remained constant. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity, and vice versa. A standard curve was prepared using trolox (250 -1250µg/ml) and the free radical scavenging ability of the extracts were expressed as mg Trolox equivalent (TE) per gram of dry leaves.

Ferric Reducing Antioxidant Potential (FRAP) assay

The FRAP assay was carried out according to the procedure of Benzie and Strain[9]. Briefly, 50µl of extract was added to 3 ml of FRAP reagents (10 parts of 300 mM sodium acetate buffer of pH 3.6, 1 part of TPTZ and 1 part of 20 mM Ferric chloride solution). The reaction mixture was incubated at 37^{0}C for 30 min and the increase in absorbance was measured at 593 nm using Spectroscan 2600 UV/Vis Spectrophotometer (Chemito). The standard curve was prepared using trolox (250 -1000µg/ml) and the results were expressed as mg Trolox equivalent (TE) per gram of dry leaves.

Total phenolic content (TPC)

The total phenolic content of the extracts were estimated by the Folin-Ciocalteau method[10]. One hundred (100) microlitres of extract was added to 1ml of 1:10 Folin-Ciocalteau's reagent and incubated at room temperature for 5 min followed by addition of 900 µl of sodium carbonate (7.5%) solution. After 1 hr incubation at room temperature, the absorbance was measured at 640 nm using Spectroscan 2600 UV/Vis Spectrophotometer (Chemito). Different volume (20-100µl) of Gallic acid (100µg/ml) was used for calibration of a standard curve. The results were expressed as mg Gallic acid equivalent (GAE) /gm of dry leaves.

Ascorbate-Iron (III) Induced Lipid Peroxidation Inhibition

The assay was performed as described by Aruoma and coworkers[11] with slight modification. Briefly, 10 mg of bovine brain extract was mixed with 2 ml of phosphate buffer saline (PBS, pH 7.4) and sonicated in an ice bath until a milk-like suspension was obtained containing phospholipids liposomes. The liposome (0.2ml) was mixed with 0.1ml of 1mM $FeCl_3$ and 0.1ml of extract (1:10 dilution) and the volume was made up to 0.5 ml with PBS buffer. The peroxidation was initiated by adding 0.1ml of 1mM ascorbate. The mixture was incubated at 37^{o}C for 60 minutes. After incubation, 1ml of 10% trichloroacetic acid was added and centrifuged at 1800 rpm for 10 minutes. After centrifugation, 1ml of supernatant was collected and mixed with 1ml of 0.67% thiobarbituric acid (TBA). The mixture was vortexed and heated in boiling water bath at 100^{o}C for 20 minutes and then rapidly cooled and the extent of oxidation inhibition was estimated from the absorbance of the organic layer at 532nm. A tube containing all the reaction mixture except the plant extract was used as control. The percent inhibition was calculated with the formula:

$$\text{Percent inhibition (\%)} = \frac{\text{(Abs control – Abs sample) X 100}}{\text{Abs control}}$$

Statistical analysis

Data on all the assays were the average of triplicate analyses and presented as mean± standard deviation (SD). Correlation analysis of DPPH, FRAP and total phenolic content assay of the extracts with their ability to inhibit Ascorbate-Iron (III) induced lipid peroxidation was carried out using the correlation and regression programme in the Microsoft EXCEL program.

RESULTS

Both matured and tender leaves of *S. parasitica* L. exhibited a high antioxidant activity as estimated by the four different *in vitro* assays. The antioxidant activity of the extracts based on DPPH free radical scavenging and FRAP assay are expressed as mg TE whereas TPC of the extracts are expressed as mg GAE per gram of the dry sample. The ability of the extracts (1:10 dilution) to inhibit Ascorbate-Iron (III) induced lipid peroxidation inhibition is expressed as percent inhibition. The antioxidant activity of the different extracts is presented in Table 1.

Table 1: Antioxidant activities of aqueous and methanolic extracts of matured and tender leaves of *Scurrula parasitica* L.

Plant material	Extracts	DPPH assay(mg TE / gm	FRAP assay(mg TE / gm	Total Phenolic contents (mg of GAE/ gm	Ascorbate-Iron Induced Lipid Peroxidation (% inhibition with 1:10 diluted extract)
Matured	Aqueous	177.17±5.59	106.99±3.23	26.48±1.20	52.09±0.72
leaves	Methanolic	190.51±6.54	134.92±8.30	36.57±3.50	51.17±1.05
Tender	Aqueous	145.39±2.03	90.17±6.76	22.84±0.33	47.39±2.89
leaves	Methanolic	144.96±5.27	93.57±8.61	21.77±1.41	47.59±0.84

In all the assays, extracts of matured leaves was found to exhibit higher activity than that of tender leaves. Water and methanolic extract of 1gm dry matured leaves showed 177.17 and 190. 51 mg TE respectively which is much higher than 145.39 and 144.96 mg TE/gm of dried tender leaves in DPPH assay. In FRAP assay, the aqueous and metabolic extracts of the mature leaves shows antioxidant activity of 106.99±3.23 and 134.92±8.30 while the tender leaves have 90.17±6.76 and 93.57±8.61 respectively. Similarly the total phenolic content of the mature leaves in aqueous and methanolic extracts were 26.48±1.20 and 36.57±3.50 while in tender leaves the observed values were 22.84±0.33 and 21.77±1.41 respectively. The percent inhibition caused by the extracts in lipid peroxidation didn't vary much though it was higher in case of matured leaves and it was found to have a high correlation (R^2= 0.8508) with their DPPH free radical scavenging ability (Fig. 1).

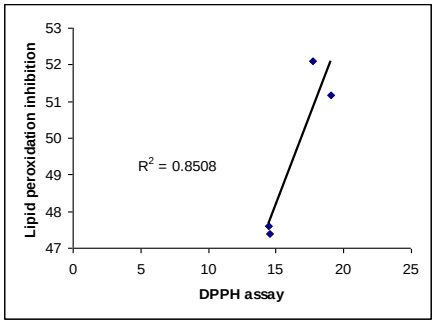

Fig. 1: Correlation between ability of the extracts to scavenge the DPPH free radical and to inhibit Ascorbate-Iron(III) induced lipid peroxidation.

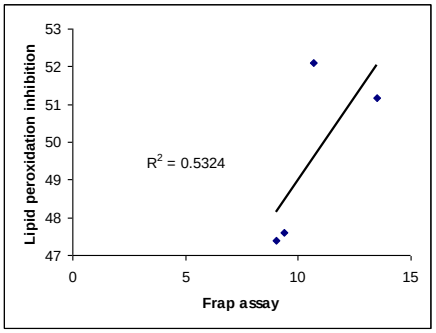

Fig. 2: Correlation between ability of the extracts to reduce ferric ions and to inhibit Ascorbate-Iron(III) induced lipid peroxidation.

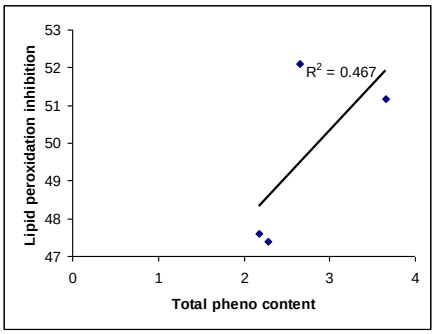

Fig. 3: Correlation between total phenol content of the extracts and their ability to inhibit Ascorbate-Iron(III) induced lipid peroxidation

The ability of the extracts to inhibit lipid peroxidation was also found to have positive correlation with ferric reducing potential (R^2= 0.5324) and total phenolic content (R^2= 0.467) as shown in Figure 2 & 3.

DISCUSSION

There is increasing evidence indicating that reactive oxygen species (ROS) and free radical mediated reactions are involved in degenerative or pathological events such as aging, cancer, coronary heart ailments and Alzheimer's disease[12]. The health promoting effect of antioxidants from plants is thought to arise from their counteracting effect on DNA/protein damage and lipid peroxidation caused by reactive oxygen species. Plants synthesize antioxidant compounds, as secondary products, which are mainly phenolics which exhibit their antioxidant activities through a number of different mechanisms[13]. Considering the chemical complexity of phytochemicals in plant extracts, an approach with multiple assays for evaluating antioxidant activities of extracts would be more enlightening and even indispensable. In this study, four antioxidant activity assays, DPPH free radical scavenging activity, Ferric Reducing antioxidant potential, total phenol content and Ascorbate-Iron (III) Induced Lipid peroxidation inhibition assay were employed.

Substances which are able to donate hydrogen or an electron to DPPH, nitrogen centered free-radical leading to formation of non radical DPPH-H, can be considered as antioxidants and therefore radical scavengers. The degree of discoloration of violet colour of alcoholic DPPH, as it gets reduced, indicates the radical scavenging potential of the antioxidant[14]. The extracts were found to reduce the colour of DPPH as soon as it was added and therefore had to be diluted 10 times before repeating the same experiment. It was an indicative of a very high ability of the extracts to scavenge the DPPH free radicals and was confirmed by the values ranging from 144.96 to 190.51 mg TE/gm of dry leaves in DPPH assay. This high free radical scavenging ability could be related to the anticancer property of this plant on human cancer cell line, HL-60[7] (Xiao *et al*., 2008) as free radicals are associated with cancer initiation.

The ability of the extracts to reduce ferric-TPTZ (Fe(III)-TPTZ) to blue ferrous-TPTZ (Fe(II)-TPTZ) was also evaluated. Fe(III) reduction is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant reaction[15]. A higher absorbance indicates a higher ferric reducing power. Like the findings of DPPH free radical scavenging assay, methanolic extracts of matured leaves exhibited highest value of 134.92 mg TE/gm and the lowest value 90.17 mg TE/gm by aqueous extract of tender leaves. Many literatures have cited that the phenolics are one of the antioxidants commonly found in most plants. Generally, extracts that contain a high amount of polyphenols also exhibit high antioxidant activity[16]. However, in the present study it was found that total phenols expressed as mg GAE/gm of dry leaves was extremely low in comparison to that of DPPH and FRAP assay. This might be due to the presence of other compounds showing activity indicating that the antioxidant activities of plant extracts are not limited to only phenolics. The activity may also come from the other antioxidant secondary metabolites, such as volatile oils, carotenoids and vitamins[17]. The diluted extracts were also checked for its ability to inhibit lipid peroxidation in bovine brain extract. Lipid peroxidation is one of the major causes of deterioration in foods that results in the formation of potentially toxic compounds. In human, lipid oxidation is also thought to induce physiological obstruction, causing aging of the cells and carcinogenesis[18,19]. The percent inhibition exhibited by the extracts of matured leaves was negligibly higher than of tender leaves as indicated by the values falling in the range 47.39- 52.09 %.

In correlation analysis, the DPPH free radical scavenging activity of the extracts was found to be highly correlated with their ability to inhibit lipid peroxidation (R^2 = 0.8508). However, correlation between lipid peroxidation inhibition and ferric reducing potential (R^2 = 0.5234) was medium and with total phenol content was lower (R^2 = 0.467). From this result, it can be hypothesized that the ability to scavenge free radicals and inhibit lipid peroxidation might be contributed by the same or related compounds which are different from those ferric reducing and phenolic compounds.

CONCLUSION

The first conclusion that can be drawn from this study is that leaves of *Scurrula parasitica* L. possess high antioxidant activity and these activities are higher in matured leaves than the tender ones. Secondly, the methanolic extracts exhibit higher antioxidant potential and should be investigated further for novel and natural anticancer drug discovery.

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