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Investigation of Antioxidant Potential of Methanolic Extract of *Swertia chirata* Buch. Ham.

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Authors' contributions

This work was carried out in collaboration between all authors. Authors LA and AM designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors MKD and VB managed the analyses of the study. Author SS managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: To evaluate the *in vitro* antioxidant potential and total phenolic contents of the methanolic extract of *Swertia chirata*.
Place and Duration: Department of Botany, Dr. H. S. Gour University (HSGVV), Sagar, between February 2010 to July 2010.
Methodology: The plant material (aerial part) was subjected to defatting with petroleum ether then successively extracted with methanol. Total phenolic contents of methanolic extract was determined using the Folin-Ciocalteau reagent method while *in vitro* antioxidant potential was evaluated by using DPPH, hydroxyl radical, nitric oxide radical scavenging as well as ferric reducing power assays.
Results: The total phenolic content in 1 mg of methanolic extract of *S. chirata* was equivalent to 4.5 µg Catechol. The IC_{50} value of the DPPH method, hydroxyl radical and nitric oxide radical scavenging activity was 222.74±0.19, 307.93±0.10 and 870.55±0.20

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µg/ml respectively. When these IC_{50} value compared with that of standard drug Butylated hydroxy anisole (BHA) the result obtained was as follows: FRSA-SCM>BHA (p=0.000246); HRSA-SCM<BHA (p=0.000507); NORA-SCM<BHA (p=2.22614). These results showed that the *S. chirata* methanolic extract exhibited significant free radical DPPH scavenging activity and Hydroxyl radical scavenging activity while it exhibited non-significant Nitric oxide radical scavenging activity. In ferric reducing power various concentrations (100, 250 and 500 µg/ml) of methanolic extract of *S. chirata* showed absorbance 0.013±0.31, 0.156±0.12 and 0.298±0.14. Phytochemical screening showed the presence of phenolic compounds such as flavonoids and tannins which may be responsible for the activity.

Conclusion: Methanolic extract of *Swertia chirata* showed significant antioxidant activity which suggest the extract may act as a natural antioxidant agent offering effective protection from free radicals.

Keywords: Antioxidant; DPPH; ferric reducing power; Swertia chirata; total phenolic content.

1. INTRODUCTION

Oxygen is essential for the survival of all on this earth. During the process of oxygen utilization in normal physiological and metabolic processes approximately 5% of oxygen gets univalently reduced to oxygen derived free radicals like superoxide, hydrogen peroxide, hydroxyl and nitric oxide radicals [1,2]. Free radicals are very detrimental in attacking lipids in cell membranes and DNA. They induce oxidations that cause DNA mutation leading to cancer and membrane damage such as membrane lipid peroxidation and a decrease in membrane fluidity [3,4]. Free radicals and oxidants activate nuclear factor-κB, a nuclear transcription factor, resulting in an upregulation of pro-inflammatory mediators such as interleukin-1, interleukin-8 and tumor necrosis factor-α [5]. This in turn stimulates the immune response; increases oxidant production and can lead to further tissue damage. Free radicals stress leads to tissue injury and progression of disease conditions, such as arthritis, hemorrhagic shock, atherosclerosis, diabetes, hepatic injury, aging and ischemia. The production of free radicals is inextricable linked to the inflammatory process. Free radicals prime the immune response, recruit inflammatory cells and are innately bactericidal [5,6].

Scavengers of free radicals are known as antioxidants. Besides playing an important role in physiological systems, antioxidants have been used in the food industry to prolong the shelf life of foods, especially those rich in polyunsaturated fats. These components in foods are readily oxidised by molecular oxygen, which is a major cause of quality deterioration, loss of nutritional value, off-flavour development and discolouration. The addition of synthetic antioxidants has been widely used industrially to control lipid oxidation in foods. However, the use of these synthetic antioxidants has been questioned due to their potential health risks and toxicity [7]. The consumption of fruits and vegetables containing antioxidants has been found to offer protection against these diseases. Dietary antioxidants can augment cellular defenses and help to prevent oxidative damage to cellular components [8]. The search for antioxidants from natural sources has received much attention and efforts have been made to identify compounds from natural sources to replace synthetic ones. Medicinal plants are a natural source of a wide variety of antioxidant molecules [9].

Swertia chirata Buch. Ham. is a herb commonly known as chirata and belongs to the family Gentianaceae. The plant is a native of temperate Himalayas, found at an altitude of 1200–3000 m (4000 to 10,000 ft), from Kashmir to Bhutan, and in the Khasi hills at 1200–1500 m

(4000 to 5000 ft). It can be grown in sub-temperate regions between 1500 and 2100 m altitudes [10,11]. It is a perennial herb. It has an erect (about 2–3 ft long) stem. The middle portion of the stem is round, while the upper is four-angled, with a prominent decurrent line at each angle. The stems are orange brown or purplish in colour, and contain large continuous yellowish pith. The root is simple, tapering and stout, short, almost 7 cm long and usually half an inch thick [10,12]. Plant is reported to contain ophelic acid, chiratin, amarogentin, gentiopicrin and swerchirin [13]. The plant has been reported to possess hypoglycemic activity [14], anti-ulcerogenic activity [15], anti-inflammatory activity [16], hepatoprotective activity [17], wound healing activity [18], antibacterial activity [19] on selected microbial strains and antimalarial activity [20].

On the basis of above background the present study was undertaken for the screening of *Swertia chirata* methanolic extract for its antioxidant potential.

2. MATERIALS AND METHODS

2.1 Plant Material

The dry aerial parts of *Swertia chirata* were obtained from Natural Remedies, Bangalore (Karnataka) and authenticated at the Department of Botany, Dr. H. S. Gour University, Sagar (M.P.) India. The Voucher specimen number is Bot/Her/B/3116.

2.2 Extract Preparation

Dried and powdered plant material (60 g) was defatted with petroleum ether (500 ml) and then successively extracted with methanol (500 ml at 40ºC) using soxhlet apparatus. The extract was cooled at room temperature, filtered and evaporated to complete dryness. The percentage yield of the extract was calculated and it was found to be 12.58%.

2.3 Phytochemical Analysis

The methanolic extract was subjected to various chemical tests to detect the presence of various phytochemicals such as tannins, flavonoids, alkaloids etc. using standard procedure [21,22].

2.4 Antioxidant Assay

2.4.1 Determination of total phenolic content

Aliquots of 0.1 to 1.0 ml of SC methanol extract were pipette out in a series of test tubes and the volume was made up to 3ml with distilled water. 0.5ml of Folin-Ciocalteau reagent was added to each tube and incubated for 3 min at room temperature. Sodium carbonate (20%; 2ml) solution was added, mixed thoroughly and the tubes were incubated for 1 min in boiling water bath. Absorbance was measured at 650nm against a reagent blank. Standard curve using different concentrations of standard phenolic catechol was prepared. From the standard curve, concentration of phenolic content in the test samples was determined and expressed as µg of catechol equivalent [23].

2.4.2 Ferric reducing power activity

Various concentrations of SC methanol extract (100, 250 and 500 µg) were each mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferric-cyanide. The mixtures were incubated at 50ºC for 20 min then 2.5 ml of 10% trichloroacetic acid (w/v) were added. 5 ml of each solution was mixed with 5 ml of distilled water and 1 ml of 0.1% ferric chloride. The absorbance was measured spectrophotometrically at 700 nm. Butylated hydroxy anisole (BHA) was used as standard antioxidant [24].

2.4.3 Free radical scavenging activity by DPPH method

Different concentrations (50, 100, 250 and 500 µg) of extract and BHA were taken in different test tubes. The volume was adjusted to 500 µl by adding methanol. 5ml of 0.1 mM methanolic solution of DPPH was added to these tubes and shaken vigorously. A control without the test compound, but with an equivalent amount of methanol was maintained. The tubes were allowed to stand at room temperature for 20 min. The absorbance of the samples was measured at 517 nm [24]. Radical scavenging activity was calculated using the following formula:

$$\%\ \text{radical scavenging activity} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \text{x } 100$$

2.4.4 Hydroxyl radical scavenging activity

Various concentrations (50, 100, 250 and 500 µg) of SC methanol extract and BHA were taken in different test tubes and made up to 250µl with 0.1M phosphate buffer. 1ml of iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5 ml of EDTA (0.018%) and 1 ml of dimethyl sulphoxide (0.85% v/v in 0.1 M phosphate buffer, pH 7.4) were added to these tubes and the reaction was initiated by adding 0.5 ml of 0.22% ascorbic acid. These reaction mixtures were incubated at room temperature for 15 min. The reaction was terminated by the addition of 1 ml of ice-cold TCA (17.5% w/v). 3 ml of Nash reagent (150 g of ammonium acetate, 3 ml of glacial acetic acid, and 2 ml of acetyl acetone were mixed and raised to 1 L with distilled water) was added to all of the tubes and left at room temperature for 15 min for colour development. The intensity of the yellow color formed was measured spectrophotometrically at 412 nm against reagent blank [25]. The percentage hydroxyl radical scavenging activity was calculated by the following formula:

$$\%\ \text{hydroxyl radical scavenging activity} = \frac{1 - \text{Difference in absorbance of sample}}{\text{Difference in absorbance of blank}} \text{x}100$$

2.4.5 Nitric oxide radical scavenging activity

Various concentrations (50, 100, 250 and 500 µg) of SC methanol extract and BHA were taken in different test tubes and made up to 3 ml with 0.1M phosphate buffer (pH 7.2). Sodium nitroprusside (5 mM) prepared in buffered saline (pH7.2) was added (1 ml) to each tube. The reaction mixture was incubated for 30 min at RT. A control without the test compound, but with an equivalent amount of methanol was maintained. After 30 min, 1.5 ml of above solution was mixed with 1.5 ml of Griess reagent (1% Sulphanilamide, 2% phosphoric acid and 0.1% N-1- Naphthylethylenediamine dihydrochloride). The absorbance

of the samples was measured at 546 nm [26]. Nitric oxide radical scavenging activity was calculated using the following formula:

$$\% \text{ radical scavenging activity} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \text{x}100$$

2.5 Statistical Analysis

The data presented as means SD were analyzed using ANOVA. Duncan's multiple range test (DMRT) was used to determine significant differences between means. The results were considered statistically significant if the P values were 0.05 or less.

3. RESULTS AND DISCUSSION

In the present study, methanol extract of *S. chirata* was tested for its antioxidant activity using DPPH, hydroxyl, nitric oxide radicals scavenging activity and ferric reducing power activity. Besides, the phenolic content was determined and preliminary phytochemical screening was carried out.

3.1 Total Phenolic Content

The total phenolic content in 1 mg of methanolic extract of *S. chirata* was equivalent to 4.5 µg Catechol (Table 1). Phenolic compounds are the most wide spread secondary metabolites in plant kingdom. These diverse groups of compounds have received much attention as potential natural antioxidant. The antioxidant activity of the plant extract is mainly due to presence of phenolic compounds since they possess redox properties, which allow them to act as hydrogen donors and singlet oxygen quenchers [27]. The interests of phenolics are increasing in the food industry because they retard oxidative degradation of lipids and improve the quality and nutritional value of food [28].

Table 1. Total phenolic contents of catechol equivalent in *S. chirata*

Test sample	µg/mg of catechol equivalent phenolics
SCM	4.5

SCM= Swertia chirata methanol extract

3.2 Free Radical Scavenging Activity By DPPH Method

The results were reported in terms of IC_{50} values. The IC_{50} value of the methanolic extract of *S. chirata* was 222.74µg/ml (Table 2). The result was compared with the IC_{50} value of BHA (152.74 µg/ml). Methanolic extract of *S. chirata* showed potent DPPH scavenging activity (12, 25.5, 62.5 and 84.98 at 50, 100, 250 and 500 µg/ml respectively). The extract showed concentration dependent DPPH activity (p=0.000246) when compared with that of standard drug Butylated hydroxy anisole (BHA). The results showed that the *S. chirata* methanolic extract exhibited significant free radical DPPH scavenging activity.

Table 2. Effect of methanolic extract of *Swertia chirata* on DPPH, hydroxyl, nitric oxide radicals-scavenging activities and ferric reducing power

Concentration (µg)	Free radical scavenging activity by DPPH method		Hydroxyl radical scavenging activity		Nitric oxide radical scavenging activity		Ferric reducing power activity	
	SCM	BHA	SCM	BHA	SCM	BHA	SCM	BHA
50	12±0.14	20.7±0.16	2.38±0.09	10.56±0.12	8.2±0.15	9.7±0.21		
100	25.5±0.23	32.2±0.14	7.14±0.11	16.77±0.14	16.5±0.24	17.1±0.10	0.013±0.31	0.42±0.23
250	62.5±0.28	70.5±0.23	30.95±0.06	42.24±0.28	20.3±0.21	20.5±0.12	0.156±0.12	0.96±0.25
500	84.98±0.16	88.4±0.03	90.95±0.13	71.34±0.21	29.1±0.11	26.7±0.14	0.298±0.14	1.74*±0.18
IC_{50}	222.74±0.19	152.74±0.14	307.93±0.10	335.43±0.21	870.55±0.20	962.6±0.16		

Values are means ± standard deviation and significant if ($p < 0.05$), SCM= Swertia chirata methanol extract; BHA= Butylated hydroxy anisole FRSA-SCM>BHA ($p= 0.000246$); HRSA-SCM<BHA ($p= 0.000507$); NORA-SCM<BHA ($p= 2.22614$)

The free radical-scavenging activity of the extract was measured in terms of hydrogen donating or radical-scavenging ability [25]. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance. The DPPH free radical scavenging of antioxidants is due to their hydrogen donating ability. The plants with higher donating capacity have shown higher DPPH free radical scavenging activity [29].

3.3 Hydroxyl Radical Scavenging Activity

The hydroxyl radicals scavenging activity of different extracts at different concentrations (50, 100, 250 and 500 µg/ml) was analyzed and the results were reported in terms of IC_{50} values. IC_{50} values of methanolic extract of *S. chirata* and BHA (standard drug) was 307.93 and 335.43 µg/ml respectively. Methanolic extract of *S. chirata* showed 2.38, 7.14, 30.95 and 90.95 % inhibition at 50, 100, 250 and 500 µg/ml (Table 2). The results showed that methanolic extract of *S. chirata* exhibited most potent activity (90.95 % inhibition at 500 µg/ml), when compared to standard drug BHA (71.34 % inhibition at 500 µg/ml). The extract showed significant antioxidant activity (p= 0.000507) in dose dependent manner.

Ethanolic extract of *Stevia rebaudiana* leaves also showed similar hydroxyl radical scavenging activity in a concentration dependent manner [30]. The ability of the extracts to quench hydroxyl radicals seems to be directly related to the prevention of propagation of the process of lipid peroxidation and scavenger of active oxygen species, thus reducing the rate of the chain reaction.

3.4 Nitric Oxide Radical Scavenging

The nitric oxide radical scavenging activity of different extracts at different concentration (50, 100, 250, 500 µg/ml) were analyzed in terms of IC_{50} values. IC_{50} value of methanolic extract *S. chirata* and BHA (standard drug) was 870.55 and 962.66 µg/ml respectively. The methanolic extract of *S. chirata* showed 8.2, 16.5, 20.3 and 29.1 % inhibition at 50, 100, 250, 500 µg/ml respectively (Table 2). The percentage inhibition of the extract was compared with BHA which showed 9.7, 17.1, 20.5 and 26.7 % inhibition at 50, 100, 250, 500 µg/ml respectively. The above results showed that IC_{50} value of methanolic extract of *S. chirata* was lower than the standard drug (BHA) which revealed the potent antioxidant activity (p= 2.22614) of methanolic extract of *S. chirata*.

Nitric oxide is an essential bio-regulatory molecule required for several physiological processes like neural signal transmission, immune response, control vasodilatation, control of blood pressure etc [31,32]. Nitric oxide is lipophilic in nature and easily diffuses between cells. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide [33]. Nitric oxide plays an important role in various types of inflammatory processes in the animal body. The plant products may have the property to counteract the effect of NO formation and in turn may be of considerable interest in preventing the ill effects of excessive NO generation.

3.5 Ferric Reducing Power

The ferric reducing power of extract and standard drug was analyzed by testing their absorbance at 700nm. The various concentrations (100, 250 and 500 µg/ml) of methanolic

extract of *S. chirata* showed absorbance 0.013, 0.156, 0.298 respectively (Table 2). The results of the extract was compared with BHA which exhibited 0.42, 0.96, 1.74 absorbance at 100, 250 and 500 µg/ml respectively. The reducing power of methanolic extract of *S. chirata* (Fe^{3+} – Fe^{2+}) was found to be increased with increasing concentration. The result obtained in the present studies showed that SC methanol extract contains hydrophilic poly phenolic compounds that cause the reducing power.

The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [34]. Tanaka et al. [35] have also observed a direct correlation between antioxidant activity and reducing power of certain plant extracts. The reducing properties are generally associated with the presence of reductones [36], which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom [37]. Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation.

The results obtained in this study are consistent with previous reports such as Saha et al. [29] observed that methanol extract of the leaves of *Mimusops elengi* Linn was evaluated for its antioxidant potential of the by using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging assay, reducing power and total antioxidant capacity. The extract showed significant activities in all antioxidant assays compared to ascorbic acid (standard drug) in a dose dependent manner. In DPPH scavenging assay the IC_{50} value of the extract was found to be 43.26µg/ml, while the IC_{50} value of the standard drug (ascorbic acid) was 58.92 µg/ml. Total antioxidant activity was also found to increase in a dose dependent manner. Similarly, Methanol and chloroform extracts of *G. arborea* were investigated for *in vitro* antioxidant activity by DPPH method and it was found that methanol extract (IC_{50} value of 15.4) has more antioxidative activity [38]. In the present study, *S. chirata* methanolic extract also showed the similar results i.e. the IC_{50} value of the DPPH method, hydroxyl radical and nitric oxide radical scavenging activity was 222.74±0.19, 307.93±0.10 and 870.55±0.20 while in case of BHA the IC_{50} value of the DPPH method, hydroxyl radical and nitric oxide radical scavenging activity was 152.74±0.14 (p= 0.000246), 335.43±0.21 (p=0.000507) and 962.6±0.16 (p= 2.22614). This showed that S. chirata methanolic extract showed better activity than BHA in hydroxyl radical and nitric oxide radical scavenging activity.

Table 3. Phytochemical analysis of methanol extract of *Swertia chirata*

Constituents/test	SCM
Alkaloids	+
Tannin	+
Saponin	-
Steroids	-
Terpenoids	-
Flavonoids	+
Glycosides	+
Carbohydrates	+
Fixed oil and fats	-
Phenolic compounds	+

Preliminary phytochemical screening of *S. chirata* methanolic extract revealed the presence of phenolic compounds, alkaloids, tannins, flavonoids, glycosides and carbohydrates (Table

3 above). So antioxidant activity shown by methanolic extract of *S. chirata* might be due to presence of these phytochemicals.

4. CONCLUSION

Above results revealed that the methanolic extract of *S. chirata* has significant antioxidant potential. Thus, it can be concluded that methanolic extracts of *S. chirata* can be used as an accessible source of natural antioxidant agent with consequent health benefits.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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