Research Article

IN VITRO EVALUATION OF ANTIOXIDANT ACTIVITY OF SKIMMIA ANQUETILIA LEAVES EXTRACTS

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DOI: 10.7897/2230-8407.050330**ABSTRACT**

The purpose of the present study is to evaluate *in vitro* antioxidant and free radical scavenging potential of different organic and aqueous fractions of *Skimmia anquetilia* Tayl. Initially the phytochemical constituents of the plant were extracted in methanol and then methanolic extract was dissolved in dist. water and fractionated with *n*-hexane, dichloromethane, ethyl acetate and *n*-butanol respectively. Eight methods were used for the determination of antioxidant potential of the fractions such as “2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical cation scavenging”, the “ferric reducing antioxidant power (FRAP)”, “2,2'-diphenyl-1-picrylhydrazil (DPPH) radical scavenging”, “total phenolic contents (TPC)”, “total flavonoid contents (TFC)”, “total antioxidant activity by phosphomolybdenum method”, “superoxide anion radical scavenging activity” and “metal chelating activity”. The results showed that in ABTS assay the trolox equivalent antioxidant capacity (TEAC) values for different fractions ranged from 8.87-0.95 µg/ml. EtOAc fraction showed highest activity as compared to other fractions, having EC₅₀ value 2.87 µg/ml. It also exhibited highest FRAP value (146.28 mM/mL FeSO₄ equivalents) as well as highest total phenolic contents (3.04 mg/ml GAE) and highest DPPH radical scavenging activity. The remaining aqueous fraction exhibited highest superoxide anion radical scavenging activity (99.5 %). Dichloromethane fraction showed highest total antioxidant activity i.e. 0.55 ± 0.25. Crude methanolic extract exhibited highest total flavonoid contents (1698 mg/g quercetin equivalents) as well as highest metal chelating activity 73.5 %. The amass results of different assays showed that *S. anquetilia* is affluent source of antioxidants.

Keywords: *Skimmia anquetilia* Tayl., DPPH assay, total phenolics, metal chelating activity, total flavonoids.

INTRODUCTION

Plants have been a rich source of therapeutic agents for many thousand years. Varieties of modern drugs have been isolated and prepared from natural sources. Higher plants contain variety of medicinal compounds and therefore play an important role for human health maintenance since ancient times¹. Man was completely dependent upon various medicinal plants, before exploration of the synthetic drugs for the treatment of many diseases². Oxidative damage of lipids, proteins, DNA and other small cellular molecules by the oxidants is the main cause for the variety of diseases as well as age related degenerative conditions³ including cancer, mutation, inflammatory neurodegenerative diseases e.g. Alzheimer's disease and cardiovascular diseases⁴⁻⁶. During some physiological conditions the free radicals are generated inside the body, causing cellular damages, which can be neutralized by antioxidants obtained from different medicinal plants. Affluent studies have predicted that antioxidant nutraceuticals derived from plants, scavenge the free radicals and modulate the oxidative stress related destructive effects^{7,8}. The dietary supplements taken by man can increase the antioxidant systems of body⁹. So far, the search for the natural antioxidants originated from the plants has remarkably enhanced in recent years¹⁰. *Skimmia anquetilia* Tayl. and Airy Shaw is a shrub which belongs to the family Rotaceae¹¹. It is distributed in Western Asia, India (Uttar Pradesh, Jammu and Kashmir, Himachal Pradesh), Nepal, Pakistan, Afghanistan^{12,13}. It is widely used as a traditional herbal medicine. It is used by local people as flock recopies. In the Rawain valley of the Uttarkashi Uttarakhand, India, the paste of its leaves made with turmeric, is used for treatment of swellings, rheumatism and therapy. Powder of its bark is used for the healing of burns and wounds^{14,15}. Its leaves are used for the treatment of headache and smallpox¹¹ as well as for freshness¹⁶. In Tons Valley Garhwal Himalaya, its leaves

are used for preparation of the traditional alcoholic beverage, locally known as soor¹⁷, which is found rich in energy as compared to proteins and carbohydrates, but is devoid of all nutrients¹⁸. The objective of this study is to evaluate the antioxidant potential of various fractions of *S. anquetilia* leaves' extract by eight methods i.e. “2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical cation scavenging”, the “ferric reducing antioxidant power (FRAP)”, “2,2'-diphenyl-1-picrylhydrazil (DPPH) radical scavenging”, “total phenolic contents (TPC)”, “total flavonoid content (TFC)”, “total antioxidant activity by phosphomolybdenum method”, “superoxide anion radical scavenging activity” and “metal chelating activity” along with the phytochemical screening of the crude fractions.

MATERIALS AND METHODS**Plant Material**

Leaves of *Skimmia anquiltilia* were obtained Swat valley (Kalam), Khyber Pakhtunkhwa, Pakistan.

Extraction and Fractionation of Antioxidants

The plant material (1.12 kg) was shade dried at room temperature and coarsely ground, then extracted with methanol and evaporated at 30°C using rotary evaporator to yield the residue which was dissolved in distilled water (1.5 L) and partitioned with *n*-hexane (1 L × 4), dichloromethane (1 L × 4), ethyl acetate (1 L × 4) and *n*-butanol (1 L × 4), respectively. These organic fractions were separately subjected to rotary evaporator to obtain their residues. The remaining aqueous layer was also concentrated in a similar way. For this study, the crude methanolic extract, organic fractions and the aqueous fraction were used to evaluate their *in vitro* antioxidant potential.

Chemicals and Standards

All the chemicals were analytical grade. Methanol, dichloromethane, *n*-butanol, *n*-hexane, ethyl acetate, distilled water, glacial acetic acid, potassium persulphate, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, iron (III) chloride, hydrochloric acid, sodium phosphate, sulphuric acid, ammonium molybdate, sodium chloride, sodium acetate were purchased from Merck. Trolox ("6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid"), ABTS ("2,2'-azinobis-(3-ethylbenzo-thiazoline-6-sulphonic), diammonium salt"), gallic acid, ascorbic acid, glutathione reduced, butylated hydroxy anisole (BHA), uric acid, 2,2'-methylene bis(6-*tert*-butyl-4-methyl phenol), butylated hydroxy toluene (BHT), kaempferol, blue chloride (NBT), phenoxazinemetho sulphate (PMS) and ferrozine were obtained from Aldrich Chemical Co., Gillingham, Dorset, UK.

Phytochemical Screening

The organic and aqueous fractions of *S. anquiliteia* leaves' extract were used for the phytochemical screening for detection of various compounds which include tannins, flavonoids, alkaloids, saponins, terpenoids, anthraquinone, sugars and steroids applying standard procedures^{19,20}.

Antioxidant Assays

The following antioxidant assays were performed to check the antioxidant potential of the studied fractions.

ABTS⁺ Assay (Trolox Equivalent Antioxidant Capacity)

The already developed ABTS⁺ assay protocol Re *et al.*²¹ was followed. The dilution of 7 mM of ABTS with water was prepared. ABTS radical cation (ABTS⁺) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowed the mixture to stand in the dark at room temperature for 12-16 hours before use. For the study of antioxidant activity of the standard reference and plant samples, the ABTS⁺ stock solution was diluted either with PBS (phosphate buffered saline) (pH 7.4) or methanol to an absorbance of 0.70 (+ 0.02) at 734 and 745 nm respectively and equilibrated at 30°C. For plant extracts, the dilution was made in the respective solvents. After addition of 10 µl of neat or diluted stock solution to 2.99 ml of diluted ABTS⁺ solution ($A = 0.700 + 0.020$), the absorbance reading was taken at 25°C, exactly 1 minute after initial mixing up to 8 minutes. Appropriate solvent blanks were run in each assay. All determinations were carried out at least three times, and in triplicate at each separate concentration level of the standards. The percentage inhibition was calculated by the following formula and was plotted as a function of concentration of antioxidants and of trolox for the standard reference data.

$$\% \text{ inhibition (at 734 nm)} = (1 - A_f/A_o) \times 100$$

Where A_o is the absorbance of radical cation solution before addition of sample/standard antioxidants while A_f is the absorbance after addition of the sample/standard antioxidants. Each measurement was made in triplicate and at least three times at each concentration level of standards and sample.

Total Phenolic Contents

Total phenolic contents of were determined according to standard method²². Stock solution of gallic acid was prepared by dissolving 0.5 g gallic acid in 10 ml of ethanol and then adding 90 ml of water. Sodium carbonate solution was prepared by dissolving 200 g of anhydrous sodium carbonate in 800 ml of double distilled water, boiling and subsequent cooling of the solution. Then a few crystals of sodium

carbonate were added. After 24 hours the solution was filtered and then the volume was raised with double distilled water up to 1 L. To prepare a calibration curve various concentrations of gallic acid i.e. 50, 100, 150, 250 and 500 mg/ml was prepared by adding double distilled water. 40 µl of each sample and blank, were taken into separate cuvettes, and to each 3.16 ml of double distilled water and 200 µl Folin-Ciocalteu's reagent was added, and mixed well. After 8 minutes, 600 µL of sodium carbonate solution was mixed and the solution was allowed to stand at 40°C for 30 minutes. At 765 nm the absorbance of each solution was noted against the blank (without phenolic solution). The concentration of total phenolic compounds of each fraction in milligram of Gallic acid equivalent (GAE) was calculated using standard equation.

$$\text{Absorbance} = 0.118x + 0.0824 \text{ (Gallic acid equivalents mg/ml)}$$

DPPH Radical Scavenging Activity

The DPPH radical scavenging abilities²³ of different fraction were compared with that of known antioxidants (BHA and trolox). DPPH solution (3 ml, 25 mg/L) in methanol was mixed with appropriate volumes of diluted sample solutions. The progress of the reaction in the mixture was monitored at 515 nm. Upon reduction, the color of the solution was changed. The percent inhibition of the DPPH radical was calculated according to the following formula:

$$\% \text{DPPH}_{\text{rem}} = (\text{DPPH})_{\text{rem}}/(\text{DPPH})_{t=0} \times 100$$

A graph was plotted showing the scavenging of DPPH radical in terms of decrease in absorbance at 515nm with time (min) for each fraction.

Metal Chelating Activity

Ferrous ion (Fe^{++}) chelating activity was done according to the method employed by Dinis *et al.*²⁴ 100 µl of sample was added to a solution of 2 mM FeCl_2 (0.05 ml). Ferrozine (0.2 ml of 5 mM) was added and total volume was adjusted to 4 mL by ethanol. Then, the mixture was shaken vigorously and left standing at room temperature for ten minutes. The absorbance of the solution was then measured at 562 nm using spectrophotometer. The percentage of inhibition of ferrozine- Fe^{2+} complex formation was calculated using the formula given below:

$$\% \text{ Chelating Activity} = ((A_{\text{Control}} - A_{\text{Sample}})/A_{\text{Control}}) \times 100$$

Where A_{Control} is the absorbance of the control, and A_{Sample} is the absorbance in the presence of the sample.

Total Antioxidant Activity

Total antioxidant activity was determined by phosphomolybdenum complex formation method²⁵. Each sample (50 µg/ml) was mixed separately with 4 ml of a reagent solution (28 mM sodium phosphate, 0.6 M sulphuric acid and 4 mM ammonium molybdate) in a test tube. The 4 ml reagent solution was used as a blank. The test tubes were capped, incubated at 95°C for 90 minutes in water bath, then cooled at room temperature. The absorbance was measured at 695 nm against blank. Samples' absorbance was noted in triplicate.

Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric reducing antioxidant power of the studied fractions was measured according to the already reported method of Benzie and Strain²⁶. Fresh working stock solution was prepared which contained 25 ml of 300 mM acetate buffer (pH 3.6), 2.5 mL of 10 mM TPTZ (2,4,6-tripyridyl-s-triazine)

solution in 40 mM HCl solution and 2.5 ml of 20 mM ferric chloride solution. The mixture was warmed at 37°C before using. Then 3 ml of FRAP reagent, 100 µl of sample and 300 µl of distilled water was mixed. The Absorbance was noted at 593 nm after every minute for 6 minutes and compared with standard curve of ferrous sulphate.

Superoxide Anion Radical Scavenging Activity

Superoxide anion radical scavenging activity was measured according to standard method of Nikishimi *et al*²⁷. Superoxide radicals generating in phenazine methosulfate-nicotinamide adenine dinucleotide (PMS-NADH) system were used against the studied fractions. The reaction mixture contained 100 µL extract, 200 µM NBT, 624 µM NADH, and 80 µM PMS in 0.1 M phosphate buffer (pH 7.4). After 2 minutes of incubation, absorbance was noted at 560 nm. The scavenging ability was measured using the following formula:

$$\text{Percent scavenging} = (1 - A_s/A_b \times 100)$$

Where A_s and A_b are the absorbance of sample and blank solutions at 560 nm, respectively.

Total Flavonoid Contents

Total flavonoid contents of the studied fractions were measured by already described method of Dewanto *et al*²⁸. The 250 µl of each fraction and quercetin standard solutions were mixed with 1.25 ml of water in a test tube and then 75 µl of 5 % NaNO₂ solution was added. After 5 minutes 150 µl of 10 % AlCl₃.6H₂O solution was added and allowed to stand for 6 minutes. Added 0.5 mL of 1M NaOH and the volume of the mixture was raised up to 2.5 ml with distilled water. Absorbance was measured immediately against blank at 510 nm and compared to a standard curve of quercetin. The flavonoid contents were expressed as mg of quercetin equivalent per gram of residue.

RESULTS

The phytochemical screening was done on all the studied fractions. Tests were performed for the detection of alkaloids, terpenoids, saponins, tannins, sugars, flavonoids and anthraquinones and the results have been shown in Table 1. ABTS assay was performed on all the organic and aqueous fractions to evaluate their free radical scavenging potential and TEAC values were obtained. Their EC₅₀ values were also calculated. The results have been shown in Figure 1. Total phenolic contents were determined and the Figure 2 shows the phenolic concentration in the six studied fractions, expressed as the mg/ml gallic acid equivalents (GAEs). DPPH assay was performed on all the studied fractions and percent scavenging of the DPPH radical was calculated along with EC₅₀ values. The values of percent scavenging of DPPH radical have been shown in Figure 3. Metal chelating activity of and the percentage of bound iron of various organic fractions as well as that of aqueous fraction was determined and the results have been represented in Figure 4. The total antioxidant activities of all the studied fractions was measured spectrophotometrically by Phosphomolybdenum complex formation method and compared with the standard antioxidant BHT and the results have been shown in Figure 5. The FRAP values of the studied fractions were calculated by comparison with a calibration curve obtained using Iron (II) sulfate as the standard reductant and results have been given in Figure 6. Superoxide anion radical scavenging activities of the various fractions were also calculated and the

results thus obtained have been represented in Figure 7. The results for total flavonoid contents have been shown in Figure 8 represented as mg/g quercetin equivalents.

DISCUSSION

Phytochemical Screening

The results for phytochemical screening (Table 1) showed that anthraquinone and tannins were present in all fractions. Alkaloids were found in *n*-butanol and remaining aqueous fraction. Saponins were present in higher concentrations in *n*-butanol while absent in dichloromethane and remaining aqueous fraction. Flavonoids were found in *n*-butanol, ethyl acetate and remaining aqueous fraction. Sugars were found in all fractions except in *n*-hexane and dichloromethane fraction. Terpenoids were absent in *n*-hexane fraction.

ABTS^{•+} Assay (Trolox Equivalent Antioxidant Capacity)

The TEAC assay developed by Miller *et al*²⁹ and later modified by Re *et al*²¹ is based upon the scavenging of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonate) (ABTS^{•+}) radical cation on addition of sample antioxidants. The extent of scavenging of radical cation, which can be detected spectrophotometrically at 734 nm, can be related to the amount of antioxidants present in the sample. TEAC value can be determined for all the compounds/samples which are able to scavenge ABTS radical cation by comparing their scavenging activity with that of trolox, a water soluble analogue of vitamin E. TEAC values obtained by using the ABTS assay of organic and aqueous fraction were compared using the graph (Figure 1) and the EC₅₀ values were calculated. *n*-Hexane fraction showed the greatest EC₅₀ value i.e. 113.03 µg/ml so, it showed lowest scavenging activity. Ethyl acetate fraction has lowest EC₅₀ value (2.87 µg/ml) thus acquiring highest scavenging activity as compared to other fractions. *n*-Butanol fraction, dichloromethane fraction and crude methanolic extract also exhibited good activity having EC₅₀ values 14.4 µg/ml, 28.5 µg/ml and 31.399 µg/ml. EC₅₀ of remaining aqueous fraction was found to be 71.6 µg/ml.

Total Phenolic Contents

Phenolic compounds have been reported to be very powerful antioxidants due to presence of hydroxyl groups in their structures. The results of total phenolic contents of the studied fractions have been shown in Figure 2. The highest phenolic contents were found in ethyl acetate fraction (3.04 mg/ml GAE) while *n*-hexane has lowest phenolic contents (0.04 mg/ml GAE). Dichloromethane and *n*-butanol fraction also exhibited good amounts of phenolic contents. High TPC values may be attributed to the already reported tannins, triterpenoid, saponins, flavonoids, gallic acid, ellagic acid, oligomeric proanthocyanidins (OPCs), phytosterols in the studied plant. The phenolic contents of the plant extracts are directly correlated to the antioxidant activity which is also in agreement with the already reported results regarding other plants.

DPPH Radical Scavenging Activity

The 1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical is one of the commercially available stable free radicals at room temperature, which has widely been used for estimating scavenging activity of pure antioxidants, natural compounds and crude mixtures such as methanolic extracts of herbs/plants. The color of DPPH radical changes from violet to colorless or somewhat yellow when it accepts an electron

or hydrogen atom from the antioxidant compounds and forms a stable diamagnetic molecule. The free radical scavenging activity of aqueous and organic fractions of leaves of *S. anquetilia* was investigated. The radical scavenging activity was observed in the descending order as ethyl acetate fraction > *n*-butanol > dichloromethane fraction > remaining aqueous fraction > crude methanolic extract > *n*-hexane fraction (Figure 3). Using similar graphs EC_{50} and T_{EC50} values for each studied fractions were determined.

Metal Chelating Activity

Ferrous ion (Fe^{2+}) chelating activity by studied fractions was measured by the Ferrozine assay. Ferrozine makes complex with iron (II) which can be measured spectrophotometrically at absorbance of 562 nm. Polyphenolic compounds compete with ferrozine for the formation of this complex and the absorbance decreases. The results were expressed as percentage of inhibition of ferrozine- Fe^{2+} complex formation. The percentage inhibition of ferrozine- Fe^{2+} complex formation in terms of % bound iron was evaluated for all the fractions and results have been shown in Figure 4. The % bound iron for organic and aqueous fractions ranged from 73.5-60.8 %. All fractions showed values higher than 60 %. Transition metals especially iron (II) found in the biological systems may act as pro-oxidants. A pro-oxidant does not act as harmful agent for bio molecules directly but facilitates production of such species which may cause damage to bio molecules. Polyphenolic compounds, thus by binding with Fe (II), do not allow them to act as pro-oxidant and thus add to an indirect defense mechanism against potentially harmful radical species. The data shows (Figure 4) that all the studied fractions contain good amounts of chelating agents which are able to engage Fe (II) in the formation of complex with them.

Total Antioxidant Activity

The total antioxidant capacity of the fractions was measured spectrophotometrically by “phosphomolybdenum complex formation” method. It is based upon the reduction of Mo (VI) to the Mo (V), by the sample analyte and the subsequent formation of the green phosphate / Mo (V) compounds, having maximum absorption at the 695 nm³⁰. This method usually detects the antioxidants such as “ascorbic acid”, “flavonoids”, some “phenolics”, “tocopherols” and “carotenoids”. From the results (Figure 5), it has been observed that dichloromethane fraction have highest total antioxidant activity i.e. 0.55 ± 0.05 followed by *n*-hexane fraction (0.32 ± 0.001), ethyl acetate fraction (0.21 ± 0.02), methanolic extract (0.19 ± 0.06) *n*-butanol fraction (0.17 ± 0.01) and remaining aqueous fraction (0.09 ± 0.01) relative to butylated hydroxytoluene (BHT), a reference standard having total antioxidant activity 1.22 ± 0.09 .

Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay, as developed by Benzie and Strain involves a single electron reduction of the Fe (TPTZ)₂ (III) complex (pale yellow) to the Fe(TPTZ)₂ (II) complex (blue) by sample antioxidants at acidic pH. Any antioxidant species with lower reduction potential than that of Fe (III) TPTZ salt (0.7 V) can reduce Fe^{3+} - TPTZ to Fe^{2+} - TPTZ contributing to FRAP value²⁰. This reduction is monitored spectrophotometrically at 593 nm. Appearance of intense blue coloration indicates reducing components in the sample. The original method of Benzie and Strain uses a 4 minute interval but we noted that the reaction/color change is in progress even after 4-minute interval. Absorbance readings, therefore, were taken at a 6 minute interval after addition of sample to TPTZ reagent

allowing the reaction to reach a steady state. The FRAP values of the studied fractions were measured by comparison with a calibration curve obtained using Iron (II) sulfate as the standard reductant (Figure 6). FRAP values for different fractions ranged from 26.24 to 146.28 mM/ml. Ethyl acetate fraction exhibited highest FRAP value (146.28 mM/ml) as compared to other fractions. *n*-butanol fraction also exhibited good FRAP value i.e. 102.31 mM/ml. Higher FRAP values were obtained for the samples extracted in more polar solvents. It is evident from (Figure 6) that the polarity of the extractive solvent has great influence on the extraction of antioxidant compounds. The order of FRAP values followed the same pattern as that of the samples with increasing polarity. However, coefficient of correlation (r^2) between phenolic compounds and FRAP values was found equal to 0.686.

Superoxide Anion Radical Scavenging Activity

The studied fractions of *S. anquetilia* frequently inhibited generation of superoxide radicals. Superoxide radical is the most representative free radical. In cellular oxidation reactions, superoxide radical is normally formed first, and its effects can be magnified because it produces other kinds of cell-damaging free radicals and oxidizing agents. Superoxide and radicals actively participate in the initiation of lipid peroxidation. Oxidation of unsaturated fatty acids in biological membranes leads to formation and propagation of lipid radicals, uptake of oxygen, rearrangement of the double bonds in unsaturated lipids, and eventual destruction of membrane lipids, which produce breakdown products. The results of superoxide anion radical scavenging activity have been shown in Figure 7. It was observed from the results that dichloromethane fraction and remaining aqueous fraction exhibited very high % scavenging activity i.e. 98.1 % and 99.5 % respectively. Ethyl acetate fraction and *n*-butanol fraction also showed good values (85.2 % and 72.4 % respectively). Crude methanolic extract showed 50.8 % scavenging while *n*-hexane fraction showed lowest value (10.6 %).

Total Flavonoid Contents (TFC)

Flavonoid compounds have been reported to be very powerful antioxidants due to presence of hydroxyl groups in their structures. High TFC values may be attributed to the already reported tannins, triterpenoids, saponins, flavonoids, gallic acid, ellagic acid, oligomeric proanthocyanidins (OPCs), phytosterols in *S. anquetilia*. The results for TFC have been shown in Figure 8. It was observed that crude methanolic extract exhibited highest total flavonoid contents (1698 mg/g quercetin equivalents) due to some synergic effect. *n*-Butanol fraction also showed good TFC value i.e. 1161 mg/g quercetin equivalents.

CONCLUSION

It was concluded from the above results that leaves of *S. anquetilia* contained good amounts of antioxidants. From the results, it is evident that ethyl acetate showed highest ABTS radical cation scavenging activity as compared to other fractions, having EC_{50} value 2.87 μ g/ml. It also exhibited highest FRAP value (146.28 mM/ml $FeSO_4$ equivalents) as well as highest total phenolic contents (3.04 mg/ml GAE) and highest DPPH radical scavenging activity. The remaining aqueous fraction exhibited highest superoxide anion radical scavenging activity (99.5 %).

Table 1: Phytochemical Screening of the various fractions of *S. anquetilia* leaves' extract

Phytochemical compounds	MeOH Extract	<i>n</i> -Hexane Soluble Fraction	Dichloromethane Soluble Fraction	EtOAc Soluble Fraction	<i>n</i> -BuOH Soluble Fraction	Remaining Aqueous Fraction
Tannins	+	++	+	+	++	+
Alkaloids	-	-	-	-	+	+
Saponins	+	++	-	+	+++	-
Flavonoids	++	-	-	+	+++	+++
Sugers	++	-	-	+	++	+
Anthraquinons	+	+	+	++	+	+
Terpenoids	+	-	+	+	+	+

Note: '+' represents presence and '-' represents absence.

Figure 1: A comparative analysis of TEAC values of various fractions of *S. anquetilia* leaves' extract using ABTS radical cation Assay

Figure 2: Comparison of TPC values of various fractions of *S. anquetilia* leaves' extract

Figure 3: DPPH free radical scavenging activity of various fractions of *S. anquetilia* leaves' extract

Figure 4: A Comparative study of Metal Chelating activity of SKM and the percentage bound Iron of various fractions of *S. anquetilia* leaves' extract

Figure 5: Total Antioxidant activity of various fractions of *S. anquetilia* leaves' extract

Figure 6: FRAP values of various fractions of *S. anquetilia* leaves' extract

Figure 7: Superoxide Anion Radical Scavenging Activity of various fractions of *S. anquetilia* leaves' extract

Figure 8: Comparison of TFC values of various fractions of *S. anquetilia* leaves' extract

Dichloromethane fraction showed highest total antioxidant activity i.e. 0.55 ± 0.25 . Crude methanolic extract exhibited highest total flavonoid contents (1698 mg/g quercetin equivalents) as well as highest metal chelating activity 73.5 %. Other polar fractions also exhibited good antioxidant potential. This study showed that this plant has different compounds with antioxidant potential and to the best of our knowledge, the detailed antioxidant activity of leaves of this plant has not reported earlier to this investigation. As some fractions of the plant assessed as having good antioxidant potential, so these may be served as very valuable sources of the therapeutic agents, that would be expected to enhance the shelf life of foods, and protect against the per oxidative damage, in the living systems, in relation to carcinogenesis and aging. These can be used in pharmaceutical industry to form potent, economical and safe drugs.

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