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Research Article

Preliminary phytochemical studies on Ethanol extract of leaves of *Ampelocissus araneosa*

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ABSTRACT

The current study is focused on determination of organoleptic character and analyses of physicochemical, fluorescence, phytochemical and GC-MS of shade dried *Ampelocissus araneosa* leaves. Through the study, the extractive value of ethanol was found to be higher than ethyl acetate and the % sulphated ash value was higher followed by water soluble ash value. The value of loss on drying indicates the presence of less amount of moisture and also results of pH of suspensions (1% and 10%) of *Ampelocissus araneosa* showed within the range of 5-7 that indicates the suitability of plant for the human usage. The appearance of green fluorescence shows the existence of chromophore containing phytoconstituents. The ethanolic extract of *Ampelocissus araneosa* shows the existence of carbohydrate, steroids, alkaloids, glycosides, tannins, flavonoids and terpenoids, and absence of gums and mucilage, sterols, proteins, and phenols. The GC-MS analysis confirms the presence of 12 phytoconstituents.

Keywords: *Ampelocissus araneosa*; Leaves; Ethanolic extract; Organoleptic character; Physicochemical; Phytochemical; GC-MS analysis.

INTRODUCTION

*Ampelocissus araneosa*is a climbing shrub (Figure 1) belonging to the family of Vitaceae. It is generally named as Asvakathara and Kauraj in Sanskrit, Ghorvel in Hindi and Kattu Thiratchai in Tamil. It is distributed in moist deciduous to evergreen forests in Tamil Nadu, Karnataka, Kerala and Maharashtra (Gamble, 1918; Prajapati*et al*., 2003; Uma Maheswari and Meerabai, 2015).Leaves are 3-foliolate, occasionally simply lobed, glabrescent on top and brownish-tomentose below (Matthew, 1995).The leaf of plant contains 22-epicalamistrin, uvaribonin and chalcone (Pettit et al., 2008).The plant has currently in traditional use for the treatment of fever, snake-bite, wounds, skin diseases, and headache (Kuru Suresh *et al*., 2011; Uma Maheswari and Meerabai, 2015).Quite recently, the methanolic extract of leaf, root and stem has been reported for its hepatoprotective, anti-inflammatory and analgesic activities (Uma Maheswari and Meerabai, 2016). Based on the above literature survey, in the current study, we are focussing on organoleptic character, physicochemical, fluorescence, phytochemical and GC-MS analyses of leaves of *Ampelocissus araneosa* plant species.

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MATERIALS AND METHODS

Plant material

The plant of *Ampelocissus araneosa* has been collected in the Shervaroyan Hills, Yercaud, Tamil Nadu in the month of November 2011 and authenticated by Dr. A. Balasubramanian, Executive Director, ABS Botanical conservation, Research & Training Centre, Kaaripatti, Salem, Tamil Nadu. The sample leaves of *Ampelocissus araneosa* were stored in Department of Pharmacology.

Figure 1: Picture of *Ampelocissus araneosa*

Preparation of plant material

The collected leaves from the whole plant of *Ampelocissus araneosa* were cleaned and washed initially with tap water followed by distilled water. The plants leaves were shade dried completely under room temperature for 2 weeks; dried leaves have been powdered by using mechanical grinder and then sieved in 60 meshes. The sieved material was stored in well closed container.

Preparation of ethanolic extract

Approximately, about 250 g of leaves powder of *Ampelocissus araneosa* had been extracted using 500 ml of ethanol in Soxhlet extractor at a temperature between 50-65°C for 72 h (Figure 5). The ethanol from the extract was removed under room temperature and dried out in the desiccators (Harborn, 1998).

Evaluation of organoleptic characters

Organoleptic characters refer to the study of morphology and other sensory characters such as odour, colour, taste, touch, texture etc. The organoleptic characters of powder of *Ampelocissus araneosa* was evaluated based on the well-established method (Saxena *et al*., 2012).

Physicochemical constants

Physicochemical constants like ash values, extractive values, pH and loss on drying have been determined based on the well-established methods and procedures (Anonymous, 1996; Sumbul *et al*., 2012).

Table 1: Organoleptic characters of leaf powder of *Ampelocissus araneosa*

S. No	Parameters	Raw
1	Appearance	Powder
2	Touch	Coarse
3	Colour	Dark greenish
4	Taste	Sweet
5	Odour	Pleasant

Table 2: Physicochemical constant values of the leaves of *Ampelocissus araneosa*

S. No	Physicochemical parameters	**Results** (Mean ± SEM, n=3)
1	Sulfated ash	11.20% ± 0.022
2	Water-soluble ash	10.22% ± 0.020
3	Total ash	9.17% ± 0.028
4	Acid-insoluble ash	3.18% ± 0.026
5	Ethanol-soluble extractive	18.21% ± 0.047
6	Acetone-soluble extractive	11.72% ± 0.052
7	Ethyl acetate-soluble extractive	10.17% ± 0.035
8	Water-soluble extractive	8.14% ± 0.043
9	Chloroform-soluble extractive	3.81% ± 0.052
10	Petroleum-soluble extractive	3.17% ± 0.024
11	Loss on drying	8.56 % ± 0.067
12	pH (1 and 10% w/v)	7.64±0.023&5.25±0.067

Table 3: Fluorescence analysis of powder of leaf of *Ampelocissus araneosa*

Light	Treatment of powder				
	Raw	10% HCl	10% aqueous NaOH	10% alcoholic NaOH	10% H_2SO_4
Day	Dark-green	Pale-brown	Brown	Chocolate- brown	Yellowish-green
Short UV (254 nm)	Pale-green	Black	Reddish- brown	Light-green	Black
Long UV (365 nm)	Green	Dark-green	Green	Light-green	Dark-green

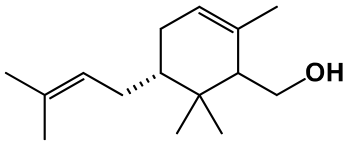
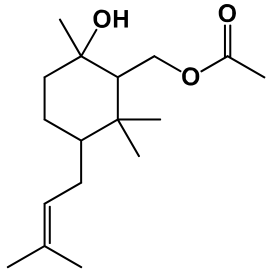
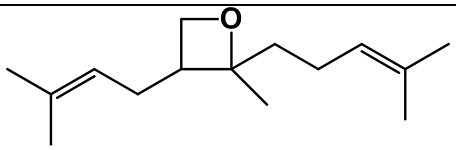
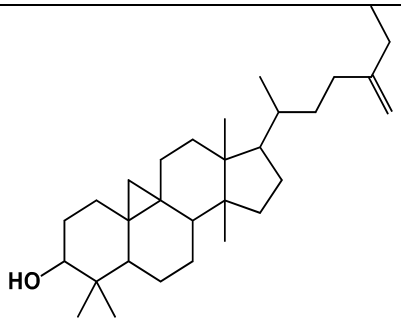
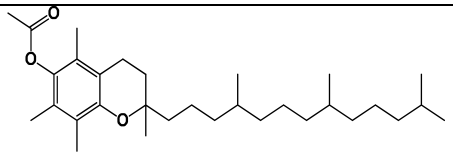
Table 4: Preliminary phytochemical screening of ethanolic extract of leaves of *Ampelocissus araneosa*

S. No	Tests	Ethanolic extract
1	Alkaloids	Present
2	Flavonoids	Present
3	Glycosides	Present
4	Terpenoids	Present
5	Gums and mucilage	Absent
6	Phenols	Absent
7	Steroids	Present
8	Sterols	Absent
9	Tannins	Present
10	Proteins	Absent
11	Carbohydrates	Present

Table 5: List of phytoconstituents in ethanolic extract of *Ampelocissus araneosa*

RT	Name of the phytoconstituents	Molecular formula	M. Wt
22.32 & 26.21	2,4,4-trimethyl-3-hydroxymethyl-5A-(3-methyl-but-2-enyl)-cyclohexene **(A)**	$C_{15}H_{26}O$	222.37
22.56, 22.94, 23.13, 23.89, 24.11, 24.53 & 27.36	2R-acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1t-cyclohexanol **(B)**	$C_{17}H_{30}O_3$	282.42
26.32	2-methyl-3-(3-methylbut-2-en-1-yl)-2-(4-methylpent-3-en-1-yl)oxetane **(C)**	$C_{32}H_{56}O_4$	504.78
26.69	9,19-cyclolanostan-3-ol, 24-methylene-, (3.beta.)- **(D)**	$C_{30}H_{50}O$	426.72
27.49	2H-1-benzopyran-6-ol, 3,4-dihydro-2,8-dimethyl-2-(4,8,12-trimethyltridecyl)-, [2R-[2R*(4R*, 8R*)]], (&-Tocopherol) **(E)**	$C_{30}H_{50}O$	426.72

Table 6: Phytoconstituents, their nature and biological activities of ethanolic extract of leaves of *Ampelocissus araneosa*

Structure of the phytoconstituents	Biological activity	References
 (A)	Apoptosis agonist, CYP2J substrate, Antieczematic, Ubiquinol-cytochrome-c reductase inhibitor; Antibacterial; Antioxidant; Anti-inflammatory	Ambritha *et al*., 2016; Karthik *et al*., 2015; Sen *et al*., 2015; Anupama *et al*., 2014
 (B)	Anti-inflammatory	Anupama *et al*., 2014
 (C)	Acaricidal activity	Nyabayo *et al*., 2015
 (D)	Anti-listerial	Penduka *et al*., 2014
 (E)	Antimicrobial, Antidiabetic, Anti-inflammatory, Antioxidant, Antitumor	Venkata Raman *et al*., 2012

Fluorescence analysis

Fluorescence analysis of leaves powder of *Ampelocissus araneosa* was performed as per the standard method and procedure (Beckett and Stenlake, 1997; Padmavathy *et al*., 2010).

Preliminary phytochemical analysis

Preliminary phytochemical screening was performed according to standard method and procedure (Harborn, 1998; Kokate, 2008).

GC-MS analysis

The GC-MS study of ethanolic extract was carried through Perkin Elmer GC-MS. Interpretation of spectra of phytoconstituents in the extract has been carried through reference database available in the NIST library. The chromatogram of unknown phytoconstituents was matched with the chromatogram of known phytoconstituents.

RESULTS

Evaluation of organoleptic characters

Organoleptic characters of leaf powder of *Ampelocissus araneosa* are presented in Table 1.

Physicochemical constants

Physicochemical constants of leaves of *Ampelocissus araneosa* are shown in Table 2.

Fluorescence analysis

The fluorescence analysis of powder leaves was viewed under the day light and short UV light and long UV light are shown in Table 3.

Preliminary phytochemical analysis

The phytochemical analysis of ethanolic extract of

leaves of *Ampelocissus araneosa* was carried out and results were tabulated in Table 4.

GC-MS analysis

The chromatogram of leaves extract of *Ampelocissus araneosa* has shown 12 phytoconstituents as shown in Figure 2 and Table 5, 6.

DISCUSSION

Medicinal plants are very important sources for biologically active constituents for design of new therapeutic agents. Initially, the leaves powder of *Ampelocissus araneosa* has been subjected to determination of organoleptic characteristics, physicochemical and fluorescence analysis. This study implies the quantity of soluble phytoconstituents for the given quantity of plant material. This helps us to judge the authenticity of the plants as well as to distinguish the plant material from the adulterants or allied species.

Report of physicochemical constants may be served as a valuable source for information and also helps in assessing the purity as well as the quality of the leaves. The extractive values provide the information for the nature of phytoconstituents present in the leaves. Water soluble extractive value is utilized for extraction of sugars, tannins, glycosides, mucilage, plant acids, etc in the medicinal plants. Ether soluble extractive value is utilized for the extraction of fixed oils, volatile oils, steroids and resins. From our study, it is observed that the extractive value of alcohol is higher than that of ethyl acetate and it is well established that alcohol is considered as the best solvent for the extraction of many phytoconstituents like alkaloids, resins, tannins, etc. indicating the presence of these constituents in our extract. The ash value can determine the inorganic matter along with some other impurities present together with the drug and percentage of ash value was in the order of acid insoluble < total ash < water soluble < sulfated ash for leaf powder. Any deviation in the ash values may indicate the adulteration of the drug. In our present findings, the ash values were found to be within expected limits indicating that there is no adulteration.

The value of moisture content or loss on drying indicates the presence of less amount of moisture in

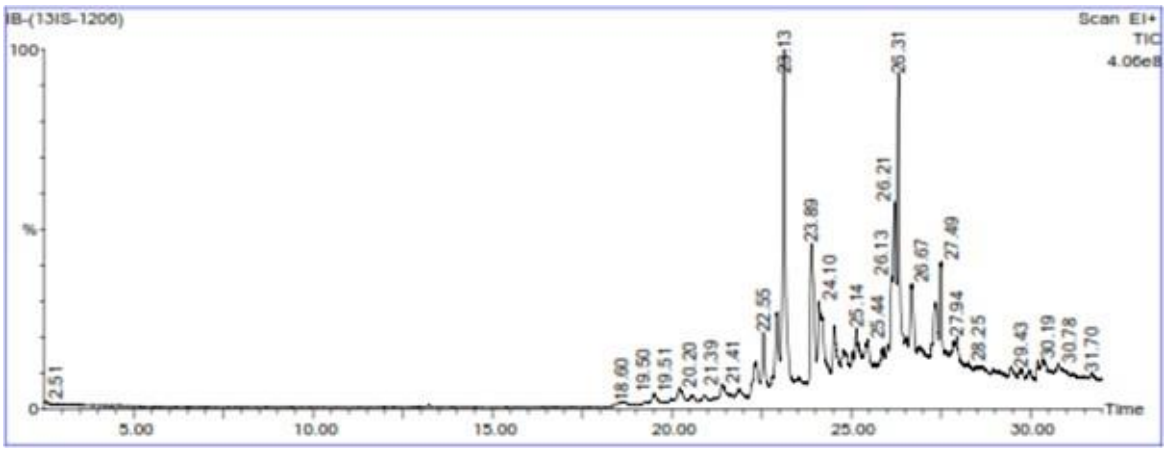


Figure 2: Chromatogram of the ethanolic extract for the leaves of *Ampelocissus araneosa*

the leaves powder. The presence of moisture is an undesirable factor favouring the growth of microorganism or enzyme hydrolysis leading to deterioration of leaves. The low moisture content prevents bacterial and fungal growth during storage and also imparts high stability.

The pH of suspensions (1% and 10%) of *Ampelocissus araneosa* showed within the range of 5-7 that indicates the suitability of plant for the human usage.

The presence of fluorescent compounds was examined in UV and daylight. The appearance of green fluorescence shows the presence of chromophore containing phytoconstituents.

The preliminary phytochemical analysis of ethanolic extract has revealed the existence of carbohydrate, steroids, tannins, alkaloids, glycosides, terpenoids, flavonoids and the absence of gums and mucilage, sterols, protein, and phenols. These types of phytoconstituents have been reported for several therapeutic and biological applications proving the medicinal significance of this species.

The GC-MS analysis of ethanolic extract shown the presence of 12 phytoconstituents and the identification of the phytoconstituents was determined on the basis of molecular formula, and retention-time with peak area. These phytoconstituents are responsible for various pharmacological activity such as phytoconstituents A for apoptosis agonist, CYP2J substrate, antieczematic, ubiquinol-cytochrome-c reductase inhibitor, antibacterial, antioxidant and anti-inflammatory; phytoconstituents B for anti-inflammatory; phytoconstituents C for acaricidal activity; phytoconstituents D for anti-listerial; phytoconstituents E for antimicrobial, antidiabetic, anti-inflammatory, antioxidant, antitumor agents. The existence of various bioactive phytoconstituents deserves the use of the leaves for various ailments by traditional practitioners.

CONCLUSION

The ethanolic extract of leaves of *Ampelocissus araneosa* species has been subjected to preliminary phytochemical studies. The organoleptic characters aimed to characterize its physical and sensory characters were determined. Further, physicochemical analysis gives information regarding the nature of the plant and valuable phytoconstituents present in it. Further, the assessment of moisture content, pH and fluorescence analysis throw more light upon its suitability as a drug for human use. The GC-MS analysis proves the presence of numerous biologically important chemical moieties responsible for various pharmaceutical applications measured based on the retention time and peak area. Thus, based on the results obtained from the above studies favours the medicinal value of this species and has greater potential to be developed as a drug candidate. This requires thorough study on various aspects to be attributed to its biological efficacy of the species and is underway.

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