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Phytochemical Profile and Efficacy of Free Radical Scavenging Activity of *Alangium salvifolium* (l.f.) Wangerin.

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Abstract: ***Alangium salvifolium (l.f.) wangerin is an important medicinal plant belonging to the family Alangiaceae commonly known as Alangi in tamil was distributed in South India. Alangium species used as natural medicine, prescribed by Ayurveda, Siddha medical practitioners for various diseases due to its wide range of biological profile. The leaves of A. salvifolium are used as astringent, laxative, refrigerant and used to treat rheumatism, leprosy, syphilis and asthma. In the present study qualitative, quantitative analysis and antioxidant studies were undertaken.***

Keywords: *Alangium salvifolium,* Ayurveda, Siddha, astringent, laxative, refrigerant, antioxidant studies, rheumatism, leprosy, syphilis and asthma.

1. Introduction

The plant *Alangium salviifolium* belongs to the family Alangiaceae is an important medicinal plant used for the treatment of various diseases. It is used in both codified and non codified system of medicines. All parts of the *Alangium salviifolium* plant are medicinally very important. Fruits are sweet, cooling and purgative and used as poultice for treating burning sensation and hemorrhage. Drug development is a complex process, and only companies with a consequent investment in research and development can afford to bring drugs for conception to market. Today, many new chemotherapeutic agents are obtained synthetically, based on "rational" drug design. The study of natural products has many advantages over synthetic drug design. The former leads to materials having new structural features with novel biological activity. In this context not only plants continue to serve as possible sources for new drugs, but chemicals derived from the various parts of these plants can also extremely used as lead structures for synthetic modification and optimization of bioactivity. The starting materials for about one half of the medicines we use today come from natural sources. Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions. There is no doubt that the future of plants as sources of medicinal agents for use in investigation, prevention, and treatment of diseases is very promising. Hence the present investigation reports phytochemical constituents and antioxidant properties of *A.salviifolium*.

2. Materials and Methodology

The plant specimens for the proposed study were collected from Bolluvampatty village, of Coimbatore District, Tamilnadu, India. Flowering shoots of the plant were also collected for identification.The collected plant material was identified and their authenticity was confirmed by the voucher specimen at the Botanical Survey of India (BSI), Southern circle, Coimbatore, Tamilnadu. The collected leaves of *Alangium salvifolium* plant were thoroughly washed in tap water and shade dried for about 15-30 days made into coarse powder with mixer grinder separately. The powder obtained was passed through 60 mesh sieve plate and then used for extraction and determination of the quality. Phytochemical analysis of the extracts of plant was carried out and their bioactive compounds [1-2]and antioxidant activity[3-6] were carried with the extracts.

3. Result and Discussion

Alangium salvifolium is a deciduous, shrub or small tree, (Plate-I) branchlets appressed – tomentose, sometimes straggling, sometimes spinous. Bark grey, orange-yellow when young; wood olive-brown, hard and close-grained, scented. Leaves oblong- or elliptic-lanceolate, entire, attenuate or sub acute, base oblique – subacute, more or less 3-5 nerved at the base. White-scented flowers in irregular axillary cymes or clusters, buds about 75 in. long, tawny-pubescent; Calyx tube cupular, five to ten lobes. Petals five to ten lobes, linearly oblong. Stamens 10 –30; anthers linear. Ovary inferior, unilocular; Ovule one, pendulous; style simple. Berries globose, covered by persistant calyx, stigma capitate. Seeds solitary and ovoid. *A. salvifolium* is distributed in Circars, Deccan and Carnatic, in dry regions, in the plains and low hills, common on roadsides

The results of qualitative phytochemical analysis of leaves of *A. salvifolium* are presented in Table- 1. The qualitative phytochemical study shows the presence of carbohydrate,protein, aminoacid, fixed oil and fat, gums and mucilage, alkaloids, saponins, glycosides, flavonoids, terpenoids, phenols, tannins and steroids in leaves. Intensity of reaction is more in methanol when compared to water.The results of quantitative phytochemical constituents of the study materials are recorded (Table-2). The water extracts contains flavonoids, tannins phenol, alkaloids, saponins and glycosides 3.51%, 2.03%, 5.03%, 2.62%, 1.83% and 1.29% respectively. The methanol contains flavonoids, tannins phenol, alkaloids, saponins and glycosides saponins and glycosides 4.87%, 4.60%, 7.9%, 3.64%,1.07% and1.15% respectively.

The antioxidant activity determination in linoleic acid emulsion by ferric thiocyanate method

The total antioxidant activity of methanolic extract of leaves of *A. salvifolium* and standard (Butylated hydroxy toluene) was determined by ferric thiocyanate method. Antioxidant activity increased steadily with increasing concentration. Methanolic extract exhibited effective antioxidant activity. The effects of extract on various concentrations are shown in Table- 3. The percentage inhibition of the methanol extract in the linoleic acid system was high when compared to the water extract (23.68% at 20µg/ml, 35.48% at 40µg/ml, 49.11% at 100µg/ml, 73.50 at 200µg/ml and 81.93% at 500µg/ml). Whereas BHT showed 32.28%, 43.27%, 65.23%, 82.80%, 97.44% at respective concentrations. The result showed that the activity of standard was more than the extract. The differences between concentrations of extract and control were statistically significant. The IC_{50} value of water extract and methanol extract of leaf and standard are 93.7µg.ml, 98µg/ml and 56.6µg/ml respectively (Table-3).

Reducing power assay by Fe^{3+}- Fe^{2+} transformation

The Table -4 depicts the reducing power of the leaf extract and standard (Butylated hydroxy toluene) using the potassium ferric cyanide reduction method. The reducing power of methanol was higher than water extract which is calculated as 17.3% at 20µg/ml, 30.26% at 40µg/ml, 40.12% at 100µg/ml, 52.37% at 200µg/ml and 63.35% at 500µg/ml. The standard (BHT) exhibited 17.19%, 28.5%, 40.79%, 44.52% and 54.19% at respective concentrations. The results exhibit increasing activity with increasing concentration. The results showed the activity of leaf extracts is more than that of standard (BHT) at higher concentration. IC_{50} values of water extract of leaf, methanol and standard are 162µg/ml, 176µg/ml and 298µg/ml respectively.

Hydrogen peroxide scavenging activity

The ability of the methanolic extract of leaf and standard to scavenge H_2O_2 was determined and shown in Table- 5. The water extract of leaf exhibited higher activity than the methanol extract 20.13%, 37.95%, 52.14%, 63.91% and 72.78% at 20µg/ml, 40µg/ml, 100µg/ml, 200 µg/ml, and 500µg/ml respectively. IC_{50} value of water extract, methanol and standard were estimated as 89µg/ml, 149µg/ml and 30.3µg/ml respectively. The standard Butylated hydroxy anisole (BHA) showed 42.04%, 61.02%, 72.27%, 81.11% and 89.18% at 20, 40, 100, 200 and 500µg/ml respectively. The standard exhibited stronger activity than both the extracts of leaf.

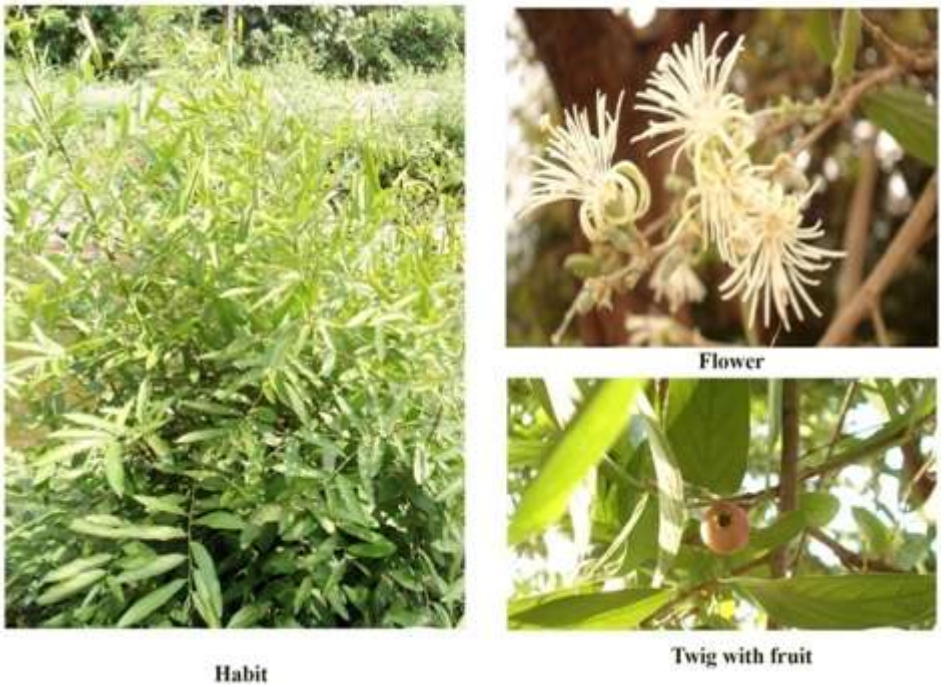
DPPH scavenging activity

The radical scavenging activity of the compounds can be measured as a decolourising effect following trapping of the unpaired electrons of 1,1 diphenyl-2-picryl hydrazyl (DPPH). illustrates the activity of extracts and standard (Butylated hydroxy anisole) against the DPPH radical. The methanolic extract of leaf exhibit higher inhibition than that of water extract 17.90%, 30.32%, 34.75%, 54.31%, 61.26% at 20, 40,100, 200 and 500µg/ml respectively. IC_{50} values of water extract, methanol extract of leaf and standard were 188µg/ml, 193µg/ml and 60.6µg/ml respectively (Table- 6). The results indicate that the standard exhibited stronger radical scavenging activity against DPPH.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of methanolic extract of leaf of *A. salvifolium* is presented in table 7. The inhibition of hydroxyl radical increase in a dose dependent manner. The hydroxyl radical scavenging activity of methanol extract showed more activity 16.04%, 21.09%, 38.59% and 48.08% at 20, 40, 100 and 500µg/ml respectively. The IC_{50} values of water extract, methanol extract of leaf and standard are 196µg/ml, 200µg/ml and 354µg/ml respectively (Table- 7). The results indicate that the methanolic extract of leaf has more activity than the standard Butylated hydroxy anisole (BHA).

PLATE - I



Fruits showing persistant calyx

Ripe fruits

Cut view of the fruits

Dry Fruits

Table 1: Phytochemical screening of leaves of *A. salvifolium*

Test	Extract	
	water	methanol
Carbohydrates Molisch test	+	++
Proteins Biuret test	+	+
Amino acids Ninhydrin test	+	+
Fixed oils and fats Spot test	+	+
Gums and Mucilages	+	+
Alkaloids Dragendroff's	+	++
Wagner's	+	++
Mayer's	+	++
Saponins Common test	+	--
Glycosides Keller-killani test	+	++
Flavonoids Shinoda test	+	++
Terpenoids Salkowski test	++	++
Phenols Lead acetate	++	+++
Tannins	+	++
Ferric chloride	--	+
Lead acetate		
Steroids Libermann-Burchard's test	++	++

The number of + indicates the intensity of reaction and compound present. – indicate the absence of compound.

Table 2: Quantitative phytochemical estimation of leaves of *A. salvifolium*

Phytochemical Constituents	Water extract (mg/g)	Methanol extract (mg/g)
Flavonoids	3.51±0.03	4.87±0.07
Tannins	2.03±0.05	4.60±0.02
Total phenols	5.03±0.05	7.9±0.11
Alkaloids	2.62±0.04	3.64±1.01
Saponins	1.83±0.03	1.07±0.06
Glycosides	1.29±0.02	1.15±0.008

Values are mean of triplicates ± SD

Table 3: The antioxidant activity of leaf of *A. salvifolium* in linoleic acid emulsion by ferric thiocyanate method

Concentration (µg/ml)	% of inhibition			IC_{50}		
	Standard (BHT)	Water extract	Methanol extract	Standard (BHT)	Water extract	Methanol extract
20	32.28± 0.33e	21.36 ± 0.06e	23.68± 0.07e	56.6 µg/ml	93.7µg/ml	98µg/ml
40	43.27± 0.21d	30.41 ± 0.04d	35.48± 0.03d			
100	65.23± 0.21c	43.68 ± 0.02c	49.11± 0.09c			
200	82.80± 0.14b	67.90 ± 0.03b	73.50± 0.02b			
500	97.44± 0.31a	83.33 ± 0.04a	81.93± 0.06a			

BHT- Butylated hydroxy toluene; Values are mean of triplicates ± SD

Table 4: Total reductive capacity of leaf of *A. salvifolium* by Fe^{3+} - Fe^{2+} transformation

Concentration (µg/ml)	% of inhibition			IC_{50}		
				Standard (BHT)	Water extract	Methanol extract
	Standard (BHT)	Water extract	Methanol extract			
20	16.19± 0.14e	15.08 ± 0.07e	17.3±0.29e	298µg/ml	162µg/ml	176µg/ml
40	28.5± 0.09d	25.13±0.51d	30.26±0.29d			
100	40.79± 0.08c	37.78 ± 0.27c	40.12±0.15c			
200	44.52± 0.04b	51.27 ± 0.44b	52.37±0.48b			
500	63.83 ± 0.38a	63.35±0.48a	54.19±0.14a			

BHT- Butylated hydroxy toluene ;Values are mean of triplicates ± SD

Table 5: Hydrogen peroxide scavenging activity of leaf of *A. salvifolium*

Concentration (µg/ml)	% of inhibition			IC_{50}		
				Standard (BHA)	Water extract	Methanol extract
	Standard (BHA)	Water extract	Methanol extract			
20	42.04± 0.04e	20.13 ± 0.43e	18..26 ± 0.74e	30.3µg/ml	89µg/ml	149µg/ml
40	61.02± 0.02d	37.95±0.58d	30.03±0.19d			
100	72.27± 0.21c	52.14±0.35c	43.94±0.75c			
200	81.11± 0.08b	63.91±0.27b	52.75±0.09b			
500	89.18± 0.13a	72.78±0.41a	74.29±0.58a			

BHA -Butylated hydroxy anisole ;Values are mean of triplicates ± SD

Table 6: DPPH scavenging activity of leaf of *A. salvifolium*

Concentration (µg/ml)	% of inhibition			IC_{50}		
				Standard (BHA)	Water extract	Methanol extract
	Standard (BHA)	Water extract	Methanol extract			
20	21.62± 0.07e	11.97±0.24e	17.90±0.19e	60.6µg/ml	188µg/ml	193µg/ml
40	42.06± 0.04d	20.36±0.50bd	30.32±0.46d			
100	66.37± 0.03c	31.99±0.17c	34.75±0.54c			
200	82.58± 0.03a	51.12±0.16b	54.31±0.09b			
500	89.5± 0.02b	56.55±0.26a	61.26±0.16a			

BHA- Butylated hydroxy anisole; Values are mean of triplicates ± SD

Table 7: Hydroxyl radical scavenging activity of leaf of *A. salvifolium*

Concentration (µg/ml)	*% of inhibition*			*IC_{50}*		
				Standard (BHA)	*Water extract*	*Methanol extract*
	Standard (BHA)	Water extract	Methanol extract			
20	11.40± 0.01e	17.07±0.16d	16.04±0.21e	354µg/ml	196µg/ml	200µg/ml
40	20.14± 0.10d	18.99±0.31d	21.09±0.15d			
100	31.38± 0.02c	35.95±0.18c	38.59±0.47c			
200	43.6± 0.07 b	52.61±0.07b	48.37±0.29b			
500	64.19± 0.14a	61.11±0.23a	63.08±0.26a			

BHA - Butylated hydroxy anisole; Values are mean of triplicates ± SD

4. Conclusion

Phytochemicals were more in methanol extract. So the methanol extract was well focused for other phytochemical analysis. Quantification studies helped to quantify the polyphenols (phenols, flavonoids, tannins), alkaloids, saponins and glycosides. Based on the traditional use of this plant and the presence of these chemicals evident for the medicinal properties of the leaf. The methanolic extract of leaf analysed for their free radical scavenging activity using various methods which include DPPH scavenging activity, hydroxyl radical scavenging activity, hydrogen peroxide activity, reducing power assay and total antioxidant activity. The extract showed maximum reducing activity and hydroxyl radical scavenging activity. Antioxidants are compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. Synthetic antioxidants are suspected of being responsible for liver damage and carcinogenesis. From this study, it can be concluded that the species is effective in scavenging free radicals and has the potential to be a powerful antioxidant.

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