ORIGINAL ARTICLE

Preliminary phytochemical screening and antioxidant activity of selected four plants

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Abstract

Aim: The present work carried out to evaluate of the phytochemicals present in the selected plants which are biologically active and *in vitro* anti-oxidant activity. **Materials and Methods:** Four selected plants *Polygonum glabrum*, *Canthium dicoccum Ochna obtusata*, *Argyreia nervosa* of Petroleum collected and plant material is dried according to the standard procedure. The dried plant materials powdered using blenders. The powdered material is subjected to extraction using different solvent systems and filtrate is collected. Finally the filtarate is dried crude used for further studies. **Results and Discussion:** Ether Extract, Ethyl Acetate Extract, and Aqueous Extracts revealed that presence of bioactive compound likes saponins, phyto-sterols and phenolic are in relatively in high concentration in preliminary phytochemical study. The quantitate estimation of phenolic compound carried out by using Folin-Ciocalteu method and flavonoid by colorimetric method using aluminum chloride to establish the relation between the antioxidant activity and phenolic and flavonoid content. The total phenol content and flavonoid content ethanol extracts are relatively more than other extracts with respect to the standard concentration of Gallic acid and rutin .In selected plants ethanol extract showed significant anti-oxidant activity of on dose dependent manner. In all the assays IC50 values are determined using ascorbic acid is a standard. **Conclusion:** The results obtained from the present investigation the selected four plants are having good bioactive compound hence the plants can be used as potential source of natural antioxidants and Pharmaceuticals.

Key words: *Argyreia nervosa*, Antioxidant activity, *Canthium dicoccum*, hydrogen peroxide-scavenging, *Ochna obtusata*, *Polygonum glabrum,* 1,1-diphenyl-2-picrylhydrazyl

INTRODUCTION

Traditional medicine has remained as the most affordable and easily accessible source of treatment in the primary health-care system of resource-poor communities. The local people have a long history of traditional plant usage for medicinal purposes. The medicinal use of plants is very old. The writings indicate that therapeutic use of plants is as old as 4000-5000 BC. Moreover, Chinese used first the natural herbal preparations as medicines. In India, however, earliest references of use of plants as medicine appear in Rigveda, which is said to be written between 1600 and 3500 BC. Later, the properties and therapeutic uses of medicinal plants were studied in detail and recorded empirically by the ancient physicians (an indigenous system of medicine) which are a basic foundation of ancient medical science in India.[1] Medicinal plant is an important element of indigenous medical systems in all over the world. The ethnobotany provides a rich resource for natural drug research and development.[2] "Traditional" use of herbal medicines implies substantial historical use, and this is certainly true for many products that are available as "traditional herbal medicines." In many developing countries, a large proportion of the population relies on traditional practitioners, and their armamentarium of medicinal plants to meet health-care needs. Although modern medicine may exist side by side with such traditional practice, herbal medicines have often maintained their popularity for historical and cultural reasons.[3] Natural products have played an important role throughout the world in treating and preventing human diseases. Natural product medicines have come from various source materials including terrestrial plants, terrestrial microorganisms, marine organisms, and terrestrial vertebrates

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and invertebrates,[4] and its importance in modern medicine has been discussed in different reviews and reports.[5] The value of natural products in this regard can be accessed from: (1) The rate of introduction of new chemical entities of wide structural diversity including serving as templates for semi-synthetic and total synthetic modification, (2) the number of diseases treated or prevented by these substances, and (3) their frequency of use in the treatment of disease. In recent years, the use of traditional medicine information on plant research has again received considerable interest.[6] The evaluation of antioxidant properties of plant extracts have been extensively performed by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assays. This is a quick, reliable, and reproducible method.[7-10] It has been observed that phenols and flavonoids contribute significantly to the antioxidant capacity of plant extracts.[11-13] That is why many research groups investigated the connection between the antioxidant capacity and polyphenol content of plants.[14-17] In most cases, the surveys have been related to traditionally used medicinal plants.[12,17]

Polygonum glabrum willd. (Polygonaceae) commonly known as Attalaree in Tamil and Niru Kanigalu in Mangalore. *P. glabrum* has been used as folk medicine and as ingredient in various Ayurvedic preparations. Traditionally, it is used as plant juice and rootstock used in pneumonia, consumption, jaundice, and fever. Leaf antispasmodic used for colic and pungent young shoots are cooked with other vegetables. The leaves are astringent, diuretic, rubefacient, and vermifuge. An infusion has been used as a treatment for gravel and stomach pain.[18-24]

Canthium dicoccum is found in Western Ghats of India. The common names in Tamil are Nanjul, Nallamandharam and in Malayalam as Nanyul. It is a medium sized tree. Trees grow up to 10 m, leaves oblanceolate, glabrous, shortly acuminate at apex, margin entire, truncate at base; stipules triangular. Fruits are edible. The plants are common in dry deciduous forests. Plant possesses antipyretic activity. In India, bark is used as febrifuge and also applied as plasters. The decoction of roots is used internally for treating diarrhea. Bark powder boiled with sesame oil is used externally for rheumatic pains.[25]

Ochna obtusata (Ochnaceae) commonly called as Tammi, Panjaram, Ramatana Champaka, or golden champak is one such medicinal plant possesses several pharmacological properties, where, few are already reported and few are yet to be investigated. It occurs throughout India, most commonly grow in forests.[26]

Argyreia nervosa (family: Convolvulaceae) plant is also known as elephant creeper or woolly morning glory. This plant is found on river banks, edges of lakes, and as undergrowth in semi-deciduous forests. Leaves of *A. nervosa* mainly contain β-sitosterol, 1-triacontanol, and quercetin. Traditionally, the plant has been used therapeutically for its wide range of clinical effects such as antiviral, antibacterial, antifungal, and anti-inflammatory properties. It has rejuvenating, age sustainer, and spermatogenic activities as well.[27] The seeds contain the highest concentration of psychoactive compounds in the entire plant. In India, usually leaves and root parts of the plant are used as antiseptic and anti-inflammatory drugs. Its seeds are used as hypotensive, spasmolytic, and tonic.[28] 50% ethanolic extract of the seeds in a preliminary biological screening showed antispasmodic activity in the isolated guinea-pig ileum[29] and antibacterial activity against *Staphylococcus aureus*. The alcoholic extract of the root exhibited statistically significant anti-inflammatory activity against granuloma technique in albino rats.[30]

MATERIALS AND METHODS

Folin–Ciocalteu reagent, DPPH radical, nitroblue tetrazolium, phenazine methosulfate, nicotinamide adenine dinucleotide, sodium nitroprusside, trichloroacetic acid, thiobarbituric acid, potassium hexacyanoferrate ($K_3Fe[CN]_6$), and l-ascorbic acid were purchased from Sisco Research Laboratories Pvt. Ltd, India. Catechin and rutin were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). All other chemicals and solvents used were of analytical grade available commercially.

Preparation of Extracts

Aerial parts of *P. glabrum, C. dicoccum, O. obtusata,* and *A. nervosa* were collected and dried. Then, the material was blended to form a fine powder and extracted using petroleum ether, ethyl acetate, and ethanol using Soxhlet apparatus for 6 h at 50°C and water by maceration the solvent was completely removed by rotary evaporator (Rotavapor® R-210, BUCHI Corporation) and respective extracts preserved for various investigations.

Phytochemical Screening

P. glabrum, C. coccum, O. obtusata, and *A. nervosa* of petroleum ether extract, ethyl acetate extract, and aqueous extract were used for screening of preliminary phytochemical using standard procedures.[31,32]

Determination of Total Phenol Content

In a test tube, 200 µl of the extract (1-0.1 mg/ml) was mixed with 1 ml of Folin–Ciocalteu reagent and 800 µl of sodium carbonate. After shaking, it was kept for 2 h reaction time. The absorbance was measured at 750 nm. Using gallic acid monohydrate, standard curve was prepared and linearity was obtained in the range of 0.78-25 µg/ml. Using the standard curve, the total phenol content was obtained. All measurements were carried out in triplicates. The total phenol content was expressed as gallic acid equivalent in mg/g of the extract.

Determination of Total Flavonoid Content

Total flavonoid content was determined by aluminum chloride method. 0.5 ml of the extract was mixed with 1.5 ml methanol, 0.1 ml 10% $AlCl_3$, 0.1 ml of 1 M potassium acetate, and 2.5 ml of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm. All determinations were carried out in triplicates. Using rutin, standard curve was prepared and linearity was obtained in the range of 1-10 µg/ml. The total flavonoid content was expressed as rutin equivalent in mg/g of the extract.

In Vitro Antioxidant Activity

DPPH radical scavenging assay

DPPH solution (0.004%), plant extracts, and standard (ascorbic acid) solution were prepared in methanol. Plant extracts and standard (ascorbic acid) solution were prepared in different concentrations 10, 20, 40, 60, 80, and 100 µg/ml. 0.5 ml of different concentrations of standard solution or plant extracts was taken in different test tubes and then 0.5 ml of DPPH (0.004%) solution was added and kept in dark for 30 min. Moreover, absorbance was recorded at 517 nm. The decrease in absorbance of the DPPH radical caused by antioxidant was due to the scavenging of the radical by hydrogen donation. It was visually noticeable as a color change from purple to yellow. The percentage inhibition activity was calculated using the formulae below:

DPPH scavenging activity (%) = $[A_0 - A_1/A_0] \times 100$

A_0 is the absorbance of the control reaction and A_1 is the absorbance in the presence of the sample of the extracts.

Hydrogen peroxide scavenging assay

The antioxidant activity was assessed on the basis of their hydrogen peroxide scavenging ability. The standard, ascorbic acid and the extracts were prepared in phosphate buffer, pH 7.4. Sample and standard (0.5 ml) were taken in different test tubes and to each test tube; 0.6 ml hydrogen peroxide solution (2 mM hydrogen peroxide in phosphate buffer, pH 7.4) was added. A control was prepared by replacing the sample/standard with phosphate buffer. These solutions were kept at room temperature for 10 min. The absorbance was measured at 230 nm against the blank solution containing phosphate buffer without hydrogen peroxide. All the samples were prepared and assayed in triplicate and averaged. The antioxidant activity was measured using the formulae below:

Scavenged H_2O_2 = $[A_c - A_s/A_c] \times 100$

A_c is the absorbance of the control reaction and A_s is the absorbance in the presence of the sample.

RESULTS

Extractive Values

The selected four plants blended and extracted with selected solvents (petroleum ether, ethyl acetate, ethanol, and water) but in the ethanol extract showed high percentage yield compare to the other solvent extracts and the values are shown in Tables 1-4, respectively; hence ethanolic extract will be the better economical product for further studies.

Preliminary Phytochemical Studies

The phytochemical screening of different crude extracts revealed that the presence of different phytochemicals such as saponins, phytosterols, and phenolic compounds are relatively found a high concentration in ethanol extraction. The phytochemicals are major responsible for the various biological activities. These compounds were act as anti-oxidants, used in treatment of inflammation, diabetes, cancer. Mainly, the phenols and flavonoids are responsible in antioxidant, inflammation, and anticancer agents. In the study, its relative phytochemicals present in the different selected solvents are respective plants are shown in Tables 5-8.

Total Phenolic Content

The total phenol content [Table 9] revealed that the ethanol extract is having high phenol content compare to the other organic solvents. The phenol continents, respectively, found 14.33 ± 0.042, 15.5 ± 0.068, 15.16 ± 0.061, and 11.6 ± 0.126 for *P. glabrum, C. dicoccum, O. obtusata,* and *A. nervosa.* Hence, the more phenolic content and more antioxidant activity and the respective values shown in Table 9 and represented graphically in Figure 1.

Total Flavonoid Content

The total flavonoid content is responsible for various biological activities. The present investigation revealed that

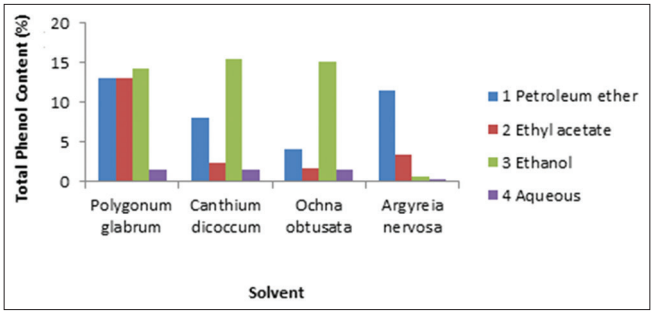

Figure 1: Estimation of total phenol content of *Polygonum glabrum, Canthium dicoccum, Ochna obtusata, and Argyreia nervosa* in extracts petroleum ether, ethyl acetate, ethanol, and aqueous

Table 1: Extractive values of *P. glabrum* PEE, EAE, and AE

Solvent	Method of extraction	Physical nature	Color	Yield (% w/w)
Petroleum ether	Continuous	Semisolid	Brownish	3.78
Ethyl acetate	Hot percolation	Semisolid	Dark brown	5.78
Ethanol		Solid	Brownish	8.56
Aqueous	Maceration	Solid	Dark brown	5.76

P. glabrum: Polygonum glabrum

Table 2: Extractive values of *C. dicoccum* PEE, EAE, and AE

Solvent	Method of extraction	Physical nature	Color	Yield (% w/w)
Petroleum ether	Continuous	Semisolid	Brownish	3.58
Ethyl acetate	Hot percolation	Semisolid	Dark brown	3.78
Ethanol		Solid	Brownish	8.56
Aqueous	Maceration	Solid	Dark brown	5.76

C. dicoccum: Canthium dicoccum

Table 3: Extractive values of *O. obtusata* PEE, EAE, and AE

Solvent	Method of extraction	Physical nature	Color	Yield (% w/w)
Petroleum ether	Continuous	Semisolid	Brownish	4.58
Ethyl acetate	Hot percolation	Semisolid	Dark brown	2.76
Ethanol		Solid	Brownish	8.56
Aqueous	Maceration	Solid	Dark brown	4.76

O. obtusata: Ochna obtusata

Table 4: Extractive values of *A. nervosa* PEE, EAE, and AE

Solvent	Method of extraction	Physical nature	Color	Yield (% w/w)
Petroleum ether	Continuous	Semisolid	Brownish	3.62
Ethyl acetate	Hot percolation	Semisolid	Dark brown	4.64
Ethanol		Solid	Brownish	6.42
Aqueous	Maceration	Solid	Dark brown	5.76

A. nervosa: Argyreia nervosa, PEE: Petroleum ether extract, EAE: Ethyl acetate extract, AE: Aqueous extract

Table 5: Preliminary phytochemicals screening of *P. glabrum* PEE, EAE, and AE

Sl.no	Test	PEE	EAE	ALE	AE
1	Carbohydrates	–	+	+	+
3	Glycosides	+	+	+	+
4	Saponins	++	+	++	+
5	Alkaloids	++	+	+	++
6	Proteins and amino acids	++	++	+	+
7	Phytosterols and triterpenoids	++	+	++	+
8	Phenolic compounds and tannins	++	+	+++	++

P. glabrum: Polygonum glabrum, PEE: Petroleum ether extract, EAE: Ethyl acetate extract, AE: Aqueous extract, ALE: Aqueous leaf extract

Table 6: Preliminary phytochemicals of *C. dicoccum* PEE, EAE, and AE

Test	PEE	EAE	ALE	AE
Carbohydrates	+	+	+	++
Glycosides	++	+	+	+
Saponins	++	+	+++	++
Alkaloids	++	+	++	-
Proteins and amino acids	+	+	+	+
Phytosterols and triterpenoids	+	+	++	+
Phenolic compounds and tannins	+	+	++	+

C. dicoccum: Canthium dicoccum, PEE: Petroleum ether extract, EAE: Ethyl acetate extract, AE: Aqueous extract, ALE: Aqueous leaf extract

ethanolic extract is having more flavonoid content shown in Table 10 and represented graphically in Figure 2.

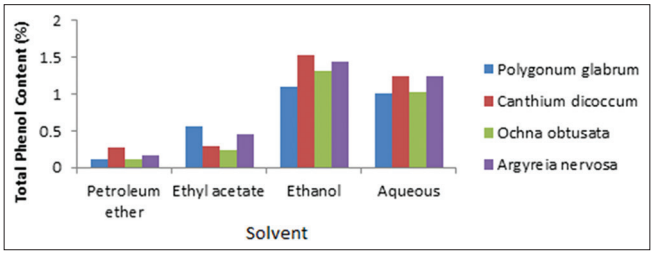

Figure 2: Estimation of total flavonoids content of *Polygonum glabrum, Canthium dicoccum, Ochna obtusata, and Argyreia nervosa* in extracts petroleum ether, ethyl acetate, ethanol, and aqueous

Table 7: Preliminary phytochemicals of *O. obtusata* PEE, EAE, and AE

Test	PEE	EAE	ALE	AE
Carbohydrates	+	+	–	+
Glycosides	++	+	+	+
Saponins	+	+	++	+
Alkaloids	+	+	++	–
Proteins and amino acids	+	+	+	+
Phytosterols and triterpenoids	+	+	+	++
Phenolic compounds and tannins	+	+	+	+

O. obtusata: Ochna obtusata, PEE: Petroleum ether extract, EAE: Ethyl acetate extract, AE: Aqueous extract, ALE: Aqueous leaf extract

Table 8: Preliminary phytochemicals of *A. nervosa* PEE, EAE, and AE

Test	PEE	EAE	ALE	AE
Carbohydrates	+	+	-	+
Glycosides	++	++	+	+
Saponins	++	++	+	+
Alkaloids	+	+	+	–
Proteins and amino acids	+	+	+	+
Phytosterols and triterpenoids	+	+++	++	+
Phenolic compounds and tannins	++	++	++	+

A. nervosa: Argyreia nervosa, PEE: Petroleum ether extract, EAE: Ethyl acetate extract, AE: Aqueous extract, ALE: Aqueous leaf extract

Table 9: Estimation of total phenol content of *P. glabrum, C. dicoccum, O. obtusata,* and *A. nervosa* in extracts petroleum ether, ethyl acetate, ethanol, and aqueous

Solvent	*P. glabrum*	*C. dicoccum*	*O. obtusata*	*A. nervosa*
Petroleum ether	13.16±0.554	8.06±0.098	4.13±0.042	8.53±0.578
Ethyl acetate	13.16±0.554	2.46±0.098	1.73±0.111	3.4±0.252
Ethanol	14.33±0.042	15.5±0.068	15.16±0.061	11.6±0.126
Aqueous	1.5±0.068	1.5±0.229	1.53±0.240	0.26±0.042

P. glabrum: Polygonum glabrum, C. dicoccum: Canthium dicoccum, O. obtusata: Ochna obtusata, A. nervosa: Argyreia nervosa

Table 10: Estimation of total flavonoids content of *P. glabrum, C. dicoccum, O. obtusata,* and *A. nervosa* in extracts petroleum ether, ethyl acetate, ethanol, and aqueous

Solvent	*P. glabrum*	*C. dicoccum*	*O. obtusata*	*A. nervosa*
Petroleum ether	0.116	0.266	0.15	0.166
Ethyl acetate	0.566	0.291	0.241	0.45
Ethanol	1.1	1.525	1.316	1.23
Aqueous	1.4	1.44	1.033	1.65

P. glabrum: Polygonum glabrum, C. dicoccum: Canthium dicoccum, O. obtusata: Ochna obtusata, A. nervosa: Argyreia nervosa

Table 11: Antioxidant activity of *P. glabrum* using DPPH scavenging model

Concentration (µg/ml)	Ascorbic acid	% Antioxidant activity *P. glabrum*			
		PEE	EE	ALE	AE
10	9	9.2	10.6	9.8	9.4
20	21.4	23.5	23.6	22.6	19.6
40	41.2	41.8	43.8	41.2	39.8
60	58.6	56.2	61.2	58.2	59.6
80	78.4	78.2	82.3	81.2	79.6
100	98.8	96.2	98.6	96.2	95.8

P. glabrum: Polygonum glabrum, PEE: Petroleum ether extract, EE: Ethyl extract, AE: Aqueous extract, ALE: Aqueous leaf extract, DPPH: 1,1-diphenyl-2-picrylhydrazyl

Table 12: Antioxidant activity of *C. dicoccum* using DPPH scavenging model

Concentration (µg/ml)	Ascorbic acid	% Antioxidant activity *C. dicoccum*			
		PEE	EE	ALE	AE
10	9	9.4	10.8	10.2	8.4
20	22.4	19.5	22.6	21.4	19.6
40	40.2	40.8	43.6	42.2	39.8
60	59.6	59.4	61.2	58.8	56.2
80	79.4	78.6	81.4	79.2	76.9
100	97.8	96.1	98.2	96.9	97.8

C. dicoccum: Canthium dicoccum, PEE: Petroleum ether extract, EE: Ethyl extract, AE: Aqueous extract, ALE: Aqueous leaf extract, DPPH: 1,1-diphenyl-2-picrylhydrazyl

Table 13: Antioxidant activity of *O. obtusata* using DPPH scavenging model

Concentration (µg/ml)	Ascorbic acid	% Antioxidant activity *O. obtusata*			
		PEE	EE	ALE	AE
10	9	9.2	10.6	9.8	9.4
20	22.1	23.5	23.6	22.6	19.6
40	42.2	41.8	43.8	41.2	39.8
60	58.6	58.8	61.6	61.2	58.8
80	77.4	79.2	81.8	76.2	78.6
100	94.8	93.8	99.6	98.4	98.2

O. obtusata: Ochna obtusata, PEE: Petroleum ether extract, EE: Ethyl extract, AE: Aqueous extract, ALE: Aqueous leaf extract, DPPH: 1,1-diphenyl-2-picrylhydrazyl

Table 14: Antioxidant activity of *A. nervosa* using DPPH scavenging model

Concentration (µg/ml)	Ascorbic acid	% Antioxidant activity *A. nervosa*			
		PEE	EE	ALE	AE
10	9	9.2	10.6	9.8	9.4
20	21.4	23.5	23.6	22.6	19.6
40	41.2	41.8	43.8	41.2	39.8
60	59.6	61.2	62.2	61.4	61.2
80	77.6	78.6	79.4	78.8	78.2
100	92.8	92.6	94.8	93.8	92.4

A. nervosa: Argyreia nervosa, PEE: Petroleum ether extract, EE: Ethyl extract, AE: Aqueous extract, ALE: Aqueous leaf extract, DPPH: 1,1-diphenyl-2-picrylhydrazyl

Table 15: Antioxidant activity of *P. glabrum* using hydrogen peroxide scavenging model

Concentration (µg/ml)	Ascorbic acid	% Antioxidant activity *P. glabrum*			
		PEE	EE	ALE	AE
10	9.8	8.2	9.6	6.8	8.4
20	21.4	19.5	23.6	18.6	16.6
40	41.2	38.8	41.8	31.2	35.8
60	58.6	52.2	62.2	49.2	56.6
80	77.6	76.2	82.3	78.2	76.6
100	92.8	86.2	96.6	92.2	89.8

P. glabrum: Polygonum glabrum, PEE: Petroleum ether extract, EAE: Ethyl acetate extract, AE: Aqueous extract, ALE: Aqueous leaf extract

Table 16: Antioxidant activity of *C. dicoccum* using hydrogen peroxide scavenging model

Concentration (µg/ml)	Ascorbic acid	% Antioxidant activity *C. dicoccum*			
		PEE	EE	ALE	AE
10	9.2	7.4	10.8	9.2	8.4
20	22.1	18.5	22.6	19.4	18.6
40	42.2	41.8	43.6	38.2	39.8
60	58.6	56.4	59.2	54.8	53.2
80	77.4	74.6	79.4	72.2	69.9
100	94.8	86.1	92.2	84.9	82.8

C. dicoccum: Canthium dicoccum, PEE: Petroleum ether extract, EE: Ethyl extract, AE: Aqueous extract, ALE: Aqueous leaf extract

Table 17: Antioxidant activity of *O. obtusata* using hydrogen peroxide scavenging model

Concentration (µg/ml)	Ascorbic acid	% Antioxidant activity *O. obtusata*			
		PEE	EE	ALE	AE
10	9.4	6.2	8.6	7.8	6.4
20	21.4	16.5	21.6	18.6	17.6
40	41.2	39.8	41.8	38.2	36.8
60	59.6	49.8	52.6	49.2	46.8
80	77.6	62.2	76.8	62.2	62.6
100	92.8	72.8	82.6	71.4	76.2

O. obtusata: Ochna obtusata, PEE: Petroleum ether extract, EE: Ethyl extract, AE: aqueous extract, ALE: Aqueous leaf extract

Table 18: Antioxidant activity of using hydrogen peroxide scavenging model

Concentration (µg/ml)	Ascorbic acid	% Antioxidant activity *A. nervosa*			
		PEE	EE	ALE	AE
10	9.8	9.2	10.6	9.8	9.4
20	21.4	19.5	21.6	18.6	16.6
40	41.2	38.8	41.8	34.2	32.8
60	59.6	56.2	59.2	54.4	52.2
80	77.6	64.6	72.4	62.8	52.2
100	92.8	82.6	84.8	81.8	79.4

A. nervosa: Argyreia nervosa, PEE: Petroleum ether extract, EE: Ethyl extract, AE: Aqueous extract, ALE: Aqueous leaf extract

Antioxidant Activity of Selected Plant Extracts

Antioxidant activity is one of main properties of plant phenols which is associated with other medicinal values. Free radical scavenging activity (antioxidant activity) of different crude extracts is measured using DPPH scavenging model and hydrogen peroxide scavenging model. Among all concentrations with four solvents, ethanolic extract was found reliable antioxidant activity which is shown in Tables 11-18.

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