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Research Article
Phytochemical Screening and Assessment of Pharmacological Properties of *Swertia chirayita* (Roxb. ex Fleming) Root Methanolic Extract

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Abstract

Background and Objective: Various parts of medicinal plants have been used to treat specific disorder from ancient times. *Swertia chirayita* (Roxb. ex Fleming) is a customary folklore medicine, used in the treatment of liver disorders, fevers, dysentery, diarrhea, stomach problems and other disorders. The present study was carried out in order to assess the antioxidant activity, to evaluate the antifungal properties of the plant's root and to observe anticancer potential of methanolic extract of *Swertia chirayita* root. **Materials and Methods:** Phytochemical analysis and different chemical tests for the screening and identification of bioactive chemical constituents in *Swertia chirayita* methanolic root extract (SCME) were carried out using the standard procedures. The plants were purchased from local herbal market. *In vitro* determination of antioxidant properties of SCME were conducted using 1,1-diphenyl-2-picrylhydrazyl (DPPH)radical scavenging activity, H_2O_2 scavenging activity, Beta-carotene bleaching assay, total antioxidant activity by phosphomolybdenum method, azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation scavenging activity and hydroxyl radical scavenging activity. Anticancer activity of SCME was determined according to the protocol of brine shrimp lethality test. Antifungal potential was determined by measuring zone of inhibition on Potato Dextrose Agar (PDA) plates. The different concentrations of SCME used were 3.0, 1.5, 0.75 and 0.37 mg mL^{-1}. The data were evaluated as Mean±Standard Deviations of 5 independent experimental responses. The results were analyzed using t-test for independent samples with SPSS version 16.0. **Results:** Phytochemical analysis of SCME showed that phlobatannins, tannins, saponins and terpenoids were present. The SCME exhibited strong antioxidant activity in a concentration dependent manner for in all six models. The SCME at dose of 3 mg mL^{-1} caused 100% death rate of brine shrimp after 72 h. The SCME showed potent activity against *Aspergillus flavus* (87%) followed by *Aspergillus niger* (88%) while the highest activity was shown against *Aspergillus fumigatus* (92%). **Conclusion:** The SCME exhibited strong antioxidant, antifungal and cytotoxic potential. Purification of different bioactive compounds should be carried out and *in vivo* studies are required for further verification.

Key words: Fungicidal activity, *Swertia chirayita*, antioxidant activity, phytochemical analysis, anti-carcinogenic activity

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Medicinal plants are conventionally used worldwide for treatment of different disorders related to human health[1]. Different preparations of these plants have been found to be greatly effective against various infections caused by micro-organisms. About 70,000 plant species have been used from time to time for curing many microbial diseases[1,2]. Various parts of medicinal plants have been used to treat specific disorder from ancient times. The indigenous systems of medicine namely Ayurvedic, Unani and Siddha have been used for several centuries. The indigenous systems of medicine used traditionally, fulfill the medical treatment requirements of 70% of village population[3]. The demands of modern pharmaceutical industries for medicinal plants has also been increased several times[2].

An imbalance between the production of free radicals and the endogenous action of antioxidant defense systems results in oxidative stress, which is divided into enzymatic and nonenzymatic systems. Compounds such as β-carotene, α-tocopherol (Vitamin E), sodium ascorbate (Vitamin C) and phenolic compounds which are widely found in medicinal plants contribute to non-enzymatic antioxidant defense systems[3-5]. The main function of antioxidant defense system is to prevent or reduce the extent of damage caused by free radicals and other reactive oxygen species. These damages may be responsible for a number of chronic diseases, including cancer, some cardiovascular and neurodegenerative diseases[6].

At present cancer is a highly lethal disease in Pakistan as well as worldwide. In the treatment of cancer medicinal plants play a crucial role[7,1]. *Swertia chirayita* (Roxb. ex Fleming) (*S. chirayita*) is from gentian family [8,9]. It is native to many areas of the northern hemisphere, comprising many regions of Eurasia and western North America[9]. *Swertia* (Gentianaceae) is a large genus of plants distributed in the mountainous regions of tropical areas. Most of the species found in India grow at high altitude in the temperate Himalayas from Kashmir to Bhutan and also in the Khasia and Western Ghats hills and Himalayan regions of Pakistan[10]. Other important species, such as *S. angustifolia*, *S. corymbosa*, *S. decussata, S. hookeri*, *S. macrosperma*, *S. petiolata*, *S. lawii*, *S. paniculata, S. punctata*, *S. calycina*, *S. purpurascens*, *S. bimaculata*, *S. ciliata*, *S. densifolia*, *S. japonica* and *S. frachetiana* are also from the customary folklore medicine and are being used as alternatives for *S. chirayita* in India, China, Pakistan, Japan and other Asian countries in the cure of liver disorders, fever, dysentery, diarrhea, stomach problems and other disorders[11].

Both the crude and purified extracts significantly inhibited ($p<0.001$) cell proliferation and induced apoptosis[12]. This is the first report of its kind and the observation suggests the chemo-preventive potential of *S. chirayita*. The ethanolic and methanolic extracts of aerial parts of *S. chirayita* exhibited anti-cancer activity against a human nasopharyngeal epidermis tumor cell line (KB) whereas in a recent study the *S. chirayita* showed 100% anticancer activity[13]. Saha and Das[14] reported that the anti cancer activity was observed when the crude extract (10-320 μg mL^{-1}) of the plant was tested against 7, 12-dimethylbenz[a] anthracene (DBMA) induced mouse skin carcinogenesis model. It has been reported that methanol extracts of both leaves and stem exhibited anticancer activity on HCT 116 cell lines in which the methanol extract of leaves exhibited more anticancer activity compared to stem[15].

Phenolic compounds are derived from secondary plant metabolism and are essential for plant growth and reproduction. Flavonoids, anthraquinones, xanthones, phenolic acids, saponines, tannins and tocopherols are the most common natural source of phenolic antioxidants. It has been showed in the present study that root of *S. chirayita* contains phlobatannins, saponines, tannins and terpenoids[16,17]. They are used for treatment of variety of disorders. In Indian medical system, these plants are used as a remedy for liver disorders, bronchial asthma, chronic fevers, anemia and diarrhea[18]. In Ayurveda, *S. chirayita* is used as antipyretic, antihelmintic, laxative and also used in treatment of asthma and leucorrhoea[19]. In Unani medicine, it is used as astringent, tonic and anti-inflammatory agent. While various parts of *S. chirayita* are considered as active remedies, the roots are one of the most potent parts. Phytochemical studies have been shown the presence of xanthone derivatives, flavonoids, iridoid glycoside and triterpenoids in this genus[20]. Because of the presence of different components, various biological activities such as hepato-protective, antimicrobial, anti-inflammatory, anticarcinogenic, hypoglycemic, antimalarial, antioxidant and CNS depressant properties have been reported for these plants[21].

The methanolic and aqueous whole plant extracts *S. chirayita*, at a dose of 200 and 300 mg kg^{-1}, produced significant ($p<0.05$) hepato-protection as shown by decrease in the activities of the serum enzymes and bilirubin while there were marked scavenging of the DPPH free radicals by the fractions[22,23].The findings of In vitro studies showed that at 25 mg mL^{-1} ($p\geq0.05$) the crude aqueous (CAE) and methanolic extracts (CME) of *S. chirayita* whole plant displayed an anthelmintic influence on live *Haemonchus contortus*.

Furthermore, when sheep naturally infected with mixed species of gastrointestinal nematodes were treated with whole plant Crude Powder (CP) of *S. chirayita,* CAE and CME at the dose of 3 g kg^{-1}, exhibited a substantial decrease in egg per gram of feces[24].

Methanolic extract showed activity against *Cladosporium oxysporum* (9 mm at dose of 800 μg mL^{-1}), moderate activity against *Aspergillus niger* (2 mm at dose of 800 μg mL^{-1}) and no activity against *Aspergillus flavus*[25]. Dichloromethane fraction from both leaf and stem of *S. chirayita* showed significant antimicrobial activities against some gram-positive and gram-negative bacteria and mild to moderate activity against some fungi[26]. Growth of *Candida tropicalis* was inhibited by *S. chirayita* extracts. The significant MIC value was 10 mg mL^{-1} against *Candida tropicalis*[2]. Methanol extract of *S. chirayita* showed only moderate antifungal activity against *A. niger* and *Penicillium*[27]. Herbal medicines are considered comparatively harmless and have been used in the management of liver maladies for a long time. The effectiveness of herbal medicines needs to be appraised through carefully designed multi center clinical studies[28]. The findings of this study about various pharmacological properties of SCME will form the basis of research study with purified phytochemicals present in SCME.

The present study was designed to carry out phytochemical analysis and to evaluate different pharmacological effects of crude extract of root *S. chirayita.*

MATERIALS AND METHODS

Chemicals: Ferric chloride, 2,2-diphenyl-1-picryl-hydrazyl, potassium persulfate, azinobis(3-ethylbenzothiozoline-6-sulfonic acid) disodium salt, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, ferrous chloride, hydrogen peroxide, ferrous ammonium sulfate, ethylenediaminetetracetic acid (EDTA) disodium salt were obtained from Hi-media, Mumbai, India. 2,2′-bipyridyl and hydroxylamine hydrochloride, Penicillin G and streptomycin [3,-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-tetrazolium bromide] (MTT) were purchased from Sigma (St. Louis, USA). Fetal bovine serum, Dulbecco's modified Eagle medium were purchased from Biochrom, Berlin, Germany. All other chemicals and reagents used were of analytical grade.

Plant collection: The roots of *S. chirayita* were purchased from local herbal market of Bannu, Pakistan, shade dried and grounded to powder which was saturated with methanol to prepare methanolic crude extract.

Extract preparation: The root powder (60 g) of *S. chirayita* was soaked in 20 mL of 80% methanol, continuously shaken for one hour and then kept at room temperature for seven days. Whatman No. 1 filter paper was used to filter the plant extract after seven days. The filtrate was further concentrated and dried on rotary evaporator under reduced pressure at 40°C, then it was stored in refrigerator at 4°C for further use.

Each sample was dissolved in 95% methanol at a concentration of 3 mg mL^{-1} and then diluted to prepare the series of concentrations for different assays.

***In vitro* determination of antioxidant activity:** Antioxidant property of plant samples was carried out according to the method described by Wojdylo *et al.*[29]. Samples (1 g) were added to 10 mL of 80% methanol and the suspensions were stirred slightly. Tubes were vortexed twice for 15 min and left at room temperature (25°C) for 24 h. The extracts were centrifuged for 10 min at 1500 rpm and supernatants were kept at 4°C prior to use within 24 h.

DPPH radical scavenging activity: The 1,1-diphenyl-2-picrylhydrazyl (DPPH) activity was assessed by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay according to the methods described by Shimada *et al.*[30]. DPPH solution (0.1 mM) was freshly prepared in ethanol and was used for the assay. Then, 1 mL of this solution was added to 2 mL of SCME solution at different doses of 3.0, 1.5, 0.75 and 0.37 mg mL^{-1}. The mixture was shaken vigorously and kept in the dark at controlled room temperature (25-28°C) for 30 min. Then the absorbance was measured at 517 nm. The degree of discoloration occurred in the samples indicates the free radical scavenging efficiency of the substances. The percentage of DPPH radical scavenging activity was calculated by the following formula[30]:

$$\text{Inhibition of DPPH radical (\%)} = \frac{\text{Abr-Aar}}{\text{Abr}} \times 100 \quad (1)$$

where, Abr is the absorbance before reaction and Aar is the absorbance after reaction.

Total antioxidant capacity: The total antioxidant capacity of the extracts was evaluated by the phosphomolybdenum method according to the procedure described by Prieto *et al.*[31]. A 0.3 mL of 3.0, 1.5, 0.75 and 0.37 mg mL^{-1} SCME solution was combined with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95°C for 90 min. Then, the

absorbance of the solution was measured at 695 nm using a UV-VIS spectrophotometer (UVmini-1240) against blank after cooling to room temperature. Methanol (0.3 mL) in the place of extract was used as the blank. The total antioxidant activity is expressed as the number of gram equivalent of ascorbic acid. The calibration curve was prepared by mixing ascorbic acid (1000, 500, 250, 125, 62.5 and 31.25 μg mL^{-1}) with methanol.

Hydroxyl radical scavenging activity: The scavenging activity of the SCME solution on hydroxyl radical was measured according to the method of Klein *et al.*[32]. Various concentrations (3.0, 1.5, 0.75 and 0.37 mg mL^{-1}) of extracts were added with 1 mL of iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5 mL of EDTA solution (0.018%) and 1 mL of dimethyl sulfoxide (DMSO) (0.85% v/v in 0.1 M phosphate buffer, pH 7.4). The reaction was initiated by adding 0.5 mL of ascorbic acid (0.22%) and incubated at 80-90°C for 15 min in a water bath. After incubation the reaction was terminated by the addition of 1 mL of ice-cold TCA (17.5% w/v). Three milliliters of Nash reagent (75 g of ammonium acetate, 3.0 mL of glacial acetic acid and 2 mL of acetyl acetone were mixed and raised to 1 L with distilled water) was added and left at room temperature for 15 min. The reaction mixture without sample was used as control. The intensity of the color formed was measured spectroscopically at 412 nm against reagent blank. The Hydroxyl Radical Scavenging Activity (HRSA) was calculated by the Eq. 2:

$$\text{HRSA}\,(\%) = \frac{(\text{A0-A1})}{\text{A0}} \times 100 \quad (2)$$

where, A0 is the absorbance of the control and A1 is the absorbance of the extract/standard.

Scavenging of H_2O_2: Scavenging of $H_2O_2$was determined by the method reported by Alam *et al.*[33]. About 4 mM H_2O_2, 0.6 mL solution (prepared in PBS) was added to 4 mL solution of extract containing 3.0, 1.5, 0.75 and 0.37 mg mL^{-1} and incubated for 10 min. The absorbance of the solution was measured at 230 nm by using spectrophotometer. Free radical scavenging potency as determined from percentage of age H_2O_2. Lower percentage of age H_2O_2 indicated strong free radical scavenging activity. Percent scavenging of the DPPH free radical was measured using the Eq. 3[34].

$$\begin{bmatrix}\text{DPPH radical scavenging}\\ \text{or DPPH inhibition (\%)}\end{bmatrix} = \left[1/\left(\text{As}/\text{Ac}\right)\right] \times 100 \quad (3)$$

where, Ac is absorbance of control and As is absorbance of sample solution.

ABTS radical cation scavenging activity: Antioxidant activity of this plant was measured using an improved ABTS method as described by Re *et al.*[35]. The ABTS•+ was produced by reacting 7 mM ABTS aqueous solution with 2.4 mM potassium persulfate in the dark for 12-16 h at room temperature. Prior to assay, this solution was diluted in ethanol (about 1:89 v/v) and equilibrated at 30°C to give an absorbance of 0.70±0.02 at 734 nm. The stock solution of the sample extracts were diluted such that after introduction of 10 μL aliquots into the assay, they produced between 20 and 80% inhibition of the blank absorbance. After the addition of 1mL of diluted ABTS solution to 10 μL of sample or Trolox (final concentration 0-15 μM) in ethanol, absorbance was measured at 30°C exactly 30 min after the initial mixing. Triplicate determinations were made at each dilution of the standard and the percentage inhibition was calculated against the blank (ethanol) absorbance at 734 nm and then was plotted as a function of Trolox concentration. The unit of total antioxidant activity is defined as the concentration of trolox having equivalent antioxidant activity expressed as μM g^{-1} sample extracts. The extract at different concentrations (3.0, 1.5, 0.75 and 0.37 mg mL^{-1}) were used to assess the antioxidant activity.

β-carotene bleaching assay: β-carotene (10 mg) was dissolved in 10 mL of chloroform. The carotene-chloroform solution (0.2 mL) was pipetted into a flask containing 20 mg of linoleic acid and 200 mg of Tween 40. Chloroform was removed using a rotary evaporator under reduced pressure at 40°C for 5 min and 50 mL of distilled water was added slowly to the residue with vigorous agitation in order to form an emulsion. A 4.8 mL aliquot of the emulsion was added to a tube containing 0.2 mL of the sample solution and the absorbance at 470 nm was immediately measured against a blank that was an emulsion without β-carotene. The tubes were placed in a water bath at 50°C and the oxidation of the emulsion was monitored spectrophotometrically by measuring the absorbance at 470 nm for 60 min. Control samples contained 0.2 mL of ethanol instead of the sample extracts. Ascorbic acid was used as the reference. The antioxidant activity was expressed as percent inhibition with reference to the control after 60 min of incubation using the following formula[36]:

$$\text{AA} = \frac{\text{DRc - DRs}}{\text{DRc}} \times 100 \quad (4)$$

where, AA is the antioxidant activity, DR_C is the degradation rate of the control {DR_C = [ln(a/b)/60]}, DR_S is the degradation rate in the presence of the sample {DR_S = [ln(a/b)/60]}, where

a is the absorbance at time 0 and b is the absorbance after 60 min[36]. The antioxidant activity of SCME was determined using different concentrations i.e. 3.0, 1.5, 0.75 and 0.37 mg mL^{-1} of SCME solution.

Cytotoxic activity assay: The cytotoxic brine shrimp lethality test was done by following procedure of Meyer-Alber *et al.*[37]. Brine shrimp lethality bioassay is a recent development in the bioassay for bioactive compounds, which indicates cytotoxicity as well as a wide range of pharmacological activities (e.g., anticancer, antiviral, pesticidal etc.) of the compounds. Bioactive compounds are almost always toxic in high doses. Here *in vivo* lethality of a simple zoological organism (brine shrimp nauplii) is used as a convenient monitor for screening of new bioactive natural products. Generally the median Effective Dose (ED_{50}) values for cytotoxicities are one tenth (1/10) of median Lethal Dose (LC_{50}) values in the brine shrimp test. The anticancer activity (cytotoxicity) of SCME was determined against brine shrimps growth using different concentrations i.e. 3.0, 1.5, 0.75 and 0.37 (mg mL^{-1}) of SCME solution.

Determination of antifungal activity: Method of Duraipandiyan and Ignacimuthu[38] was followed to assess antifungal activity of *S. chyrayita* methanolic extract (SCME). Similarly for the antifungal activity, the stock cultures of fungi (*Aspergillus niger* (MTCC 1881), *Aspergillus flavus* (MTCC 1883), *Cladosporium oxysporum* (MTCC 1777), were revived by inoculating in broth media and grown at 27°C for 72 h. The agar plates of the Potato Dextrose Agar (PDA) media were prepared. Each plate was inoculated with an aliquot (0.1 mL) of the fungal suspension (103 spores mL^{-1}), which was spread evenly on the plate. After 20 min, wells were made and filled with test samples of different concentrations. The positive control plates with Terbinofine (standard drug) and negative control plates with DMSO were also prepared. All the plates were incubated at 27°C for 72 h and then the diameter of zone of inhibition was noted. The antifungal activity of SCME was determined against above mentioned 3 different fungal strains using different concentrations i.e. 3.0, 1.5, 0.75 and 0.37 mg mL^{-1} of SCME solution.

Preliminary phytochemical screening: Chemical tests were carried out on the methanolic extract to identify the constituents using standard procedures as described by Harborne[39], Sofowora[40] and Trease and Evans[41].

Test for tannins: About 2 mL of them ethanolic extract was stirred with 2 mL of distilled water and few drops of FeCl3 solution were added. The formation of a green precipitate was an indication for the presence of tannins.

Test for saponins: About 5 mL of methanolic extract was shaken vigorously with 5 mL of distilled water in a test tube and warmed. The formation of stable foam was taken as an indication for the presence of saponins.

Test for phlobatannins: About 2 mL of methanolic extract was added to 2 mL of 1% HCl and the mixture was boiled. Deposition of a red precipitate was taken as an evidence for the presence of phlobatannins.

Test for terpenoids: About 2 mL of the methanolic extract was dissolved in 2 mL of chloroform and evaporated to dryness. Then 2 mL of concentrated sulphuric acid was added and heated for about 2 min. A greyish color indicates the presence of terpenoids.

Statistical analysis: The data were evaluated as Mean±Standard Deviations of five independent experimental responses. The results were analyzed using t-test for independent samples with SPSS version 16.0. The significance of the results is statistically satisfactory with significance level less than 0.1%.

RESULTS

In vitro antioxidant activities

DPPH radical scavenging activity: The DPPH radical scavenging activity of SCME was assessed at different concentrations 3.0, 1.5, 0.75 and 0.37 mg mL^{-1}. It was observed that SCME was capable of neutralizing the DPPH free radicals via hydrogen donating activity in a dose dependent manner (Table 1). The maximum DPPH radical scavenging activity of SCME was recorded at a dose of 3.0 mg mL^{-1} which was 88.1±0.1%.

Total antioxidant capacity: The total antioxidant capacity of SCME was assessed at different concentrations 3.0, 1.5, 0.75 and 0.37 (mg mL^{-1}). Maximum activity; 92±0.2% was found in 3.0 mg mL^{-1} of SCME extract (Table 2).

Hydroxyl radical scavenging activity: The scavenging activity of the SCME solution on hydroxyl radical was measuredat various concentrations 3.0, 1.5, 0.75 and 0.37 (mg mL^{-1}). The concentration of 3.0 mg mL^{-1} of SCME extract exhibited maximum activity; 84.33±0.2% (Table 3).

Table 1: Scavenging activities of ascorbic acid and SCME for DPPH free radicals

Samples (mg mL^{-1})	Ascorbic acid (%)	p-values	SCME (%)	p-values
3.0	94.59±0.05	0.000	88.10±0.07	0.000
1.5	91.37±0.05	0.000	83.35±0.05	0.000
0.75	89.00±0.05	0.000	78.05±0.41	0.000
0.37	87.35±0.05	0.000	75.33±0.05	0.000

SEM: *Swertia chirayita* methanolic root extract, Data are presented as Mean±SD

Table 2: Scavenging activities of ascorbic acid and SCME against phosphomolybdate free radical

Samples (mg mL^{-1})	Ascorbic acid (%)	p-values	SCME (%)	p-values
3.0	97.05±0.66	0.000	91.58±0.81	0.000
1.5	96.20±0.5	0.000	84.88±0.56	0.000
0.75	94.00±0.51	0.000	78.05±0.80	0.000
0.37	92.00±0.54	0.000	72.58±0.73	0.000

Data are presented as Mean±SD

Table 3: Scavenging activities of ascorbic acid and SCME against OH free radicals

Samples (mg mL^{-1})	Ascorbic acid (%)	p-values	SCME (%)	p-values
3.0	90.10±0.07	0.000	84.33±0.16	0.000
1.5	87.23±0.11	0.000	78.12±0.1	0.000
0.75	84.98±0.05	0.000	72.23±0.07	0.000
0.37	81.03±0.4	0.000	68.65±0.04	0.000

Data are presented Mean±SD

Table 4: Scavenging activities of ascorbic acid and SCME against H_2O_2 free radicals

Samples (mg mL^{-1})	Ascorbic acid (%)	p-values	SCME (%)	p-values
3.0	90.10±0.04	0.000	80.33±0.03	0.000
1.5	87.23±0.03	0.000	78.12±0.04	0.000
0.75	84.98±0.04	0.000	73.23±0.03	0.000
0.37	81.03±0.03	0.000	69.65±0.03	0.000

Data are presented as Mean±SD

Table 5: Scavenging activities of ascorbic acid and SCME against ABTS free radicals

Samples (mg mL^{-1})	Ascorbic acid (%)	p-values	SCME (%)	p-values
3.0	85.24±0.04	0.000	80.88±0.03	0.000
1.5	74.73±0.03	0.000	69.25±0.04	0.000
0.75	68.1±0.03	0.000	64.56±0.03	0.000
0.37	61.03±0.03	0.000	57.35±0.06	0.000

Data are presented as Mean±SD

Table 6: Scavenging activities of ascorbic acid and SCME against β-carotene free radicals

Samples (mg mL^{-1})	Ascorbic acid (%)	p-values	SCME (%)	p-values
3.0	93.12±0.03	0.000	87.03±0.03	0.000
1.5	87.93±0.03	0.000	85.12±0.04	0.000
0.75	84.17±0.03	0.000	73.34±0.04	0.000
0.37	76.46±0.04	0.000	68.12±0.04	0.000

Data are presented as Mean±SD

Scavenging of H_2O_2: Scavenging activity of the SCME solution againstH_2O_2 free radicle was determined at various concentrations i.e. 3.0, 1.5, 0.75 and 0.37 (mg mL^{-1}). The maximum activity; 80.33±0.2% was shown by 3.0 mg mL^{-1} (Table 4).

ABTS radical cation scavenging activity: Scavenging activities of SCME against ABTS free radicals were estimated using different Concentrations i.e. 3.0, 1.5, 0.75 and 0.37 (mg mL^{-1}). The 3.0 mg mL^{-1} demonstrated maximum activity; 80.88±0.1% (Table 5).

β-carotene bleaching assay: Scavenging activities of SCME against beta carotene free radicals were determined at various concentrations i.e. 3.0, 1.5, 0.75 and 0.37 (mg mL^{-1}). The maximum activity; 87.03±0.3% was shown by 3.0 mg mL^{-1} (Table 6).

Cytotoxic activity: The anticancer activity (cytotoxicity) of SCME was determined against brine shrimps growth using different concentrations i.e. 3.0, 1.5, 0.75 and 0.37 (mg mL^{-1}) of SCME solution. It was observed that at concentration of 0.37, 0.75, 1.5 and 3 mg mL^{-1}, 30, 20, 10 and 0% survival and 70, 80, 90 and 100% death occurred, respectively (Table 7).

Table 7: Survival and death rate against SCME of brine shrimps

Samples (mg L^{-1})	Total No. of brine shrimps	After 24 h		After 48 h			Survival (%)			Death (%)		
		Live	Dead	Live	Dead	After 72 h	After 24 h	After 48 h	After 72 h	After 24 h	After 48 h	After 72 h
3	10	2	8	0	10	All dead	20	0	0.0	80	100	100
1.5	10	3	7	1	9		30	10		70	90	
0.75	10	4	6	2	8		40	20		60	80	
0.37	10	4	6	3	7		40	30		60	70	

Data are mean values of triplicate experiments

Table 8: Antifungal activity of SCME (Inhibition %)

Fungal strains	Samples (mg mL^{-1})	SCME (%)	p-value	Terbinafine (%)	p-value
Aspergillus flavus	3.00	87±0.04	0.000	100±0.04	0.000
	1.50	81±0.04	0.000	98±0.04	0.000
	0.75	76±0.04	0.000	91±0.05	0.000
	0.37	70±0.04	0.000	85±0.27	0.000
Aspergillus niger	3.00	88±0.03	0.000	100±0.04	0.000
	1.50	82±0.85	0.000	96±0.04	0.000
	0.75	77±0.04	0.000	87±0.04	0.000
	0.37	71±0.05	0.000	88±0.04	0.000
Aspergillus fumigatus	3.00	92±0.23	0.000	100±0.04	0.000
	1.50	84±0.05	0.000	97±0.05	0.000
	0.75	75±0.04	0.000	89±0.04	0.000
	0.37	71±0.05	0.000	85±0.04	0.000

Data are presented as Mean±SD

Antifungal activity: The antifungal potential of SCME is highly significant statistically as indicated by p-value (p = 0.000), against fungal strain *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigates* (Table 8). The percentage inhibition of SCME is 90, 89 and 94%, respectively at high 3 mg mL^{-1} concentration. Negative and positive controls are parts of a well-designed scientific experiment. The negative control group is a group in which no response is expected and positive control, in which a known response is expected. When both are successful, are usually sufficient to eliminate most potential confounding variables, it means that the experiment produces a negative result when a negative result is expected and a positive result when a positive result is expected. Terbinafine was positive control which shows 100% inhibition against these fungal strains, while the DMSO was negative control which shows no inhibition (Table 8).

Phytochemical screening: The phytochemical screening of SCME was also performed with the aim to investigate the presence of various bioactive compounds. This investigation showed the presence of tannins, saponins, phlobatannins and terpenoids in SCME.

DISCUSSION

Free radicals which are produced inside our body during different reactions are scavenged by naturally produced antioxidants and prevent us from the serious effect of tissue or cell damage caused by oxidants. The crude methanolic extract of SC was found highly reactive against DPPH as it showed 88% inhibition by 3.0 mg mL^{-1}. Mahmood *et al.*[23] reported significant scavenging activity against DPPH (51%) by very low dose (35 μg mL^{-1}) of crude extract of whole plant of *S. Chirayita.* Methanol extract of *S. Corymbosa* exhibited the highest activity in DPPH radical scavenging activity as mentioned by Mahendran and Bai[42]. It has been shown that the reducing power and scavenging effects on H_2O_2, increases sharply with the increasing concentration of the samples and standards to certain extent[23]. Methanolic root extracts of *S. chirayita* found to have potential antioxidant activity (56%)[43].

The acetone: water (8:2) extract showed the highest Total Phenolic Content (TPC) and DPPH radical-scavenging activity as reported by Singh *et al.*[44]. Nair *et al.*[45] reported that a hydro-alcoholic extract of *S. chirayita* showed highly significant ABTS and DPPH radical-scavenging activity (98 and 93%), respectively. Kshirsagar *et al.*[22] stated that *S. chirayita* showed better antioxidant activity than other species with highest content of phenolic and flavonoids.

The cytotoxic activity assay of SCME showed a maximum death rate against brine shrimps at 3 mg mL^{-1} concentration which supports the presence of bioactive element against cancer causing pathogen. It was reported that methanol extracts (10-320 μg mL^{-1}) of both leaves and stem exhibited anticancer activity on HCT 116 cell lines in which the methanol extract of leaves exhibited more anticancer activity compared to stem[15]. Both the crude and purified extracts significantly

inhibited cell proliferation and induced apoptosis as observed by Saha *et al.*[12]. The ethanolic and methanolic extracts of aerial parts of *S. chirayita* exhibited anti-cancer activity against a human nasopharyngeal epidermis tumor cell line (KB) whereas in another study the *S. chirayita* showed 100% anticancer activity[13]. Prosenjit and Sukta reported that the anticancer activity was observed when the crude extract (10-320 μg mL^{-1}) of the plant was tested against DBMA induced mouse skin carcinogenesis model[14]. Shrestha *et al.*[46] reported that the brine shrimp bioassay of methanol and ethyl acetate extract of *S. chirayita* showed cytotoxicity. The extract showed LC_{50} of 128.82 ppm. Purified fraction (AJ-1) from rectified spirit extract of *S. chirayita* also showed significant activity against the brine shrimp (LC_{50} value of 9.34 μg mL^{-1}), in which the mortality rate increased with the increasing concentration of the compound, suggesting a positive correlation between brine shrimp toxicity and cytotoxicity[47].

The SCME exhibited a highly significant antifungal activity against fungal strain *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigatus.* The percent inhibition of SCME was 90, 89 and 94%, respectively at a high 3 mg mL^{-1} concentration. Laxmi *et al.*[25] revealed that methanolic extract showed significant activity against *Cladosporium oxysporum* (9 mm at 800 μg mL^{-1}), moderate activity against *Aspergillus niger* (2 mm at 800 μg mL^{-1}) and no activity against *Aspergillus flavus*. *Candida tropicalis* was inhibited by *S. chirayita* extracts. The significant MIC value was 10 mg mL^{-1} against *Candida tropicalis*[26]. Methanol extract of *S. chirayita* showed only moderate antifungal activity against *A. niger* and *Penicillium*[27]. Methanolic extract of *S. chirayita* exhibited significant antifungal with the zone of inhibition comparable to amphotericin[46]. Only methanolic extract was used in the study, if extract with other solvent e.g. ethanol, acetone and water would have been used, the comparative results were obtained.

CONCLUSION

Thus it is concluded that the various valuable biological activities of the SCME may be due to the presence of phlobatannins, tannins, saponins and terpenoids. Therefore, further analysis of the methanolic crude root extract of *S. chirayita* for isolation and characterization of bioactive compounds is indispensable.

SIGNIFICANCE STATEMENT

The *Swertia chirayita* methanolic root extract showed an excellent potential as a therapeutic agent for cancer and other infectious diseases due to the potent antioxidant, antifungal and ant-carcinogenic properties as shown in this study.

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