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PHYTOCHEMICAL SCREENING AND *IN VITRO* EVALUATION OF ANTIOXIDANT ACTIVITY OF VARIOUS EXTRACTS OF *SCURRULA PARASITICA L.*

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ABSTRACT

Scurrula parasitica is a parasitic shrub belonging to the family Loranthaceae growing on Holoptelia integrifolia was collected from Western Ghats of Karnataka. The present study was designed to investigate the phytochemicals and antioxidant activities of petroleum ether, chloroform, ethyl acetate and methanol extracts. Phytochemical screening revealed the presence of phytoconstituents such as alkaloids, flavonoids, saponins, tannins, phenolic compounds, sterols, terpenoids, glycosides and carbohydrates. The total phenolic content of 30, 21, 38 and 42 µg of GAE in 100 µg of petroleum ether, chloroform, ethyl acetate and methanol extracts respectively. Analysis of the antioxidant activities of all the extracts revealed a concentration dependent activity, which increased gradually with increasing concentration of the extracts.

KEY WORDS

Antioxidant activity, phytochemicals, Scurrula parasitica, total phenols.

INTRODUCTION

Traditional knowledge has helped the recent decade regarding the therapeutic properties of plants [1]. Medicinal plants are used for curing many diseases as they contain phytochemicals of various biological activities [2,3]. Phenolic compound is an important phytochemical which possess anti-inflammatory, antiallergic, antithrombotic, antimicrobial and anticancerous activities [4,5,6].

Free radicals are molecules which contain one or more unpaired electrons spinning around the nucleus, when they are produced in large amount they are called as oxidative stress/ oxidative damage and they cause many diseases like atherosclerosis, diabetes, cancer, aging and neurodegenerative disorders [7,8]. A substance which are effective against oxidative damage caused by this free radicals in human body are antioxidants [9,10]. As the synthetic antioxidants are carcinogenic and less effective [11], the antioxidants of plant origin are important in functional foods, nutraceutical, dietary, cosmetic, research and pharmaceutical areas [12,13]. The phytochemical compounds obtained from plants can be altered synthetically to enhance their therapeutic properties and this may give a lead in the development of new potential drugs [14,15].

Scurrula species were used in Indonesia and Java to treat cancer and as an infusion for fatigue. The stems and leaves of *S. atropurpurea* are used for the treatment of cancer [16] and *S. ferruginea* has antibacterial activity [17] and is used for the treatment of gastrointestinal complaints, hypertension, ulcer and cancer [18,19]. *S. parasitica* has anticancerous and analgesic activities [20,21]. The present study was undertaken to screen the phytochemical constituents and antioxidant activity of *Scurrula parasitica*.

MATERIALS AND METHODS

Collection of Plant materials

S. parasitica growing on *Holoptelia integrifolia* was collected from Western Ghats of Karnataka. The collected plant was brought to the laboratory and disinfected. The herbarium specimens were prepared and deposited in the Herbarium of Department of Studies in Botany, University of Mysore, Manasagangotri, Mysore. The collected plant was identified with the help of Flora of Presidency of Madras [22].

Preparation of Extracts

The *S. parasitica* (leaves) was washed under running tap water and air dried under shade at room temperature. The dried leaves were then crushed

into fine powder (in blender) and stored in an air tight container until further use. A total of 40 gm dried leaf powder was successively extracted with 250 ml each of organic solvents in the following sequence, *viz*., petroleum ether, chloroform, ethyl acetate and methanol using Soxhlet apparatus. After complete extraction, respective solvent extracts were evaporated under reduced pressure and the dried extracts thus obtained were stored in air tight vials at 4° C for further studies [23].

Phytochemical screening

The extracts were subjected to qualitative phytochemical screening for identification of various classes of active phytochemical constituents using the methods described by Harborne [24] and Trease and Evans [25].

Determination of Total Phenolic Content

The total phenolic content in the petroleum ether, chloroform, ethyl acetate and methanol extracts of *S. parasitica* was determined by following the method of Singleton *et al*. [26]. A stock solution of extract (5 mg/ ml) was prepared and from this 20 µl of the extract (1 mg/ ml) was mixed with 0.75 ml of 20% sodium carbonate solution and 0.25 ml of Folin-Ciocalteu reagent. The reaction mixture was allowed to stand in light for 3 min and incubated for 2 h in dark. In the presence of Folin-Ciocalteu reagent the change in the colour of the solution holding the extracts was measured using spectrophotometer at 765 nm using UV-Visible Spectrophotometer. Total phenolics were quantified by calibration curve obtained from measuring the absorbance of known concentrations of Gallic acid standard (0- 100 µg/ ml). Total phenolic content were expressed as µg of Gallic Acid Equivalent (GAE) in 100 µg of plant extract. The experiment was repeated thrice with three replicates each.

Antioxidant Activity

DPPH (2, 2- diphenyl-1-picrylhydrazyl) Scavenging Activity

The free radical scavenging activity of petroleum ether, chloroform, ethyl acetate and methanol extracts of *S. parasitica* was determined by DPPH method [27]. The stock solution was prepared by dissolving 1 mg of petroleum ether, chloroform, ethyl acetate and methanol extracts in 1 ml of respective solvents (1 mg/ ml). The reaction mixture contained 5 µl of plant extract and 95 µl of DPPH (300 µM) in methanol. Different concentrations (100- 1000 µg/ ml) of test sample were prepared, while the concentration of DPPH remained same. These reaction mixtures were incubated at 37º C for 30 min and the absorbance was measured at 517 nm. Per cent DPPH radical scavenging activity upon sample treatment was determined by comparison with a methanol control. Ascorbic acid was used as a positive control. The experiment was repeated thrice with three replicates each. The per cent radical scavenging activity (RSA) was calculated using the formula below.

$$\% \text{ DPPH scavenging activity} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Nitric oxide Scavenging Activity

Nitric oxide radical scavenging activity of petroleum ether, chloroform, ethyl acetate and methanol extracts of *S. parasitica* was carried out as described by Marcocci *et al*. [28] with slight modifications. One millilitre of sodium nitroprusside (5 mM) in 0.5 M phosphate buffer was mixed with 3 ml of different concentrations (100- 1000 µg/ ml) of the plant extracts, incubated at 25º C for 150 min and the absorbance was measured at 546 nm. Ascorbic acid was used as a positive control. The experiment was repeated thrice with three replicates each. The capability to scavenge NO radical was calculated using the formula below.

$$\% \text{ NO scavenging activity} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Hydrogen peroxide Scavenging Activity

Hydrogen peroxide scavenging activity of petroleum ether, chloroform, ethyl acetate and methanol extracts of *S. parasitica* was carried out as described by Czochra and Widensk [29]. Hydrogen peroxide (40 mM, 0.6 ml) solution in phosphate buffer (pH 7.4) was mixed with different concentrations (100- 1000 µg/ ml) of the plant extracts (2.4 ml), incubated for 10 min and the absorbance was measured at 230 nm. Ascorbic acid was used as a positive control. The experiment was repeated thrice with three replicates each. The per cent hydrogen peroxide scavenging activity was calculated using the formula below.

$$\% H_2O_2 \text{ scavenging activity} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Total antioxidant capacity by Phosphomolybdenum Method

Total antioxidant capacity of petroleum ether, chloroform, ethyl acetate and methanol extracts of *S. parasitica* (1 mg/ ml) was evaluated by phosphomolybdenum method according to Prieto *et al*. [30]. Plant extracts (0.3 ml) at different concentrations (100- 1000 µg/ ml) was mixed with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixtures were incubated at 95º C for 90 min and the absorbance was measured at 695 nm. Ascorbic acid was used as a standard. The experiment was repeated thrice with three replicates each.

Reducing Power Assay

Reducing power estimation of petroleum ether, chloroform, ethyl acetate and methanol extracts of *S. parasitica* was carried out as described by Nagulendran *et al*. [31] with slight modifications. Petroleum ether, chloroform, ethyl acetate and methanolic plant extracts solution [0.75 ml from the stock solution (1 mg/ ml)] was mixed with 0.75 ml of 0.2 M phosphate buffer (pH 6.6) and 0.75 ml of 1% potassium ferricyanide and incubated at 50º C for 20 min. Then, 0.75 ml of 10% trichloroacetic acid was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 1.5 ml of the supernatant solution was mixed with 1.5 ml of distilled water and 0.5 ml of 0.1% $FeCl_3$. Absorbance was measured at 700 nm in UV- Visible Spectrophotometer using phosphate buffer as a blank and butylated hydroxytoluene (BHT) as a standard. The experiment was repeated thrice with three replicates each.

Statistical Analysis

Data from three replicates were analyzed for each experiment and analysis of variance (ANOVA) using SPSS Inc. 16.0. Significant effects of treatments were determined by F values ($P \leq 0.05$). Treatment means were separated by Tukey's Honestly Significant Differences (HSD) test.

RESULTS

Phytochemical analysis

The methanol extract of *S. parasitica* revealed the presence of saponins, alkaloids, tannins, phenolic compounds, sterols, flavonoids, glycosides and carbohydrates and absence of terpenoids, proteins, amino acids, fixed oils and fats. ethyl acetate extract contained only saponins, alkaloids, sterols, flavonoids, glycosides and carbohydrates. Alkaloids and carbohydrates were present in both chloroform and petroleum ether extracts, saponins and sterols were present only in petroleum ether extract while terpenoids was present only in chloroform extract. Proteins, amino acids, fixed oils and fats were absent in all the four solvent extracts of the plant **(Table 1)**.

Table 1: Qualitative Phytochemical Analysis of *S. parasitica* extracts

Phytochemical compounds	Extracts Petroleum ether	Chloroform	Ethyl acetate	Methanol
Saponins				
a. Foam Test	++	--	++	++
Alkaloids				
a. Wagner's Test	++	++	++	++
b. Dragendorff's Test	--	--	--	++
c. Mayer's Test	--	--	--	++
d. Hager's Test	--	--	++	++
Tannins and Phenolic compounds				
a. Ferric chloride Test	--	--	--	++
b. Gelatin Test	--	--	--	--
Sterols				
a. Libermann-Burchard's Test	++	--	++	++
Terpenoids				
a. Libermann-Burchard's Test	--	++	--	--
Anthraquinones	--	--	--	--
Flavonoids				
a. Ferric Chloride Test	--	--	--	--
b. Alkaline reagent Test	--	--	++	++
Cardiac glycosides				
a. Keller-Killani Test	--	--	--	--
Glycosides				
a. Borntrager's Test	--	--	--	--
b. Baljet Test	--	--	++	++
Proteins and Amino acids				
a. Ninhydrin Test	--	--	--	--
b. Biuret Test	--	--	--	--
Carbohydrates				
a. Benedict's Test	--	--	++	++
b. Fehling's Test	++	++	++	++
Fixed Oils and Fats				
a. Saponification Test	--	--	--	--

++ : Present; -- : Absent

Determination of total phenolic content

The total phenolic content in the petroleum ether, chloroform, ethyl acetate and methanol extracts of *S. parasitica* was determined as Gallic Acid Equivalent (GAE) and expressed as µg of GAE in 100 µg of plant extracts. Among the four extracts, 100 µg of methanol extract had highest phenolic content of 42 µg of GAE followed by 38, 30 and 21 µg of GAE in 100 µg of ethyl acetate, petroleum ether and chloroform extracts respectively.

Antioxidant activity

DPPH, Nitric Oxide and Hydrogen Peroxide Scavenging Activity

The various extracts of *S. parasitica* was subjected for DPPH activity and it revealed that methanol (110.91 µg/ ml) > petroleum ether (115.39 µg/ ml) > ethyl acetate (199.36 µg/ ml) > chloroform (222.41 µg/ ml). In nitric oxide and hydrogen peroxide activity, among the extracts the highest activity was shown by petroleum ether extract (105.79 and 108.17 µg/ ml) followed by methanol (110.76 and 115.55 µg/ ml), ethyl acetate (120.94 and 198.72 µg/ ml) and

chloroform (211.95 and 304.13 µg/ ml) respectively. Ascorbic acid standard showed highest activity when compared to extracts in all the activity **(Figure 1, 2 and 3)**.

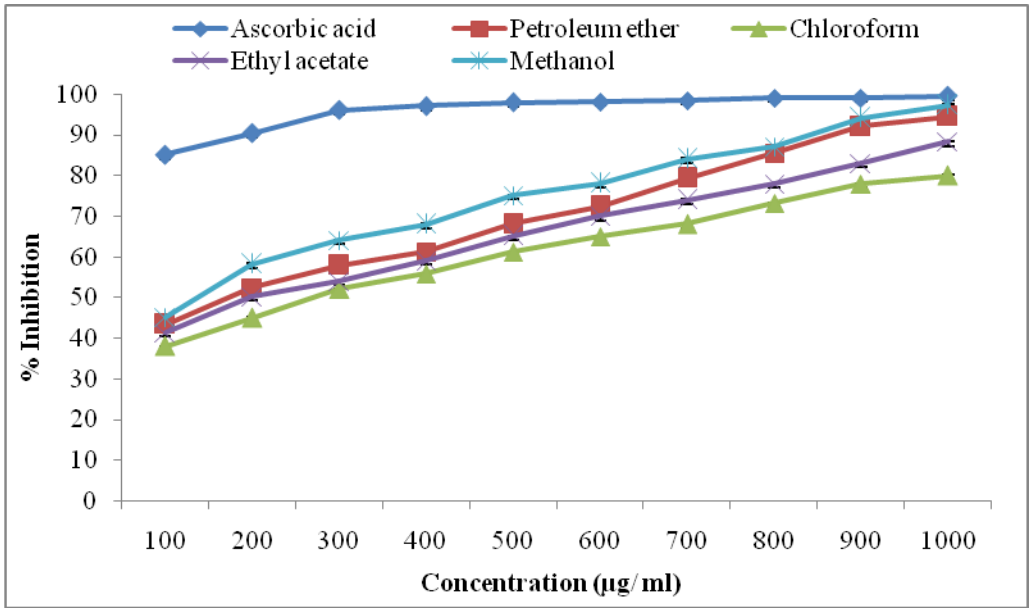

Fig. 1: DPPH scavenging activity of different solvent extracts of *S. parasitica*. Values are mean of three independent replicates. ± indicate Standard Error

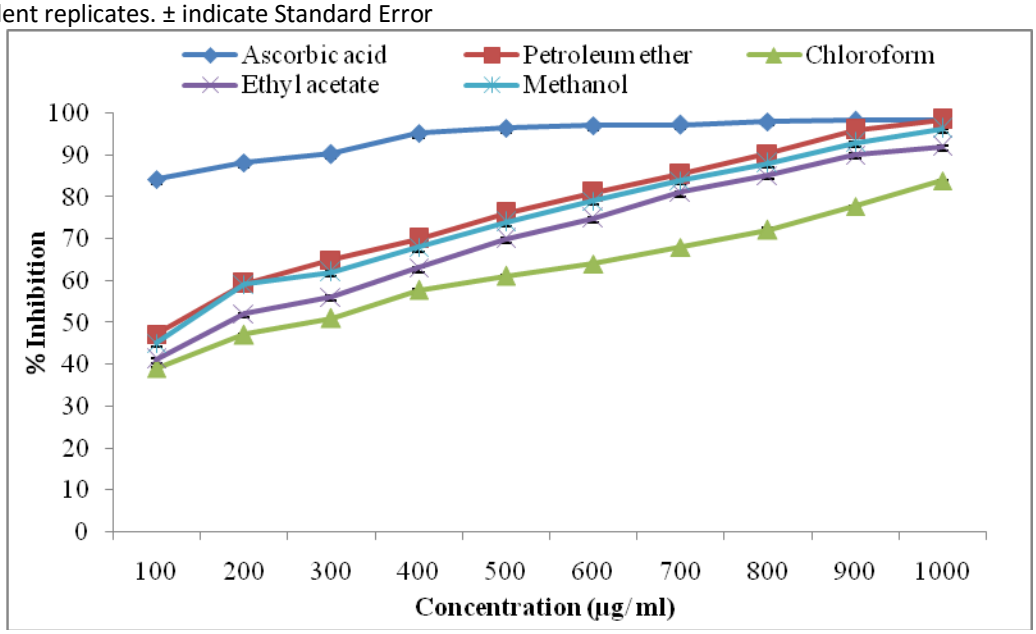

Fig. 2: Nitric oxide scavenging activity of different solvent extracts of *S. parasitica*. Values are mean of three independent replicates. ± indicate Standard Error

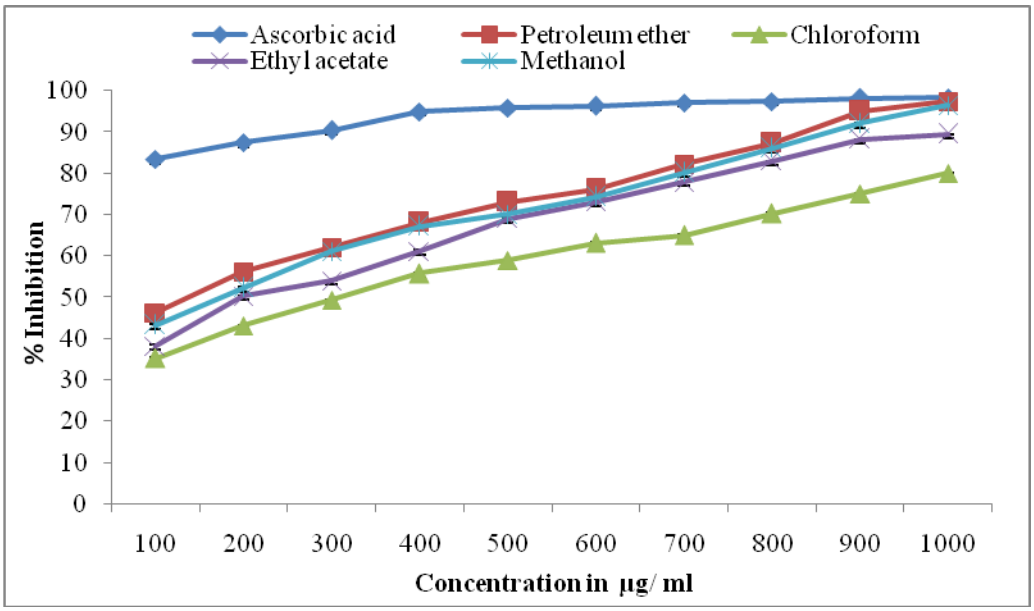

Fig. 3: Hydrogen peroxide scavenging activity of different solvent extracts of *S. parasitica*. Values are mean of three independent replicates. ± indicate Standard Error

Total antioxidant capacity by Phosphomolybdenum Method and Reducing Power Assay

The total antioxidant capacity and reducing power ability of *S. parasitica* leaves in different solvent extracts is shown in **Figure 4 and 5**. Methanol extract showed highest total antioxidant capacity and reducing power ability with an increase in absorbance from 0.152 at 100 µg/ ml to 0.449 at 500 µg/ ml and 0.765 at 100 µg/ ml to 1.817 at 500 µg/ ml respectively compared to ethyl acetate, chloroform and petroleum ether.

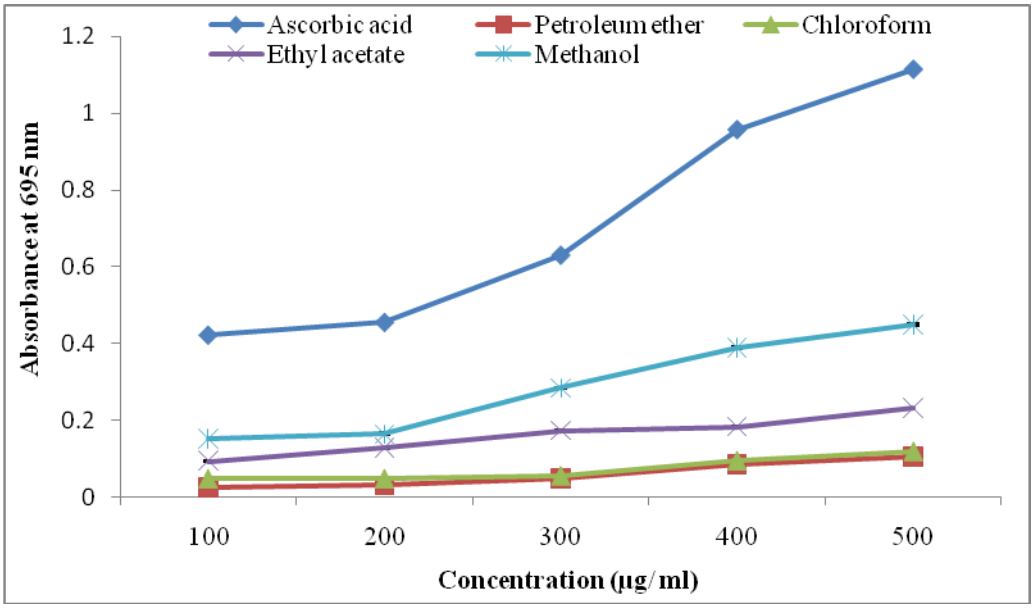

Fig. 4: Total antioxidant capacity of different solvent extracts of *S. parasitica*. Values are mean of three independent replicates. ± indicate Standard Error.

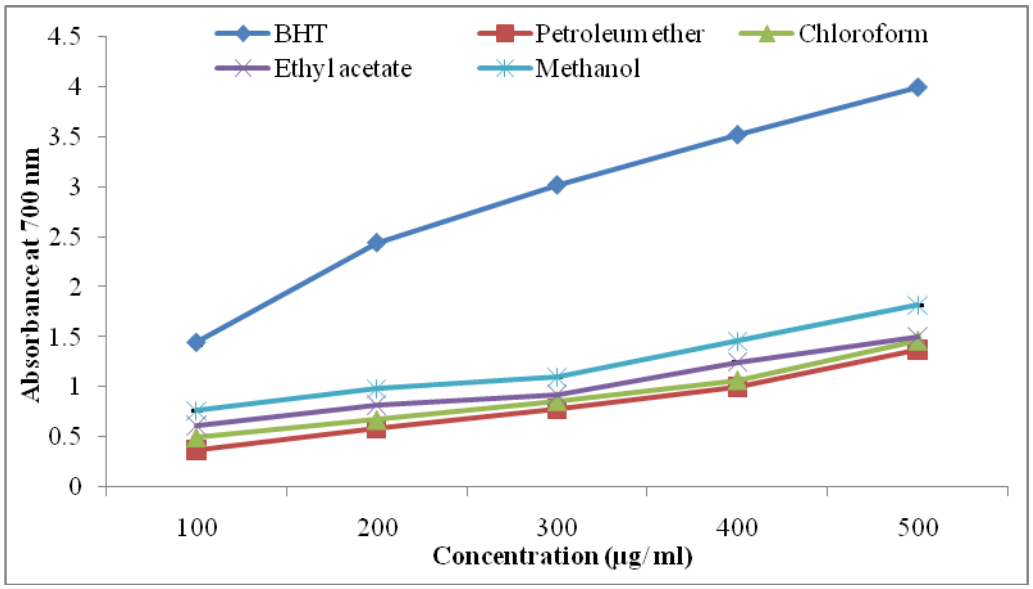

Fig. 5: Reducing power assay of different solvent extracts of *S. parasitica*. Values are mean of three independent replicates. ± indicate Standard Error.

DISCUSSION

The present study on phytochemical screening of the leaves extracts of *S. parasitica* revealed the presence of saponins, alkaloids, tannins, phenolic compounds, sterols, terpenoids, flavonoids, glycosides and carbohydrates. Phytochemicals have the ability to cure many diseases due to their medicinal properties [32]. Saponins have antibiotic, chlolesterol lowering, cardiac depressant and hypotensive properties and they are also used to treat hyperglycaemia [33,34]. Among the phytochemicals alkaloids are the largest group, they have the capability to reduce pain in humans such as headache and they have antimicrobial activity [35]. Terpenoids have anticancerous, anti-inflammatory, antimalarial, antibacterial and antiviral activities [36]. It has been reported that tannins have antimicrobial, astringent and haemostatic properties and they are used for healing burns, ulcers and wounds [37]. Tannin is a phenolic compound and plant phenols are important due to their free radical scavenging ability so they contribute to the antioxidant activity [38]. Flavonoids have analgesic, antipyretic, spasmolytic, antiallergic, anti-inflammatory, antimicrobial and antioxidant activities [39,40].

The results of the present investigation showed that the antioxidant activity was dose dependent and enhanced with the increase in concentration of the extracts. Similar reports on increased antioxidant activity with increased extract concentration have been reported from other parasites such *Viscum nepalense* [41], *Dendrophthoe trigona* [42], *Taxillus cuneatus* [43], *T. tomentosus* [44], *H. elastica* [45] and *T. dodoneifolius* [46].

It is known that during oxidative stress and exposure to radiation, excessive free radicals are produced that cause damage to the biomolecules [47]. Redox properties of phenolic compounds are responsible for antioxidant activity that take part in absorption and neutralization of free radicals, quenching singlet and triplet oxygen or decomposing peroxides [48]. In this regard, polyphenolic compounds like flavonoids and phenolic acids in plants have been reported to comprise multiple biological effects, together with an antioxidant activity [49]. In the present study an attempt was made to measure the antioxidant activity of all the solvent extracts of *S. parasitica* using five different methods.

The method used to evaluate the ability of the plant extracts to scavenge free radicals is the DPPH free radical scavenging assay [50]. Nitirc oxide is a smallest and most diffusible signal molecule synthesized in major group of organisms [51]. It is active molecule concerned with diverse biological pathways. Nitric oxide is known to contribute for negative and positive effects depending on the concentrations reached in the diseases [52]. Hydrogen peroxide is a weak oxidizing agent that rapidly crosses the cell membrane and reacts with Fe^{2+} and possibly Cu^{2+} ions to form the damaging toxic hydroxyl radical [53]. The ability of the extracts to scavenge hydrogen peroxide is due to their phenolics, which donate electrons to hydrogen peroxide, thus

neutralizing it to water [54]. The total antioxidant activity of many medicinal plants depends on the presence of polyphenols and flavonoids of plants [55]. The reducing ability of the plant is due to the presence of reductones which break the free radical chain by donating a hydrogen atom and result in the antioxidant activity [56].

The results obtained in the present study suggest that leaves extracts of *S. parasitica* could be used as a potential source of drugs. Henceforth considering the data on total phenolic content and antioxidant activity, further studies to isolate the bioactive principles implicated in biological activity is necessary.

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