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Research Article

ANTIOXIDANT AND ANTI-PROLIFERATIVE ACTIVITY OF *SWERTIA CORYMBOSA* (GRISEB.) WIGHT EX C.B. CLARKE

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ABSTRACT

Objective: To investigate the *in vitro* antioxidant and anti-proliferative activity of aerial parts extracts of *Swertia corymbosa* in HeLa and HepG2 cancer cell line.

Methods: The successively extracted aerial parts of *S. corymbosa* using various solvents were analyzed for their total phenolics, tannins and flavonoid contents. *In vitro* antioxidant activity was assayed by using DPPH, ABTS, superoxide, hydroxyl radical scavenging assays, FRAP and metal chelating ability. Further, antiproliferative activity of *S. corymbosa* methanolic extract was analyzed by MTT assay.

Results: Methanol extract recorded higher phenolic, tannins and flavonoid content and showed comparable antioxidant activity with standard reference. The methanol extract showed a dose –dependently decreased the cell viability in HeLa and HepG2 cancer cell lines.

Conclusions: The results indicate that methanol extract possessed potent antioxidant and cytotoxic activity. Further, the study validates the therapeutic benefits of the folklore medicine.

Keywords: *Swertia corymbosa,* Antioxidant activity, Phenolics, Flavonoids, Free radicals cytotoxicity, Antiproliferation.

INTRODUCTION

The use of traditional medicine is widespread and plants provide a large source of natural antioxidants that might serve as leads for the development of novel drugs. Therefore, investigations of natural antioxidants and bioactive compounds for preservation of traditional medicines and use in treating certain human diseases have received much attention[1]. Reactive Oxygen Species (ROS) effect oxidative damage to various biomolecules including proteins, lipids, lipoproteins and DNA[2]. This oxidative damage is a critical etiological factor implicated in several chronic human diseases including cardiovascular dysfunctions, atherosclerosis, inflammation, carcinogenesis, drug toxicity, reperfusion injury, neurodegenerative diseases and cancer. Recently, traditional medicine plants have received much attention as sources of biological active substances including antioxidants that might serve as leads for the development of novel drugs[3].

The synthetic antioxidants such as butylated hydroxytoluene and butylated hydroxyanisole are very effective and are used for industrial processing but they possess potential health risks and toxic properties to human health and should be replaced with natural and safe sources of food antioxidants has been created and the search for natural antioxidants, especially of plant origin has notably increased in recent years. Phenolic antioxidants can inhibit free radical formation and/or interrupt propagation of autoxidation. Fat-soluble vitamin E and water-soluble vitamin C are both effective in the appropriate matrix. Plant extracts, generally used for their flavoring characteristics, often have strong H-donating activity thus making them extremely effective antioxidants. This antioxidant activity is most often due to phenolic acids, phenolic diterpenes, flavonoids, and volatile oils[4].

Cancer is a notorious disease that now becomes the major cause of human mortality in the world. Almost half of the incidence and mortality happen in Asia, with lung and bronchus, breast, and colorectal cancers in women to be the most common fatal cancers [5]. The aetiology of cancer is primarily from unhealthy lifestyle and pollution. Carcinogenesis or production of cancer involves mainly three steps, namely initiation, promotion, and progression. The implication of free radicals in different steps of carcinogenesis is well documented. Some of the reactive oxygen and nitrogen species and their pathways could facilitate cancer development by DNA or other biomolecules damaging[6]

Chemotherapeutic drugs are still considered as the most important treatments for cancer, however, this kind of treatments trigger enormous side effects. Regarding this dilemma, ongoing research on natural medicine sources in the form of functional foods or nutraceuticals has been attracting many scientists. Phytochemicals containing antioxidant properties showed capacity to inhibit carcinogenesis[7]. Several studies discovered these antioxidant compounds are related to other bioactivities. For instances, *in-vitro* cytotoxicity in tumor cells[8] *in vivo* cytotoxicity in experimental animals and anti- carcinogenesis[9,10].

Based on World Health Organization data, more than 80% of world inhabitants depend on using plant for their medicine[11]. Plants have a long history in the treatment of different cancer cells. *Swertia corymbosa* (Griseb.) Wight ex C.B. Clarke belongs to family Gentianaceae, commonly known as Shirattakuchi by Irulars. This plant has a long history of being used by Irulars and Paliyan ethnic medical practitioners have been used for medicinally as diarrhea, fever, jaundice, diabetic, inflammation, nervous disorders and also as an antidote for all poisons except cobra venom[12,13]. *Swertia*, an important genus of the family Gentianaceae, are rich sources of xanthonoids, flavonoids, irridoids, terpenoids and secoiridoid glycoside[14]. Xanthonoids, the major class of compounds among the chemical constituents of this genus have been reported to show significantly inhibit proliferation of cancer cells[15]. In order to intensively explore the potency of *S. corymbosa* for cancer medication, the study of antioxidant and cytotoxic activity of *S. corymbosa* was undertaken.

MATERIALS AND METHODS

Chemicals and reagents

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, ethylenediamine tetracetic acid (EDTA) disodium salt, 2,2'-bipyridyl and hydroxylamine hydrochloride, Penicillin G and streptomycin [3,-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-tetrazolium bromide] (MTT) were obtained from Hi-media (Mumbai, India), Sigma (St. Louis, USA) and fetal bovine serum, Dulbecco's modified Eagle medium-Biochrom (Berlin, Germany). All other chemicals and reagents used were of analytical grade.

Plant material

The fresh aerial parts of *Swertia corymbosa* were collected during the month of August from Vellingiri hills, Coimbatore, Tamilnadu, India. Freshly collected aerial parts were washed with tap water to remove adhering dust and then dried under shade. The dried samples were powdered in a Willy mill to 60-mesh size and used for solvent extraction.

Preparation of extracts

The dried, powdered plant samples were successively extracted in Soxhlet with Petroleum ether, Chloroform, Ethyl acetate, Methanol and hot water. Each time before extracting with the next solvent, the material was dried in hot air oven at 40°C. The different solvent extracts were concentrated by rotary vacuum evaporator (Yamato BO410, Japan) and then dried vacuum evaporator (Yamato RE300, Japan) and then dried. The dried extract obtained with each solvent was weighed. The percentage yield was expressed in terms of air dried weight of plant material. The evaporated extracts thus obtained were dissolved in the respective solvents at the concentration of 1 mg/mL and used for assessment of total phenolics and antioxidant activity through various *in vitro* assays.

Determination of total phenolics, tannins and flavonoids content

The total phenolic content was determined according to the method described by Siddhuraju and Becker[16] and the results were expressed as gallic acid equivalents (GAE). The tannin content of the tested samples was calculated by Siddhuraju and Manian[17] method in terms of GAE. Total flavonoids in the extracts were estimated as rutin equivalent according to the method of Zhishen et al[18].

***In vitro* antioxidant assays**

Free radical scavenging activity on diphenylpicrylhydrazyl radical (DPPH·)

The antioxidant activity of the extract was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH, according to the method of Blios[19]. Sample extracts at various concentrations (20 µL, 40 µL, 60 µL, 80 µL and 100 µL) were taken and the volume was adjusted to 100 µL with methanol. About 5 mL of a 0.1 mM methanolic solution of DPPH was added to the aliquots of samples and BHT and rutin were used as a standard compounds. Negative control was prepared by adding 100 µL of methanol in 5 mL of 0.1 mM methanolic solution DPPH. The tubes were allowed to stand for 20 min at 27 °C. The absorbance of the sample was measured at 517 nm against the blank (methanol). Radical scavenging activity of the samples was expressed as IC_{50} which is the concentration of the sample required to inhibit 50% of $DPPH^{\cdot}$ concentration.

ABTS radical cation scavenging activity

Antioxidant activity of this plant was measured using an improved ABTS method as described by Re et al[20]. $ABTS^{\cdot+}$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 sample extracts.

Ferric reducing/antioxidant power (FRAP) assay

The antioxidant capacity of solvent extracts was estimated according to the procedure described by Pulido et al[21]. FRAP reagent (900 µL), prepared freshly and incubated at 37°C, was mixed with 90 µL of distilled water and 30 µL of test sample or methanol (for the reagent blank). The test samples and reagent blank were incubated at 37°C for 30 min in a water bath. The final dilution of the test sample in the reaction mixture was 1/34. The FRAP reagent was prepared by mixing 2.5 mL of 20 mM TPTZ in 40 mM HCl, 2.5 mL of 20 mM $FeCl_3.6H_2O$ and 25 mL buffer (pH-3.6). At the end of incubation, the absorbance readings were taken immediately at 593 nm against the reagent blank, using a spectrophotometer. Methanolic solutions of known Fe (II) concentration, ranging from 100 to 2,000 µM, ($FeSO_4{\cdot}7H_2O$) were used for the preparation of the calibration curve. The parameter equivalent concentration was defined as the concentration of antioxidant having a ferric-TPTZ reducing ability equivalent to that of 1 mM $FeSO_4{\cdot}7H_2O$. Equivalent concentration was calculated as the concentration of antioxidant giving an absorbance increase in the FRAP assay equivalent to the theoretical absorbance value of a 1 mM concentration of Fe (II) solution.

Hydroxyl radical scavenging activity

The scavenging activity of the *S. corymbosa* extracts on hydroxyl radical was measured according to the method of Klein et al[22]. Various concentrations (20, 40, 60, 80 and 100 µg/mL) 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 sulphoxide (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 (75g of ammonium acetate, 3.0 mL of glacial acetic acid, and 2 mL of acetyl acetone were mixed and raised to 1L 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) is calculated by the following formula: % HRSA = [(A0 - A1)/A0] ×100, where A0 is the absorbance of the control and A1 is the absorbance of the extract/standard.

Superoxide anion scavenging activity

The effect of scavenging superoxide radical was determined by the nitroblue tetrazolium reduction method[23]. The assay was based on the capacity of various extracts to inhibit formazan formation by scavenging the superoxide radicals generated in riboflavin-light-NBT system. Each 3 mL reaction mixture contained 50 mM sodium phosphate buffer (pH-7.6), 20 µg riboflavin, 12 mM EDTA, 0.1 mg NBT and 100 µL sample solution or standards (BHA and BHT). Reaction was started by illuminating the reaction mixture with sample extract for 90 sec. Immediately after illumination, the absorbance was measured at 590 nm against the reagent blank (reaction mixture without plant sample). Identical tubes with reaction mixture kept in the dark served as negative control. The scavenging activity on superoxide anion generation was calculated as: Scavenging activity (%) = [(A0 - A1) / A0] × 100, where, A0 is the absorbance of the control, and A1 is the absorbance of the sample extract/standard.

Metal chelating activity

The chelating of ferrous ions by various extracts of *S. corymbosa* was estimated by the method of Dinis et al[24]. Initially, about 100 µL the extract sample was added to 50 µL solution of 2 mM $FeCl_2$. The reaction was initiated by the addition of 200 µL of 5 mM ferrozine and the mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm against the blank (deionized water). The metal chelating capacities of the extracts were evaluated using the following equation: Metal chelating capacity (%) = [(A0 - A1) / A0] ×100, where, A0 is the absorbance of the control, and A1 is the absorbance of the sample extract/standard. The chelating activity of the extracts was evaluated using EDTA as standard and results were expressed as mg EDTA equivalent/g extract.

Assay of nitric oxide radical (NO•) scavenging activity

The procedure is based on the method, where sodium nitroprusside in aqueous solution at physiological pH spontaneously generates NO, which interacts with oxygen to produce nitrite ions that can be estimated using Greiss reagent. Scavengers of NO compete with oxygen leading to reduced production of nitrite ions. For the experiment, sodium nitroprusside (10 mM) in phosphate buffered saline (PBS) was mixed with different extracts and incubated at room temperature for 150 min. The same reaction mixture without the extracts but with the equivalent amount of methanol served as the control. After the incubation period, 0.5 mL of Griess reagent (1% sulfanilamide, 2% H3PO4, and 0.1% N- (1-naphthyl) ethylenediamine dihydrochloride) was added. The absorbance of the chromophore formed was read at546 nm[25].

Antiproliferative activity

Cell culture

Human cervical cancer cell line (HeLa) and hepatocellular carcinoma (HepG2) were purchased from National Center for Cell Science, Pune, India, and were maintained in Duelbacco Minimum Eagles Medium supplemented with 100U/mL of Penicillin G, 100µg/mL of Streptomycin, 0.25µg/mL of Amphotericin and 10% heat inactivated fetal Bovine Serum at 37C 5% CO2 and 90 % humidity through the study.

Cytotoxicity

Antiproliferative activity of *S.corymbosa* methanol extract (dissolved in 0.5% dimethyl sulphoxide) was tested in HeLa and HepG2 cells, using the MTT assay as described earlier [26]. The cells were seeded in 96-well plates (Sarstedt, USA) with 1×106 cells/well and incubated 72 h at 37°C. The cells were treated with five different concentrations of the plant extract (31.25, 62.50, 125, 250 and 500 µg/mL). After incubation, 20 µL/ well (50 mg/10 mL PBS) of MTT in phosphate buffered saline was added and incubated for 1 h. 100 µL of Stop mix solutions [10 g SDS (w/v), 0.6 mL aectic acid (v/v) and set volume to 100 mL with DMSO, pH, 4.5] was added each well to stop the reaction and shaked for half an hour. Absorbance was read at 550 nm using an Elisa reader. The percentage growth inhibition and percentage viability of the culture were calculated according to the following equation. % Cell viability = Mean absorbance in test wells/ Mean absorbance in control wells ×100

Statistical analysis

The data were subjected to a one way analysis of variance and the significance of the difference between means was determined by Duncan's multiple range tests using the SPSS 19. Values expressed were means± SD or SE. *P*-values < 0.05, < 0.01 were regarded to be significant.

RESULTS

The percentage of yield, total phenolics, tannins and flavonoid contents of aerial extracts of *S. corymbosa* are presented in Table 1. The highest extract yield (25.71%) was obtained in methanol followed by ethyl acetate (18.85%) and petroleum ether (14.49%). Similarly, methanolic extract recorded the highest total phenolics (124.05± 11.55 mg GAE/ g extract), tannins (92.45±4.38mg GAE/g extract) and flavonoid (79.81± 5.13 mg RE/100 g extract) contents followed by petroleum ether and ethyl acetate extracts. Chloroform extract displayed very low phenolics and tannin contents, whereas hot water extract gave lowest level of flavonoids.

The proton-radical scavenging action has been known as an important mechanism of antioxidation. DPPH radical is a stable organic free radical which has been extensively used for evaluating the free radical scavenging potential of natural antioxidants.

Table 1: Yield percent, total phenolics, tannins and flavonoid contents of different solvent extracts of *S. corymbosa*

Solvents	Yield (%)	Total phenolics (mg GAE/g extract)	Tannin (mg GAE/g extract)	Flavonoids (mg RE/g extract)
Petroleum ether	14.49	83.30± 4.04^{b}	46.17±5.33^{b}	55.42± 3.33^{b}
Chloroform	9.12	27.17± 2.57^{e}	7.87±1.32^{e}	24.81± 2.49^{d}
Ethyl acetate	18.85	56.98± 2.23^{c}	27.24±2.45^{c}	33.65± 2.00^{c}
Methanol	25.71	124.05± 11.55^{a}	92.45±4.38^{a}	79.81± 5.13^{a}
Hot water	11.89	42.42±1.47^{d}	17.78±3.31^{d}	11.01± 2.82^{e}

GAE, gallic acid equivalents/g extract, RE, rutin equivalents/g extract.

Values are means of three replicate determinations (n=3) ±standard deviation, values followed by different superscript in a column are significantly different (*P* < 0.05).

In the present study, concentration of the sample extract necessary to decrease initial concentration of DPPH• by 50% (IC_{50}) under experimental condition has been calculated and expressed in Figure 1. A lower value of IC_{50} indicates higher antioxidant activity. Higher DPPH• scavenging activity was shown by methanol extract (23.54µg/mL) followed by petroleum ether extract (32.56µg/mL). These values were significantly higher over positive standards BHA (34.59µg/mL) and BHT (45.56µg/mL).

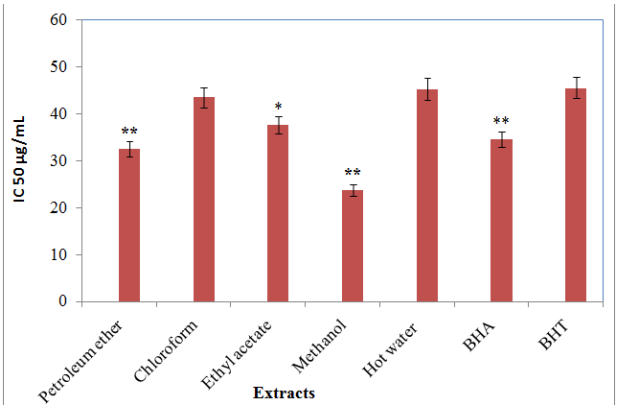

Fig. 1: DPPH radical scavenging activity of different solvent extracts of *S. corymbosa*

Values represent mean ± SE of three repeated experiments. Error bars indicates ±SE

*, ** Significant at *P*<0.05, *P*<0.01 respectively.

$ABTS^{\bullet+}$ scavenging is based on the ability of antioxidants to quench the long-lived ABTS radical cation, a blue/green chromophore with characteristic absorption at 734 nm. Trolox, a water-soluble analog of vitamin E (α-tocopherol), is used as a positive control. The activity is expressed in terms of the Trolox-equivalent antioxidant capacity of the extract (µmol/g sample extracts). The efficacy of ABTS cation radical scavenging activity of various solvent extracts of *S. corymbosa* is shown in Table 2. The methanol extract showed as highest scavenger [(32711.05± 339.94) µmol TAA/g extract] of $ABTS^{\bullet+}$.

Table 2: FRAP, ABTS cation radical scavenging and metal chelating activity of different solvent extracts *S. corymbosa*

Sample	FRAP mmol Fe(II)/g extract[a]	ABTS µmol Trolox/g extract[b]	Metal chelating activity mg EDTA/g extract[c]
Petroleum ether	1600.61±103.45[b]	29307.47± 723.32[b]	97.35±1.50[b]
Chloroform	1356.01±90.14[c]	17386.46± 535.56[d]	45.85±3.45[d]
Ethyl acetate	1410.98±63.47[c]	21833.01± 625.95[c]	67.24±1.82[c]
Methanol	1955.29±35.14[a]	32711.05± 339.94[a]	127.93±10.05[a]
Hot water	1227.66±21.18[d]	12786.82± 361.36[f]	61.98±4.68[c]
BHA	1213.89±80.86[d]	21682.08± 359.47[c]	62.37±2.14[c]
BHT	1097.87±10.76[e]	16814.31±512.37[e]	45.04± 1.65[d]

[a]mmol of ferrous equivalents / g extract; [b] µmol of Trolox equivalents/ g extract; [c] mg of EDTA equivalent/g extract; BHA- Butylated hydroxyanisole; BHT- Butylated hydroxytoluene

Values are means of three replicate determinations (n=3) ±standard deviation, values followed by different superscript in a column are significantly different ($P < 0.05$).

The FRAP values for different solvent extracts of *S. corymbosa* are shown in Table 2. Among the various solvent extracts, methanol extract (1955.29±35.14 mmol Fe (II)/mg extract) showed higher ferric reducing antioxidant activity. All the extracts exhibited significant reducing ability higher than that of positive controls BHA (1213.89±80.86 mmol Fe (II)/mg) and BHT (1097.87±10.76 mmol Fe (II)/mg). This might be due to the presence high phenolic, tannins and flavonoids content of these plant extracts.

The scavenging ability of *S. corymbosa* extracts on superoxide radicals are shown in Figure 2. Methanolic extract showed highest superoxide radicals scavenging activities (75.74 %) at a level of 100µg in the reaction mixture, which is comparable to BHA and BHT. Petroleum ether extract showed next higher (64.56 %) scavenging activity, whereas water extracts revealed low scavenging activity.

The chelating effect on the ferrous ions by the various solvent extracts of *S. corymbosa* is shown in Table 2. Metal chelating activity increased with increasing concentration of the extracts. All the sample extracts exhibited the ability to cheated metal ions. Among the different extracts tested, the methanol extract showed higher activity (79.81± 5.13 mg EDTA/ g extract) followed by petroleum ether and ethyl acetate extracts.

The hydroxyl radical scavenging potential of various solvent extracts of *S. corymbosa* is shown in Figure 3. Each extract showing hydroxyl radical scavenging activity increased with increasing concentration of sample extracts. In our study, methanol (52.42%) extract is found to be comparable with reference standards BHA (65.66%) and BHT (78.11%). Petroleum ether extract showed (45.82%) hydroxyl radical scavenging activity and hot water extract exhibited lowest scavenging activity (19.32 %).

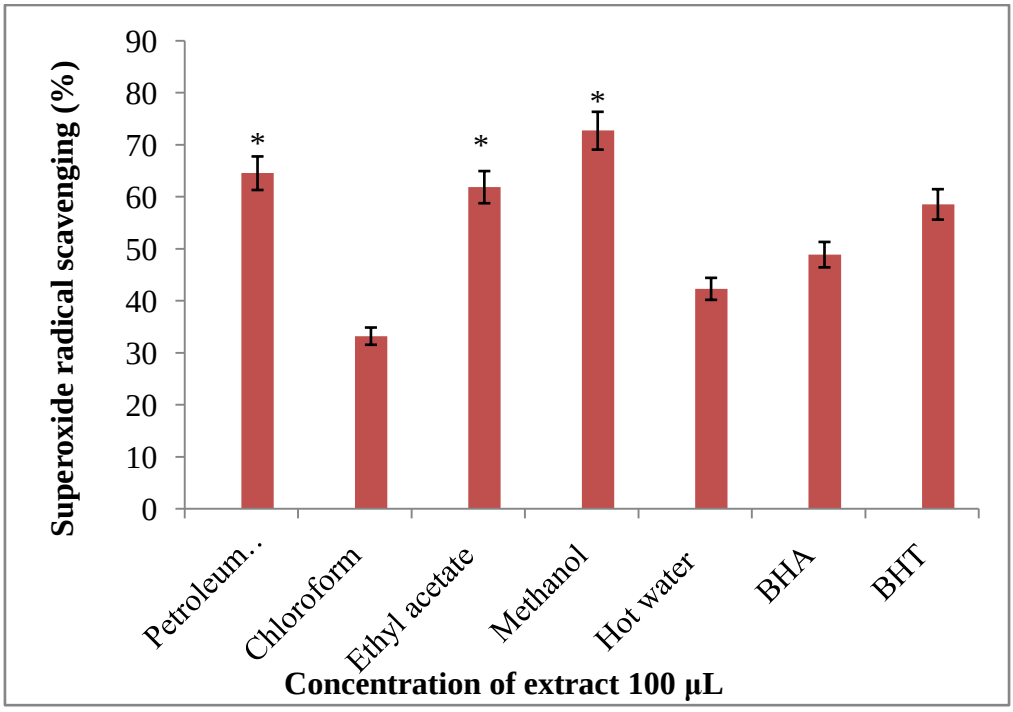

Fig. 2: Superoxide radical scavenging activity of different solvent extracts *S. corymbosa*

Values represent mean ± SE of three repeated experiments. Error bars indicates ±SE

* Significant at $P<0.05$

The scavenging activity of the extract against nitric oxide released by sodium nitroprusside was investigated and percentage inhibitory is shown in Figure 4. Among the various solvent extracts tested, methanol extract (70.15%) registered higher antioxidant activity. All extracts showed reducing power but not at the same level. The order of nitric oxide activity of various sample extracts is as follows: BHA>ME>PE>BHT>EA>CH>HW.

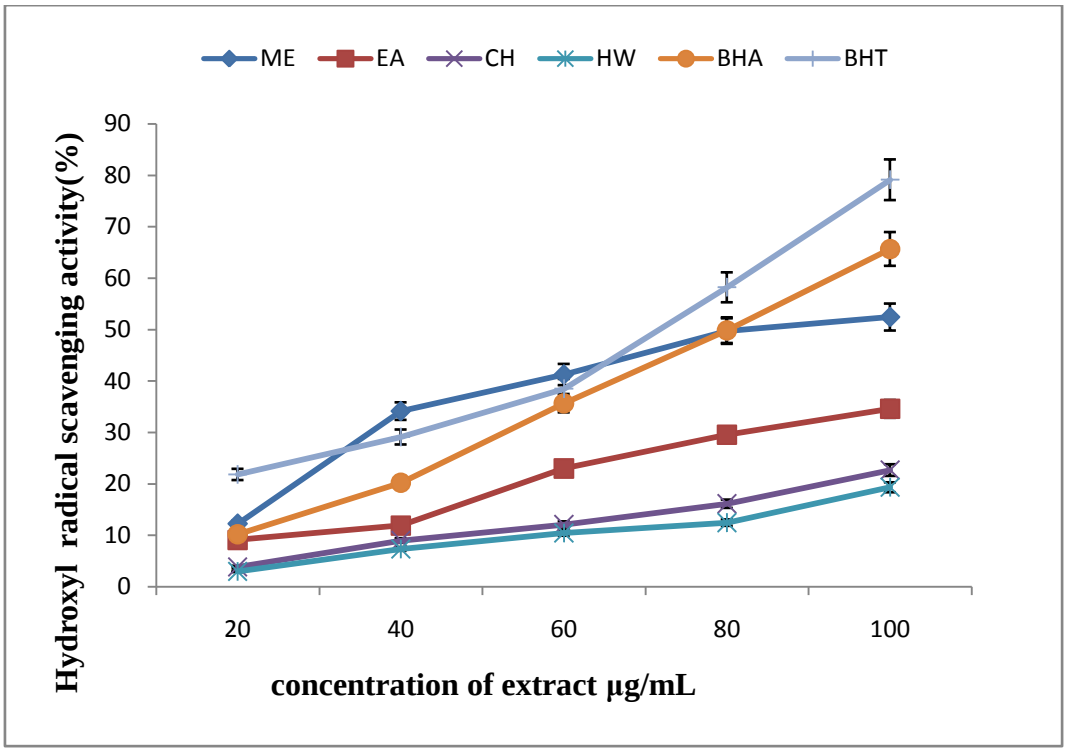

Fig. 3: Hydroxyl radical scavenging activity of different solvent extracts *S. corymbosa*

Values represent mean ± SE of three repeated experiments.

PE- petroleum ether extract, CH - chloroform extract, EA - ethyl acetate extract, ME - methanol extract, HW-hot water extract, BHT- Butylated hydroxytoluene, BHA-Butylated hydroxyanisole.

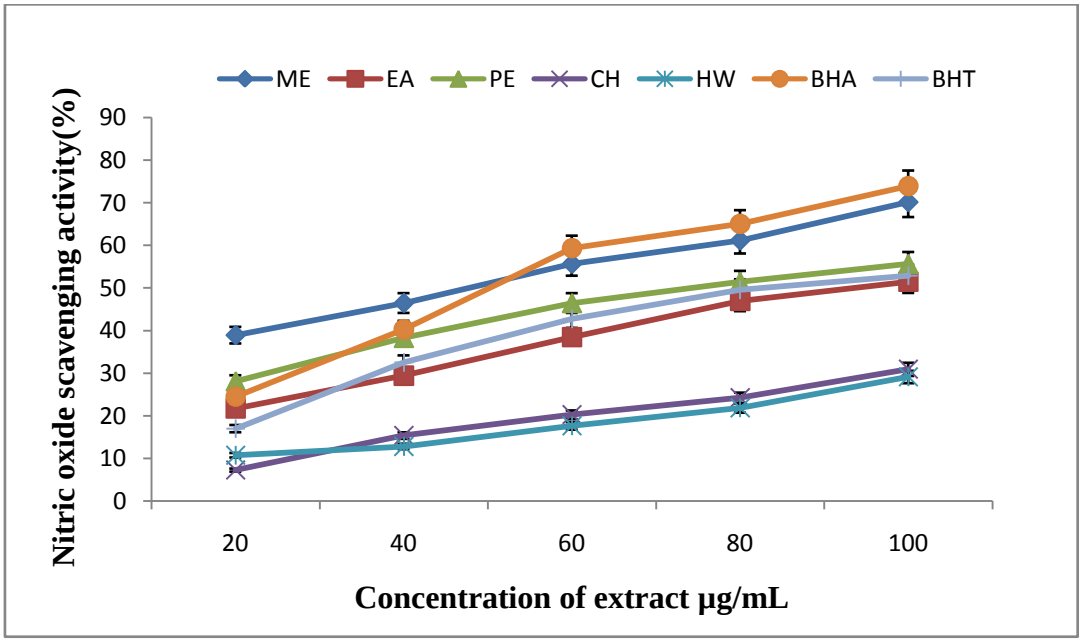

Fig. 4: Nitric oxide radical (NO•) scavenging activity of different solvent extracts *S. corymbosa*

Values represent mean ± SE of three repeated experiments.

PE- petroleum ether extract, CH - chloroform extract, EA - ethyl acetate extract, ME - methanol extract, HW-hot water extract, BHT- Butylated hydroxytoluene, BHA-Butylated hydroxyanisole.

S. corymbosa methanol extract was selected to study the antiproliferative activity on HeLa (human cervical cancer cell line) HepG2 (hepatocellular carcinoma) cells based on the antioxidant activity results. The assay data showed that methanol extract strongly inhibited the proliferation of HeLa and HepG2 cell lines with the IC_{50} values of 87.90 and 61.64µg/mL respectively and the cytotoxic activity increased in a dose dependent manner (Table 3).

Table 3: Anti-proliferative activity of *S. corymbosa* methanol extract

HeLa			HepG2		
Concentration µg/mL	**% inhibition**	**IC_{50} value µg/mL**	**Concentration µg/mL**	**% inhibition**	**IC_{50} value µg/mL**
31.25	30.75		31.25	37.26	
62.50	43.91	87.90	62.50	55.59	61.64
125.00	56.94		125.00	62.48	
250.00	67.89		250.00	65.68	
500.00	80.44		500.00	66.91	

Values are means of three replicate determinations (n=3)

DISCUSSION

Polyphenols compounds are well known as antioxidant and scavenging agents against free radicals associated with oxidative damage[27]. The presence of these compounds such as tannins, flavonoids and phenols in *S. corymbosa* extract may give credence to its local usage for the management of oxidative stress induced ailments. Tannins have been used traditionally for the treatment of diarrhea, hemorrhage and detoxification[28, 29]. The composition of tannins as observed in this study may justify its traditional usage for the management of diarrhea. Flavonoids are important secondary metabolite of plant modulating lipid peroxidation involved in atherogenesis, thrombosis and carcinogenesis. It has been confirmed that pharmacological effect of flavonoids is correlating with their antioxidant activities[30,31]. Furthermore, the ethanomedicinal usage of *S. corymbosa* extract might be attributed to the high concentration of flavonoids and therefore it could support its usage for the management of obesity and diabetes. The phenolic compounds have direct antioxidative activity due to their hydroxyl groups and were found to play an important role in stabilizing lipid peroxidation[32]. Therefore, the concentration of this compound as shown in this study could contribute synergistically to the significant antioxidant potency of this plant and thus may support the local usage for the treatment of radical related diseases.

The reaction of plant extract with purple coloured DPPH radical converted the radical to α, α diphenyl-β-picryl hydrazine due to the extract antioxidant property. The degree of discoloration indicates the potential of the plant extract to scavenging free radical due to its ability to donate hydrogen proton. In DPPH radical scavenging activity, the highest activity was observed in methanol extract. These values were significantly higher over positive standards BHT and BHA. The result obtained from the present study concurred with the findings of Loganayaki and Manian[33], Senthilkumar et al[34] and Ganie et al[35] on *Crataeva magna*, *Rhodiola imbricatea* and *Arnebia benthamii* respectively who have attributed the antioxidant potential of these plants to high concentration of phenolics compounds. Consequently, the strong antioxidant activity of *S. corymbosa* as shown in the present study might be related to the high contents of phenolics compounds.

$ABTS^{\bullet+}$ decolourization assay measures the total antioxidant capacity in both lipophilic and hydrophilic substances. The effect of the antioxidant concentration and the duration of the inhibition of the radical cation's absorption are taken into account when the antioxidant activity is determined. The advantages of this radical are its water-solubility and high absorption coefficient at long wavelengths, allowing the determination of its rate of consumption with minimal interferences[36]. In this assay, total antioxidant activity (TAA) reflects the ability of hydrogen donating antioxidants to scavenge $ABTS^{\bullet+}$, comparable with that of Trolox. Though all the extracts exhibited considerable $ABTS^{\bullet+}$ scavenging activity, the methanol extract exhibited stronger TAA (32711.05± 339.94 µmol/g) followed by petroleum and ethyl acetate extracts with TAA values of 29307.47± 723.32 and 21833.01± 625.95 µmol/ g respectively. In the present study, the order of scavenging activity of sample extracts is methanol extract > petroleum ether > ethyl acetate extract > BHA > chloroform extract > BHT > Hot water. These results suggest that the investigated extracts may prevent or retard the *in vitro* formation of radical species related with oxidative stress. The extensive investigations on antiradical and antioxidant activities of small phenolics, including flavonoids and phenolic acids have been reported[31,37]. Apart from these, Hagerman et al[38] have reported that the high molecular weight phenolics (tannins) have more ability to quench free radicals ($ABTS^{\bullet+}$) and that effectiveness depends on the molecular weight, the number of aromatic rings and nature of hydroxyl groups substitution than the specific functional groups. In similar lines, the present study also reveals higher $ABTS^{\bullet+}$ scavenging activity in the methanol extract of *S. corymbosa* that has registered the highest contents of tannins (Table 1). This is in agreement with the reports of Chen et al[39] that the higher ABTS•+ scavenging activity of ethanolic extract of *Swertia chirayita* is due to its high phenolic content.

The simple and reliable test measures the reducing potential of an antioxidant reacting with a ferric-TPTZ (Fe (III)-TPTZ) complex and producing a colored ferrous-TPTZ (Fe (II))-TPTZ) complex by a reductant at low pH, was adopted. A higher absorbance power indicates a higher ferric reducing power. In the present study, methanol extract showed high (1955.29±35.14 mmol/g) reducing activity fallowed by petroleum ether extract also exhibited a high (1600.61±103.45mmol/g) reducing activity (Table 2). The antioxidant efficiency determined by the present FRAP assay depends on the redox potentials of the compounds under study, characterized by the complexity of their molecules. From our results, it is apparent that the reducing ability of *S. corymbosa*, as determined by the FRAP assay, seems to depend on the degree of hydroxylation and extent of conjugation of the phenolic compounds.

The cellular damage resulting from hydroxyl radicals is strongest among free radicals, hydroxyl radicals can be generated by biochemical reaction. Superoxide radical is converted by superoxide dismutase to hydrogen peroxide, which can subsequently produced extremely reactive hydroxyl radicals in the transition metal ions such as iron and copper or by UV photolysis. DNA is susceptible to oxidative damage and hydroxyl radicals oxidize guanosine or thymine to 8-hydroxyl-2-deoxyguanosine and thymine glycol which change DNA and lead to mutagenesis and carcinogenesis[34]. In the present study, ascorbic acid iron-EDTA was used to generate hydroxyl radical. All the extract exhibited •OH scavenging activity ranging between 33.21% and 72.74 % at 100µg/mL concentration in the reaction mixture. Generally, higher levels of •OH scavenging activity exhibited by methanol extract in the present study could be attributed to the active hydrogen donor ability of hydroxyl substitution provided by phenolic flavonoids extracted in the early stages of successive extraction. It may be concluded that the ability of different solvent extracts of *S. corymbosa* aerial parts to quench hydroxyl radicals seems to be directly related to the prevention of lipid peroxidation, thus reducing the rate of chain reaction.

Photochemical reduction of nitroblue tetrazolium (NBT) in the presence of riboflavin. When the riboflavin is photochemically activated, it reacts with the NBT to give NBTH that leads to formazan according to the reaction. In presence of oxygen, concentrations of

radical species are controlled by the quasi equilibrium. Thus, superoxide anions appear indirectly when the test is performed under aerobic conditions. In the presence of an antioxidant that can donate an electron to NBT, the purple color typical of the formazan decays, a change that can be followed spectrophotometrically at 560 nm. All of the extracts had a scavenging activity on the superoxide radicals in a dose dependent manner.

The method of metal chelating activity is based on chelating of Fe^{2+} ions by the reagent ferrozine, which is quantitative formation of a complex with Fe^{2+} ions. The formation of a complex is probably disturbed by the other chelating reagents, which would result in the reduction of the formation of red colored complex. In this assay, *S. corymbosa* extracts and standard antioxidant compounds interfered with the formation of ferrous and ferrozine complex, suggesting that they have chelating activity and capture ferrous ion before ferrozine. Among the different solvent extracts tested, methanol extracts showed better scavenging ability compared to other solvent extracts. The extracts may be able to play a protective role against oxidative damage by sequestering iron (II) ions that may otherwise catalyze Fenton-type reactions or participate in metal-catalyzed hydroperoxide decomposition reactions[40]. The iron (II) chelating properties of the additives may be attributed to their endogenous chelating agents, mainly phenolics. Certain phenolic compounds have properly oriented functional groups, which can chelate metal ions. Chelating agents may serve as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ion[41]. Accordingly it is suggested that the low to moderate ferrous ion chelating effects of the different solvent fractions of *S. corymbosa* would be somewhat beneficial to protect against oxidative damages.

Nitric oxide (•NO) is an abundant reactive species that acts as an important biological signaling molecule in a large variety of diverse physiological processes, including neurotransmission, blood pressure regulation, defense mechanisms, smooth muscle relaxation and immune regulation[42]. However, when present in high concentrations, it can be associated with several types of biological damage, such as lipid peroxidation, protein oxidation and nitration, enzymes inactivation and DNA damage[43]. In addition, it quickly reacts with the superoxide radical to form peroxy nitrite; a major damaging oxidant produced *in vivo*[44]. In the performed method NO was generated from sodium nitroprusside (SNP) and was measured by the Griess reagent. In aqueous solution at physiological pH, SNP spontaneously generates NO, which interacts with oxygen to produce nitrite ions that can be estimated by the use of Griess reagent. Scavengers of NO compete with oxygen leading to reduce the production of NO. As shown in Figure 4, methanol extract also exhibited concentration dependent activity for NO radical scavenging capacity. Thus, besides the scavenging capacity observed for both superoxide radical and nitric oxide, *S. corymbosa* may also prevent the formation of other deleterious species, like peroxynitrite.

The IC_{50} of extract on cell line less than100 µg/mL is categorized as a potential cytotoxic and toxic substance [45,46]. In the present study, methanol extract of *S. corymbosa* was found to be best cytotoxic towards human cervical cancer cell line (HeLa) and hepatocellular carcinoma (HepG2) cells in 72 h MTT assay and the concentration required for 50% cell death were 87.90 and 61.64 µg/mL respectively. The methanol extract of *S. corymbosa* exhibited both the highest antioxidant and anticancer activities in compare to other extracts. The cytotoxicity of methanol extract can be related to the antioxidant activity and synergism effect of multi-component in extract. Several active compounds contained in the aerial parts of this species have been revealed as a scavenging radical which may be able to inhibit carcinogenesis. xanthone is major compound in the genus and showed its cytotoxicity in HeLa cells through both mitochondrial dysfunction and ER stress cell death pathways[47]. Imai et al[48] determined that flavonoid effectively suppressed the proliferation of a human colon carcinoma cell line, COLO 201, through apoptosis induction while phenolics showed anticancer activity on cancer colon cell by arresting the cell cycle[49].

In the present investigation, the overall results from *in vitro* experiments, including determination of total phenolics, tannins and flavonoids contents, DPPH, ABTS radical scavenging activity, FRAP assay, hydroxyl radical, superoxide anion scavenging activity, metal chelating activity, antiproliferative activity on HeLa and HepG2 cells demonstrated that methanol extract of *S. corymbosa* have a significant effect on antioxidant and antiproliferative activities. Further investigation is currently underway to figure out the mode of action and to identify specific phytochemicals responsible for their antioxidant and anti-proliferative activities.

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