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RESEARCH ARTICLE

ANTIOXIDANT ACTIVITY OF METHANOL EXTRACT OF *AMPELOCISSUS ARANEOSA* PLANCH, (VITACEAE)

[1]Uma Maheswari, B. and *[2]Meerabai, R.S.

[1]Department of Botany, Sri Sarada College for Women (Autonomous), Salem -636016, Tamil Nadu, India
[2]HOD and Associate Professor, Department of Botany, PSGR Krishnammal College for Women (Autonomous), Coimbatore-641004, Tamil Nadu, India

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ABSTRACT

The antioxidant potency of methanol extracts of *Ampelocissus araneosa* leaf, stem and root was investigated. Total antioxidant activity was determined using Free radical scavenging activity (DPPH) and Ferrous ion chelating activity. In addition, the content of phenols and total flavonoids were measured in the extracts. The free radical scavenging capacity and ferrous ion chelation activity were found to be increasing with increase in extract concentration from 50 to 250 μg/ml and 1000 to 5000 μg/ml respectively. The result showed that the root extract has higher antioxidant activity than stem and leaf extract. GC-MS profile of these extracts were investigated to find out the possible phytochemical involved in antioxidant activity.

Key words: Free radicals, phenolic content, flavonoid content, GC-MS.

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INTRODUCTION

Oxidation is essential to many living organisms for the production of energy for biological activities. However, the uncontrolled production of oxygen-derived free radicals is involved in the onset of many diseases such as cancer, rheumatoid arthritis and atherosclerosis as well as in degenerative processes associated with aging (Turkoglu et al., 2007). Almost all organisms are well-protected against free radical damage by enzymes such as superoxide dismutase and catalase or compounds such as ascorbic acid, tocopherols and glutathione (Elmastas et al., 2005). When the mechanism of antioxidant protection becomes unbalanced by factors such as ageing, deterioration of physiological functions may occur, resulting in diseases and accelerated ageing. However, antioxidant supplements or antioxidant-containing foods can be used to help the human body to reduce oxidative damage (Cazzi et al., 1997). Natural products with their potential to act as antioxidants, play a major role in the prevention of various pathological conditions (Kirby, 1996). In fact, polyphenols, particularly flavonoids, which are widely distributed in the plant kingdom and are present in considerable amounts in fruits, vegetables, spices, medicinal herbs, and beverages, have been used to prevent many human diseases, such as diabetes, cancers, and coronary heart diseases (Broadhurst et al., 2000).

Corresponding author:* **Meerabai, R.S.,
HOD and Associate Professor, Department of Botany, PSGR Krishnammal College for Women (Autonomous), Coimbatore-641004, Tamil Nadu, India.

Moreover, flavonoids have been shown to exhibit antioxidative, antiviral, antibacterial, and antitoxic activities (Middleton and Kandaswami, 1993). The biological activities of these polyphenols in different systems are believed to be due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygens, or decomposing peroxides (Oswa, 1994). In view of the above, we designed a study to evaluate the antioxidant potential in *Ampelecissus araneosa* in terms of phenolic and flavonoid contents. This plant is a climbing shrub, found in moist deciduous to evergreen forests in Kerala, Karnataka and Tamil Nadu. It is called Kattu thiratchai in Tamil, Asvakathara in Sanskrit and Kauraj, Grovel in Hindi. The roots are used in Ayurveda, Siddha and Unani system of medicine as a cooling agent and astringent (Narayan et al., 2003).

MATERIALS AND METHODS

Collection of plant material

The fresh whole plants of *Ampelocissus araneosa* were collected from Yercaud, Salem district, Tamil Nadu. The plant was identified and authenticated by Botanical survey of India, Coimbatore. The leaves, stem and root collected were washed and cleaned to remove foreign organic matter, cut into small pieces and then kept for drying in shade. The dried plant parts were made into coarse powder. These powders were stored in

air tight container and used for further extraction. Methanol extracts was obtained by Soxhlet apparatus (6 h). They were concentrated to dryness and kept at 4 °C in refrigerator.

Determination of total phenolic content

Total phenolic contents of methonolic extracts of *Ampelocissus araneosa* leaves, stem and root were determined using Folin–Ciocalteu assay (Meda et al., 2005). To 0.1 ml of extract, 2.5 ml of 10-fold diluted Folin–Ciocalteau reagent, and 2.0 ml of 7.5% sodium carbonate were mixed. After incubation at 40ºC for 30 min, the absorbance of the reaction mixtures were measured at 760 nm using a spectrophotometer. Gallic acid was used as a standard and total phenolic content of the extracts were expressed in milligram gallic acid equivalents (mg GAE/g extract).

Determination of total flavonoid content

Total flavonoid content was determined by the aluminium colorimetric method (Quettier-Deleu et al., 2000), using quercetin as a standard. Known test samples were individually dissolved in dimethyl sulphoxide. Then, the sample solution (150 µl) was mixed with 150 µl of 2% Aluminium chloride. After 10 min of incubation at ambient temperature, the absorbance of the supernatant was measured at 435 nm using a spectrophotometer. The total flavonoid content was expressed as quercetin equivalents in miligram per gram extract (mg QRT/g extract).

Statistical analysis

All the analysis were performed in triplicate and the results were statistically analyzed and expressed as mean (n = 3) ± standard deviation.

Antioxidant activity

Free radical scavenging activity

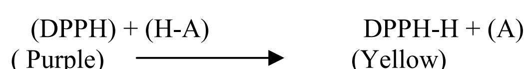
The free radical scavenging activity of methanol extract of *Ampelocissus araneosa* leaves, stem and root was measured by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method (Blois, 1958), 0.2 mM solution of DPPH in methanol was prepared and 100µl of this solution was added to various concentrations of, methanol extract of leaf, stem and root at the concentrations of 50, 100, 150, 200 and 250 µg/ml. After 30 minutes, absorbance was measured at 517nm. Butylated hydroxyl toluene (BHT) was used as the reference material. All the tests were performed in triplicate and percentage of inhibition was calculated by comparing the absorbance values of the control and test samples.

$$\% \text{ inhibition} = \left[\frac{(AC - AE)}{AB}\right] \times 100$$

where AC = absorbance of the control, AE = absorbance of tested samples and

AB = absorbance of the blank (without extract)
The scavenging reaction between DPPH and an antioxidant, H-A is

(DPPH) + (H-A) DPPH-H + (A)
(Purple) (Yellow)



Ferrous ion chelating activity

The chelating of ferrous ions by methanol extract of *Ampelocissus araneosa* leaves, stem and root was estimated (Singh and Rajini, 2004). The different concentrations of methanol extracts (1000, 2000, 3000, 4000 and 5000 µg/ml separately for leaf, stem and root) were mixed with 100µl of 2mM ferrous sulphate solution and 300µl of 5mM ferrozine. The mixture was incubated at room temperature for 10 minutes. The absorbance of the solution was measured at 562nm. Ethylene diamine tetra acetate (EDTA) was used as standard. All the tests were performed in triplicate and percentage of inhibition was calculated by using the formula,

$$\text{Percentage of inhibition} = \frac{(AB - AE)}{AB} \times 100$$

where AB = absorbance of the blank (without extract) and AE = absorbance of tested samples.

GC-MS

1 µl of the methanol extract of leaf, stem and root of *Ampelocissus araneosa* was employed for GC-MS analysis. It was carried out on a Thermo GC-Trace Ultra ver: 5.0, Thermo MS DSQ II and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: DB 5-MS capillary standard Non-Polar column, Dimension-30Mts, ID: 0.25 mm, Film -0.25 µm, helium was used as carrier gas at a constant flow of 1 ml/min. The oven temperature was programmed from 70°C raised to 260°C at 6°C / min. The eluted component is detected in the mass detector and the components were compared with components stored in the library and determined the name, molecular weight etc.

Identification of phytocomponents

Identification of compounds was conducted using the database of Wiley9 library combined with the National Institute of Standards and Technology (NIST) library. The name, molecular weight, molecular formula, and area under peak of the components of the test materials were ascertained.

RESULTS AND DISCUSSION

The results of total phenolic and flavonoids content are shown in Table 1. The total phenolic content was found to be high in the root extract (39.46 ± 1.65) followed by stem extract (34.44 ± 2.62) and leaf extract (31.67 ± 2.49). The total flavonoid content was found to be high in the root extract (24.64 ± 1.49) followed by the stem (19.58 ± 1.24) and leaf extract (16.76 ± 1.98). In the present study, the percentage of scavenging effect on the DPPH radical was concomitantly increased with the increase in the concentration of both leaf, stem and root methanolic extracts from 50 to 250 µg/ml. The percentage of inhibition was increased from 56.53 at 50 µg/ml to 78.67 at 250 µg/ml for leaf extract, 59.84 at 50 µg/ml to 80.11 at 250 µg/ml for stem extract, 58.78 at 50µg/ml and 82.72 at 250 µg/ml for root extract (Table 2). Furthermore, it was noticed that the methanol extract of leaf, stem and root extract has pronounced scavenging activity as with that of the standard, Butylated hydroxyl toluene.

Table 1. Total phenolic and flavonoid content of *Ampelocissus araneosa*

S. No	Phytochemicals	Plant parts used	Extract (g/gm)
1.	Phenol	Leaf	31.67 ± 2.49
		Stem	34.44 ± 2.62
		Root	39.46 ± 1.65
2.	Flavonoid	Leaf	16.76 ± 1.98
		Stem	19.58 ± 1.24
		Root	24.64 ± 1.49

Table 2. Free radical scavenging activity (DPPH) of methanol extract of *Ampelocissus araneosa*

S. No.	Sample Concentration (µg/ml)	BHT % of Inhibition	Leaf Extract % of Inhibition	Stem Extract % of Inhibition	Root Extract % of Inhibition
1	50	49.39 ± 0.34	56.53 ± 0.33	59.84 ± 0.27	58.78 ± 0.36
2	100	57.15 ± 0.23	59.63 ± 0.31	63.52 ± 0.24	64.48 ± 0.24
3	150	65.56 ± 0.25	68.55 ± 0.25	69.48 ± 0.37	71.74 ± 0.29
4	200	77.79 ± 0.35	71.59 ± 0.28	72.68 ± 0.31	75.69 ± 0.31
5	250	91.74 ± 0.42	78.67 ± 0.26	80.11 ± 0.25	82.55 ± 0.19

Table 3. Ferrous ion chelating activity of methanol extract of *Ampelocissus araneosa*

S. No.	Sample Concentration (µg/ml)	EDTA % of Inhibition	Leaf Extract % of Inhibition	Stem Extract % of Inhibition	Root Extract % of Inhibition
1	1000	52.28 ± 0.16	38.62 ± 0.23	40.64 ± 0.18	41.43 ± 0.29
2	2000	74.55 ± 0.37	43.47 ± 0.26	42.22 ± 0.42	43.82 ± 0.33
3	3000	84.48 ± 0.24	47.59 ± 0.32	45.56 ± 0.28	47.53 ± 0.37
4	4000	92.14 ± 0.28	52.84 ± 0.21	49.83 ± 0.33	53.65 ± 0.28
5	5000	95.53 ± 0.18	56.73 ± 0.31	54.66 ± 0.26	59.57 ± 0.30

Table 4. Phytochemicals identified in *Ampelocissus araneosa* by GC-MS analysis possessing antioxidant property

S. No.	RT	Area %	Name of Compound	Nature of compound
			Methanol extract of leaf	
1.	10.93	5.35	1,2,3-Benzenetriol	Pyrogallol[21]
2.	20.24	3.37	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Terpene alcohol[22]
3.	20.24	3.37	Neophytadiene	Terpene[23]
4.	22.66	7.05	Hexadecanoic acid (CAS)	Palmitic acid[24]
5.	22.66	7.05	n-Hexadecanoic acid	Palmitic acid[25]
6.	25.98	8.12	9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	Fatty acid[26]
7.	38.66	1.57	Stigmasta-5,22-dien-3-ol, (3á,22E)- (CAS)	Steroidal compound[27]
8.	38.66	1.57	Stigmasterol	Steroidal compound[28]
			Methanol extract of stem	
9.	20.24	0.84	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Terpene alcohol[22]
10.	20.24	0.84	Neophytadiene	Terpene [23]
11.	22.68	11.10	Hexadecanoic acid (CAS)	Palmitic acid [24]
12.	22.68	11.10	l-(+)-Ascorbic acid 2,6-dihexadecanoate	Ascorbic acid [27]
13.	22.68	11.10	n-Hexadecanoic acid	Palmitic acid [25]
			Methanol extract of root	
14.	7.97	10.37	Methyl salicylate	Carboxylic acid [29]
15.	22.03	0.2	Hexadecanoic acid, methyl ester (CAS)	Fatty acid methyl ester [30]

Iron binding capacity in terms of percent inhibition of the methanol extract of *Ampelocissus araneosa* at 5000µg/ml was higher for root (59.73%) than the leaf (56.96%) and stem (54.66%) (Table 3). However, it was comparable to that of the reference standard, Ethylene diamine tetra acetate. The antioxidant properties of medicinal plants are due to phenolic compounds. The methanol extract of *Ampelocissus araneosa* showed higher level of phenols (root 39.46 ± 1.65, stem 34.44 ± 2.62 and leaf 31.67 ± 2.49) than the other secondary metabolites. The higher amount of phenol is important in regulation of plant growth, development and disease resistance. Earlier reports revealed that plant phenolic compounds including flavonoids are potent antioxidants with reported antimutagenic and anticarcinogenic effects (Middleton and Kandaswami, 1994). The abundance of flavonoids in the stem and root is also indicative of its potent antioxidant effect, which suggests that the plant may be very useful as an antibacterial, antiinflammatory, antiallergic, antiviral, antithrombotic, antimultagic and vasodilatory compound (Alan and Miller, 1996). The antioxidative properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metalions, such asiron and copper and inhibition of enzymes responsible for free radical generation (Shahriar et al., 2013). Several studies have described the antioxidant properties of different parts of various medicinal plants which are rich in phenolic compounds (Prabhakaran and Kavitha, 2012; Lima et al., 2010). Many supportive reports emphasize the positive correlation between phenolic content and antioxidant efficacy (Krishnamoorthy et al., 2011). DPPH test, which is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants, is a direct and reliable method for determining radical scavenging action (Raquibul Hasan et al., 2009). The reducing capacity of compounds could serve as indicator of potential antioxidant property (Meir et al., 1995). Thus, the results of the antioxidant activity showed that methanol extract of root with increasing concentration in both the methods were higher comparing to stem and leaf, proving

that phenolic and flavonoid contents were also higher in root than stemand leaf. GC-MS analysis revealed the phytochemicals such as 1,2,3-Benzenetriol; 3,7,11,15-Tetramethyl-2-hexadecenol; Neophytadiene; Hexade canoic acid (CAS); n-Hexadecanoic acid; 9,12,15-Octade catrien-1-ol, (Z,Z,Z); Stigmasta-5,22-dien-3-ol, (3á,22E)- (CA S); Stigmasterol ; l-(+)-Ascorbic acid2,6-dihexadecanoate; Methyl salicylate; Hexadecanoic acid, methyl ester (CAS) which could be responsible for the antioxidant activity along with their other biological activities (Table 4).

Conclusion

The present study has demonstrated that *Ampelocissus araneosa* extracts possess potent antioxidant activities, which could be due to phenols and flavonoids. The free radical scavenging capacity and ferrous ion chelation activity suggested that activity with increase in concentration of the extract. These antioxidant properties may be due to the phytochemical compounds revealed through GC-MS analysis.

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