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Phytochemical screening, total phenolic content and in vitro antioxidant studies of leaf, bark and flower extracts of *Schefflera* spp. (Araliaceae)

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ABSTRACT

In the present study, the phytochemical constituents and antioxidant activity of various extracts from leaves, bark and flower of *S. venulosa* and *S. wallichiana* was investigated by different *in-vitro* methods. Phytochemical investigation revealed the presence of saponins, tannins, flavonoids, alkaloids, cardiac glycosides and reducing sugars in both plant species. Estimation of total phenolic content and free radical assays such as 1,1-diphenyl-2-picryl hydrazyl (DPPH), Ferric reducing antioxidant power (FRAP) and Reducing power assay were performed. The phenolic content varied from 0.36±0.05 to 93.41±0.22 mg GAE/g extract in both plant species. Aqueous flower extracts of *S. venulosa* (IC_{50} = 13.16 µg/ml) and aqueous leaf extract of *S. wallichiana* (14.13±0.01µg/ml) showed potent antioxidant activity. The maximum reducing ability in the FRAP assay was observed in aqueous flower extracts of *S. venulosa* (195.77±0.14 µM Fe (II) /g) and aqueous leaf extract of *S. wallichiana* (193.43±0.14 µM Fe (II) /g). OD at 700 nm for reducing power assay also showed similar results for the aqueous flower extracts of *S. venulosa* (2.05±0.25 OD) and aqueous leaf extract of *S. wallichiana* (2.00±0.18 OD). The results provide evidence that the studied plants might indeed be potential sources of natural antioxidants.

INTRODUCTION

Medicinal plants constitute the major constituents of most indigenous medicines. A large number of western medical preparations contain one or more ingredients of plant origin. Recently, interest has increased considerably in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants (Ito *et al*., 1983). In addition, natural antioxidants have the capacity to improve food quality and stability and also act as nutraceuticals to terminate the free radical chain reaction in biological systems, and thus may provide additional health benefits to consumers (Nahak and Sahu, 2010). There is currently an upsurge of interest in phytochemicals as a new source of natural antioxidants to be used in food and pharmaceutical industries to replace synthetic antioxidants, which have restricted use due to their potential health risks and toxicity.

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As plants have been widely screened for antioxidants to prevent the oxidative stress caused by photons and oxygen, they represent a potential source of new compounds with antioxidant activity. Plants like *Polyscias fruticosa* (Araliaceae), *Gymnema indorum* (Asclepiadaceae), *Mentha arvensis* (Labiatae), *Piper sarmentosum* (Piperaceae) have shown potent antioxidant activity (Chanwitheesuk *et al*., 2005). The term 'antioxidants' refers to compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of the oxidative chain reaction which can thus prevent damage done to the body's cell by reactive oxygen species (Moussa *et al*., 2011). Araliaceae (the ginseng family) comprise approximately 55 genera and 1500 species. The family is broadly distributed in the tropics and sub-tropics. The family includes a number of important medicinal plants, such as *Panax* (ginseng) and *Eleutherococcus* (Siberian ginseng), and several well known ornamentals, including *Hedera* (English ivy), *Schefflera* (the umbrella trees) and *Polyscias* (Wen *et al*., 2001). The genus *Schefflera* is an epiphyte. *Schefflera* probably comprises over 650 species and is widely distributed in the tropics and subtropics.

The leaves and bark of several *Schefflera* species are used as a remedy for cough and as a diuretic. The ethnomedicinal uses of *Schefflera* include the treatment of asthma, liver diseases, rheumatism, arthritis, sprains, fracture, stomach pain, antipyretic, anti-inflammatory, analgesic, migraine and general tonic (Ragasa *et al*., 2005). *S. venulosa* extract contains mainly caffeoyl acids, quercetin glycoside and oleanolic acid glycoside which helps in blood circulation and prevents cardiac and cerebral vascular diseases (Purohith *et al.,* 1991). Phytochemical and antioxidant studies on *S. venulosa* and *S. wallichiana* have not been reported. Therefore, the present study was carried out to explore the antioxidant potential of various solvent extracts of *S. venulosa* and *S. wallichiana* and to compare their total phenolic content and their ability to target multiple radical species.

MATERIALS AND METHODS

Plant material

Schefflera species were collected from the natural forests of Kodagu, southern Karnataka. The plant parts such as leaves, bark and inflorescence were excised with plier as well as machete and placed in ziplock polythene bags, labeled, brought to the laboratory and processed for further use. A herbarium specimen of the plant is maintained in the Herbarium collection of the Department of Botany, University of Mysore, Manasagangotri, Mysore.

Preparation of extracts for phytochemical screening

The collected plant parts were dried under shade as well as at 40˚C in a hot air oven prior to blending to remove the water content and powdered. The powdered materials weighing approximately 500g were placed in polythene zip lock covers for further use. Fifty grams of shade dried leaf and flower powder were extracted with soxhlet apparatus in the order of polarity (Hexane> Chloroform> Ethyl acetate>Methanol>Water).

Phytochemical Screening

Qualitative phytochemical analysis of the crude powder and different solvent extracts were determined using standard procedures (Harborne, 1984; Trease and Evans 2002). The extracts were tested qualitatively for the presence of phytochemical constituents such as tannins, saponins, terpenoids, flavonoids, alkaloids, steroids, cardiac glycosides, phlobatannins, anthraquinones and reducing sugars.

Estimation of total phenolic content

The total phenolic content of plant extracts was estimated by Folin-Ciocalteau (FC) method as per the procedure of Volluri *et al*. (2011) with some modifications. Different concentrations of the plant extracts (50-250 µg/mL) and the standard gallic acid (5-25 µg/mL) were taken in test tubes and 1.0 mL of FC reagent was added, after 3-5 min 2.0 mL of sodium carbonate (20%; w/v) was added and the mixture was allowed to stand for 30-45 min under dark. After the prescribed period of incubation the absorbance was taken at 765 nm in a spectrophotometer (T-60 UV-visible spectrophotometer, TTL-Technologies.). The concentration of total phenolics was expressed in terms of mg GAE/g gallic acid equivalents.

Radical scavenging activity by DPPH assay

Radical scavenging activity by DPPH method was evaluated according to the procedure of Pannangpetch *et al*. (2007). Aliquots of standard (5-25µg/mL) and plant extracts (20-100 µg/mL) were taken and the volume was made up to 250 µL using distilled water or methanol. To this one mL of DPPH was added and the tubes were kept under dark for 10 min. The incubated mixture was read at the absorbance of 517 nm using spectrophotometer. Percent radical scavenging was calculated based on the extent of reduction in the color.

Per cent radical scavenging activity = $A_c - A_s / A_c \times 100$

Where A_c = absorbance of the control; A_s = absorbance of the sample.

Total antioxidant power by Ferric reducing antioxidant power (FRAP) assay

Total antioxidant activity by FRAP assay was determined by the modified method of Benzie and Strain (1996). The stock solutions of Acetate buffer (300 mM), 2,4,6-tripyridyl-S-triazine (TPTZ) 10 mM in HCl 40 mM and $FeCl_3.6H_2O$ (20 mM) were prepared. From this stock, a fresh working solution was prepared by adding 25 mL of acetate buffer, 2.5 mL of TPTZ and 2.5 mL of $FeCl_3.6H_2O$.

The temperature of the solution was raised to 37°C before use. Different concentration of plant extracts (20-100 µg/mL) as well as the standard ascorbic acid (5-25 µg/mL) was taken in test tubes and the volume was made up to 3000 µL with a freshly prepared FRAP solution and incubated for 30 min under dark condition. The absorbance was measured at 593 nm using spectrophotometer based on the concentration of the plant extract the color changes from light brown to various shades of blue.

Determination of reducing power

The reducing power of the bark extracts was evaluated according to the procedure of Yen and Chen (1995) with some modifications. The concentration of plant extracts and the standard ascorbic acid were prepared as mentioned earlier. Different concentrations of standard (5-25 µg/mL) and plant extracts (20-100 µg/mL) were taken in test tubes and the volume was made up to 500 µL by using 2.0 M phosphate buffer and 1% potassium ferricyanide, then the tubes were kept for incubation at 50°C for 20 min. After this period, 2.5 mL of 10% trichloroacetic acid was added and then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride were added. The absorbance was measured at 700 nm against a blank sample. The reduction of yellow color to the various shades of green and blue depends on the reducing power capacity of each tested extract.

Table. 1: Phytochemical constituents of *S. venulosa* in different solvent extracts.

Solvents Tests	Hexane			Chloroform			Ethyl acetate			Ethanol			Methanol			Aqueous		
	L	B	F	L	B	F	L	B	F	L	B	F	L	B	F	L	B	F
Saponins	+	-	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+
Tannins	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
Flavonoids	-	-	-	-	-	-	-	-	-	+	-	+	+	-	+	+	+	+
Terpenoids	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alkaloids	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+	+	+	+
Steroids	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phlobatannins	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cardiac Glycosides	-	+	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-
Anthraquinones	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Reducing Sugars	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	+	+	+

Legend: + = present, - = absent, L = leaf, B = bark, F = flower

Table. 2: Phytochemical constituents of *S. wallichiana* in different solvent extracts.

Solvents Tests	Hexane		Chloroform		Ethyl acetate		Ethanol		Methanol			Aqueous	
	L	B	L	B	L	B	L	B	L	B	F	L	B
Saponins	+	-	+	+	+	-	+	-	+	+	+	+	+
Tannins	-	-	-	-	-	-	+	-	+	-	+	+	-
Flavonoids	-	-	-	-	-	-	-	-	+	-	+	+	-
Terpenoids	-	-	-	+	-	-	+	-	+	-	+	-	-
Alkaloids	-	-	-	-	-	-	-	-	-	-	+	+	-
Steroids	+	-	-	+	-	-	+	-	-	-	+	-	-
Phlobatannins	-	-	-	-	-	-	-	-	-	-	-	-	-
Cardiac Glycosides	+	-	-	-	-	-	-	-	-	-	+	+	+
Anthraquinones	-	-	-	-	-	-	-	-	-	-	-	-	-
Reducing Sugars	-	-	-	+	-	-	-	-	-	+	-	+	-

Legend: + = present, - = absent, L = leaf, B = bark, F = flower

Table 3. Total phenolic content and antioxidant assays of *S. venulosa* in different solvents.*

	TPC (mg GAE/g extract)	DPPH (IC_{50}=µg/ml)	FRAP (µM Fe (II) /g extract)	Reducing Power assay (OD at 700nm)
Leaf extracts				
Hexane	**0.36±0.05**	129.03±0.08	27.16±0.14	0.04±0.07
Chloroform	0.71±0.05	127.60±0.30	23.90±0.28	0.04±0.07
Ethyl acetate	13.88±0.04	126.60±0.30	36.10±0.20	0.19±0.12
Ethanol	7.35±0.03	154.60±0.20	23.50±0.20	0.15±0.12
Methanol	17.26±0.03	104.67±0.35	50.20±0.11	0.51±0.13
Aqueous	89.33±0.22	15.16±0.12	185.07±0.14	1.08±0.03
Bark extracts				
Hexane	2.35±0.03	133.10±0.15	24.60±0.36	0.05±0.07
Chloroform	1.65±0.02	134.53±0.29	31.50±0.20	0.05±0.06
Ethyl acetate	24.94±0.03	71.40±0.26	118.20±0.20	1.52±0.19
Ethanol	23.10±0.02	72.46±0.26	110.63±0.14	0.95±0.19
Methanol	19.38±0.04	63.50±0.28	137.93±0.20	0.99±0.12
Aqueous	57.50±0.28	15.40±0.26	163.20±0.28	1.05±0.12
Flower extracts				
Hexane	0.57±0.05	135.77±0.14	24.43±0.20	0.05±0.07
Chloroform	1.78±0.04	134.77±0.14	27.10±0.20	0.04±0.08
Ethyl acetate	23.72±0.04	20.06±0.12	140.33±0.17	0.92±0.35
Ethanol	24.79±0.04	18.20±0.12	151.33±0.14	1.63±0.26
Methanol	53.76±0.02	16.26±0.17	145.00±0.20	0.05±0.07
Aqueous	93.41±0.22	13.16±0.03	195.77±0.14	2.05±0.25

Table. 4: Total phenolic content and antioxidant assays of *S. wallichiana* in different solvents.*

	TPC (mg GAE/g extract)	DPPH (IC_{50}=µg/ml)	FRAP (µM Fe (II) /g extract)	Reducing Power assay (OD at 700nm)
Leaf extracts				
Hexane	0.38±0.04	158.40±0.23	21.63±0.20	0.04±0.07
Chloroform	0.80±0.05	152.40±0.20	24.60±0.11	0.07±0.29
Ethyl acetate	36.28±0.01	106.83±0.12	30.53±0.60	0.21±0.12
Ethanol	11.98±0.04	28.36±0.20	34.70±0.21	0.41±0.12
Methanol	82.25±0.14	86.03±0.08	31.10±0.11	0.31±0.12
Aqueous	92.33±0.22	14.13±0.01	193.43±0.14	2.00±0.18
Bark extracts				
Hexane	3.42±0.03	146.87±0.08	22.26±0.14	0.04±0.07
Chloroform	21.90±0.05	96.20±0.17	21.50±0.20	0.06±0.07
Ethyl acetate	84.50±0.14	18.36±0.20	133.73±0.20	0.92±0.08
Ethanol	35.00±0.14	39.00±0.11	121.27±0.20	0.85±0.08
Methanol	5.08±0.22	45.76±0.14	59.20±0.20	0.53±0.13
Aqueous	49.02±0.03	19.05±0.16	137.93±0.27	1.58±0.20
Flower extracts				
Methanol	20.38±0.11	17.46±0.20	130.80±0.26	0.71±0.08

* All values are expressed as mean ±standard error mean (SEM) (n=3).

Statistical analysis

All the experiments were done in triplicates. Statistical analysis was done using SPSS program (16.0 version). One way ANOVA and post hoc tests were conducted and Probability (*P*) value less than 0.05 was considered as significantly different.

RESULTS AND DISCUSSION

Phytochemical screening

In the present investigation, the phytochemical constituents and antioxidant evaluation of *S. venulosa* and *S. wallichiana* was carried out. Phytochemicals are the plant secondary metabolites which are not directly involved in the growth and development of a plant but also serve as a defensive agent against any pathogen. The results provided in tables 1 and 2, indicate that both the plant species studied contain saponins, tannins, flavonoids, alkaloids, cardiac glycosides and reducing sugars. *S. wallichiana* showed positive results for the presence of steroids and terpenoids. Phytochemical analysis of both the plant species revealed the presence of saponins being a major phytochemical in all the parts of both plant species. Saponins are a class of chemical compounds, one of many secondary metabolites found in natural sources. In plants, saponins may serve as anti-feedants, and to protect the plant against microbes (Hostettmann and Marston, 1995).

Total phenolic content

Phenolics are ubiquitous secondary metabolites in plants possessing a wide spectrum of biochemical activities such as antioxidant, antimutagenic and anticarcinogenic activities. It is reported that the phenolics are responsible for the variation in the antioxidant activity of the plant (Cai *et al*., 2004). They exhibit antioxidant activity by inactivating lipid free radicals or preventing the decomposition of hyperoxides into free radicals (Pokomey, 2001; Pitchaon *et al*., 2007). The total phenolic content and antioxidant activity of *S. venulosa* and *S. wallichiana* are represented in table 3 and 4 respectively. The total phenolic content varied significantly between the two species of Araliaceae i.e. *S. venulosa* and *S. wallichiana*. The amount of total phenolics was measured by the Folin-Ciocalteu method. High phenolic content was seen in the aqueous flower extract of *S. venulosa* (93.41±0.22 mg GAE/g extract) and aqueous leaf extracts of *S. wallichiana* (92.33±0.22 mg GAE/g extract), whereas low content was seen in hexane leaf extracts of both *S. venulosa* (0.36±0.05 mg GAE/g extract) and *S. wallichiana* (0.38±0.04 mg GAE/g extract). Among the plant extracts, the aqueous extracts of *S. venulosa* and *S. wallichiana*, showed high phenolic content and the antioxidant activity (Tables 3 and 4).

Antioxidant assays

DPPH scavenging assay is one of the most preferred antioxidant method for determining the radical scavenging activity of plant material. DPPH is a stable free radical that possesses a characteristic absorption maximum at 517 nm, which is diminished in the presence of a compound (i.e. antioxidants) capable of reducing it to its hydrazine form by donating hydrogen/electron. Free radical scavenging of phenolic compounds is an important property underlaying their various biological and pharmacological activities (Mayakrishnan *et al*., 2012). Aqueous extracts of both the plant species showed free radical scavenging activity (Tables 3 and 4), lower IC_{50} values indicating higher antioxidant activity of the extract. The aqueous flower extract of *S. venulosa* (IC_{50} value=13. 16±0.03µg/ml) and aqueous leaf extracts of *S. wallichiana* with IC_{50} value of 14.13±0.01µg/ml showed DPPH radical scavenging activity, in comparison with the standard ascorbic acid (IC_{50} value=11.5±0.00µg/ml). Hexane and chloroform extracts with weak total phenolic content, exhibited weak radical scavenging activity.

The FRAP assay (Ferric Reducing Ability of Plasma) evaluates total antioxidant power and is chosen to assess the presumable effects of medicinal plants (Szollosi and Varga, 2002). FRAP assay depends upon the ferric tripyridyltriazine (Fe (III) – TPTZ) complex to the ferrous tripyridyltriazine (Fe (II) – TPTZ) by a reductant at low pH. Fe(II) – TPTZ has an intensive blue color and can be monitored at 593 nm (Benzie and Strain, 1996). The reducing ability of various solvent extracts in different parts of both *S. venulosa* and *S. wallichiana* were examined and the values were presented in Tables 3 and 4. Maximum reducing ability at 100 µg/ml was seen in aqueous flower extracts *S. venulosa* (195.77±0.14 µM Fe (II) /g of extract) and aqueous leaf extract of *S. wallichiana* (193.43±0.14 µM Fe (II) /g of extract), whereas low reducing power was observed in the ethanolic leaf extract of *S. venulosa* (23.50±0.20 µM Fe (II) /g of extract) and chloroform extract of bark of *S. wallichiana* (21.50±0.20 µM Fe (II) /g of extract).

The reducing power of a compound is related to its electron transfer ability and may therefore, serve as a significant indicator of its antioxidant activity (Ajila *et al*., 2007). In the reducing power assay, the extracts of *S. venulosa* and *S. wallichiana* showed a concentration dependent antioxidant potential. In this assay, the presence of antioxidants in the extracts causes the reduction of the ferric cyanide complex in the ferrous form, leading to a color change of the test solution from yellow to different shades of green and blue, depending on the reducing power capacity of each tested extract. Therefore, Fe^{2+} concentration can be monitored by measuring the formation of Pearl's Prussian blue at 700 nm. Increased absorbance at 700 nm indicates an increase in reducing power. In the present study, it depicts that the reductive effect of *S. venulosa* and *S. wallichiana* increased with concentration of extracts. High absorbance at 700 nm indicates high reducing power. The highest reducing power activity was seen in aqueous flower extracts of *S. venulosa* (2.05±0.25 OD) and aqueous leaf extract of *S. wallichiana* (2.00±0.18 OD).

Antioxidants are compounds which interfere with the oxidative processes by scavenging free radicals, cheating free catalytic metals and by acting as electron donors (Gulchin *et al*.,

2005). Reducing the capacity of a compound may serve as an indicator of its potential antioxidant capacity (Meir *et al*., 1999). In the present study, the aqueous extracts of both plant species exhibited antioxidant activity, which has potential application to reduce oxidative stress with consequent health benefits. Phenolic compounds act as free radical acceptors and chain breakers. They interfere with the oxidation of lipids and other molecules by rapid donation of the hydrogen atom to radicals (Dai and Mumper, 2010). Aqueous extracts of *S. venulosa* and *S. wallichiana* showed higher amounts of phenolic contents indicating strong antioxidant activity.

CONCLUSION

In the present work, high antioxidant activity was observed in aqueous extracts of flower of *S. venulosa* and aqueous extracts of leaf of *S. wallichiana* when compared to other solvents. This may be attributed to the presence of hydrophilic antioxidants and the compounds present may play a role in preventing human diseases in which free radicals are involved, such as cancer, ageing and cardiovascular diseases. Further studies on the fractionation of solvent extracts and characterization by spectroscopy techniques may reveal the compounds responsible for the antioxidant potentials.

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