



ANTIFUNGAL, PHYTOCHEMICAL, PROTEIN AND FT-IR ANALYSIS OF *AMPELOCISSUS LATIFOLIA* (ROXB.) PLANCH.

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ABSTRACT

In recent times, focus on plant research has increased all over the world. The therapeutic effect of these plants for the treatment of various diseases is based on the chemical constituents present in them. Medicinal plants provide affordable means of health care for poor and marginalised people. *Ampelocissus* is a genus of Vitaceae family. The FT-IR analysis of stem and fruit powder showed the alkanes, alkenes, alcohols, esters, amines, ketones, aldehydes. Despite its well-recognised medicinal and economic potential, there are no commercial plantations worldwide. Wild plants have continuously been used to meet the growing commercial demand in terms of their socio-economic value. *Ampelocissus latifolia* is the plant which may not be freely available in future due to over exploitation, habitat destruction or lack of domestication and cultivation. Since this plant species is an important ingredient of several medicines due to its usefulness, phytochemical investigation for isolation of important active ingredients through cell culture will be helpful.

KEY WORDS

Ampelocissus latifolia, Vitaceae

INTRODUCTION

In recent times, focus on plant research has increased all over the world. The therapeutic effect of these plants for the treatment of various diseases is based on the chemical constituents present in them. Medicinal plants provide affordable means of health care for poor and marginalised people. *Ampelocissus* is a genus of Vitaceae family. This family is very interested because of their wide geographical and ecological range. *Ampelocissus* having more species found in tropical region, Africa, Asia and throughout the world. *Ampelocissus* was first discovered by Planchon in 1887. It is native to Indian subcontinent is used extensively for its medicinal values [1-2].

Ampelocissus latifolia (Roxb) is a large twining herbaceous climber [3]. Fruits are spherical and reddish brown in colour, it is similar to cultivated grape and the stem is hollow shaped and it is mainly used for bone fracture and stem ash is applied abdominally for easy

delivery[4]. More species of *Ampelocissus latifolia* reported to have medicinal value and the plants in Ayurveda used as kushtha, kamala, sotha and Varna [5] and traditionally plant is used for wound healing [5]. Mainly fruit and stem extract can be used as a raw drug and they possess varied medicinal properties[6]. Dried fruit, stem, leaf and root powder of *Ampelocissus latifolia* extract with ethanol, methanol, acetone and hexane were tested for antifungal activity [7].

MATERIALS AND METHODS

Plant collection

The plant *Ampelocissus latifolia* was collected from Salem. The fruit stem, leaf and root were shade dried and made into coarse powder with mechanical grinder and the fine powder was passed through sieve.

Preparation of plant extract

The fruit stem, leaf and root powder of *Ampelocissus latifolia* (20gms) was extracted with various solvents such as ethanol, methanol, acetone and hexane by soxhlet extraction for 8hrs. The extracts obtained were kept for evaporation to remove the excess solvent. These extracts were then stored in plastic bottle in refrigerator for further studies.

Antifungal analysis

The antifungal analysis was carried out by Disc Diffusion method. The disc were dipped in various extracts, dried and kept on Potato Dextrose Agar medium, swabbed with test organism and plates were incubated at 30°C for 48hours.

Phytochemical analysis

1gm of fruit, stem, leaf and root powder was extracted with 10ml of methanol and these extracts were subjected to qualitative tests to determine the presence of various phytoconstituents such as alkaloids, reducing sugar and flavonoids.

Protein analysis

The fruit stem, leaf and root extract was tested with ninhydrin solution and change in colour indicates the presence of protein in the sample. The quantification of protein was tested by ELISA using Brad Ford method to quantify the amount of protein present in the sample.

FTIR analysis

The fruit, stem, leaf and root powder of *Ampelocissus latifolia* was examined by infra-red spectrum at the spectral range of 500 – 4000 cm⁻¹ by Fourier Transform Infra-Red Spectroscopy to recognize the functional groups.

of basal position within the Rosids. Morphologically, the family is well delimited and easily recognized.

Antifungal analysis

Antifungal activity of *Ampelocissus latifolia* on *Aspergillus Niger* and *Penicillium candidum* has been investigated in ethanol, methanol, acetone and hexane extracts of fruit, stem, leaf and root of *Ampelocissus latifolia*. The extract of the plant showed no inhibition zone against all tested fungi by Shahat *et al.*, [8]. In our present study there was no antifungal activity recorded in ethanol, methanol, hexane and acetone extracts of fruit, stem, leaf and root, when *Aspergillus niger* & *Penicillium candidum* was used.

Phytochemical studies

The fruit, stem, leaf and root extracts were subjected to qualitative tests to determine the presence of various phytoconstituents such as alkaloids and reducing sugars. Fruit, stem, leaf and root extract of *Ampelocissus latifolia* showed the presence of phytochemicals like alkaloids, flavonoids, reducing sugars and gums (Table 1-4) and the same were reported by Parag *et al.*, [9].

Protein analysis

The stem, fruit, leaf and root extracts were tested with Ninhydrin solution and change in colour indicates the presence of protein in the sample. The quantification of protein by ELISA using Brad Ford method showed the presence of high amount of protein in fruit compared to others. (Table 5).

FTIR analysis

The FTIR analysis revealed the similarity and variation between the stem, fruit, leaf and root parts of *Ampelocissus latifolia* based on the functional group present. The FTIR analysis of stem, fruit, leaf and root powder showed the presence of amines, amides, alcohols, carboxylic acid, esters and nitro compounds and phenols (Table 6-9) and the same was reported by Pednekar *et al.*, [10-11].

RESULTS AND DISCUSSION

Ampelocissus latifolia belongs to the family vitaceae having more species found in tropical region. The evolutionary history of the grape family is of interest because of the wide geographical and ecological range

Table 1: Phytochemical studies of *Ampelocissus latifolia* stem extract

S NO	REAGENT/TEST	TEST FOR	COLOUR CHANGE	STEM
1	Benedict's reagent	Reducing sugars	Brick red	+
2	Fehling's reagent	Reducing sugars	Brick red	+
3	Molisch's test	Reducing sugars	-	-
4	10%NaOH	flavonoids	-	-
5	Mayer's reagent	alkaloids	-	-

[Ppt – Precipitate, + - Present, - - Absent

Table 2: Phytochemical studies of *Ampelocissus latifolia* fruit extract

S NO	REAGENT/TEST	TEST FOR	COLOUR CHANGE	FRUIT
1	Benedict's reagent	Reducing sugars	Brick red	+
2	Fehling's reagent	Reducing sugars	Brick red	+
3	Shinoda's test	Flavonoids	-	-
4	Mayer's reagent	Alkaloid	Cream	+

+ - Present, - - Absent

Table 3: Phytochemical studies of *Ampelocissus latifolia* leaf extract

S NO	REAGENT/TEST	TEST FOR	COLOUR CHANGE	LEAF
1	Benedict's reagent	Reducing sugars	Brick red ppt	+
2	Fehling's reagent	Reducing sugars	Brick red ppt	+
3	Molish's test	Gums	Red-violet ring	+
4	10% NaOH	Flavonoids	-	-
5	Wagner's reagent	alkaloids	Orange brown ppt	+

Ppt- Pricipitate , + - Present , - - Absent

Table 4: Phytochemical studies of *Ampelocissus latifolia* root extract

S NO	REAGENT/TEST	TEST FOR	COLOUR CHANGE	ROOT
1	Benedict's reagent	Reducing sugars	-	-
2	Fehling's reagent	Reducing sugars	-	-
3	Shinoda's test	Flavonoids	Pink colour	-
4	Wagner's reagent	Alkaloids	-	-

+ - Present, - - Absent

Table 5: Protein analysis of *Ampelocissus latifolia* stem and fruit extract

S NO	SAMPLE	PROTEIN $\mu\text{g}/\mu\text{l}$
1	STEM	5.22
2	LEAF	7.6
3	ROOT	7.7
4	FRUIT	13.77

Table 6: FT-IR peak values of *Ampelocissus latifolia* stem extract

S NO	STANDARD	BOND	WAVE NUMBER	FUNCTIONAL GROUP ASSIGNMENT
1	3500 – 4000	O-H stretching of α -cellulose	3906.98	√
			3718.92	
2	3500 – 3300	N- H stretching	3641.76	√
			-	
3	2960 – 2850	C-H stretching	2928.07	√
			2861.52	
4	1740 – 1720	C=O stretching of hemicellulose	1737.94	√
			-	
5	1600, 1580, 1500, 1450	C=C stretching	1614.49	√
			-	
6	Above - 1500	C=O, NH ₂ , NH, C=C, C=N functional group stretching	-	√
7	1350 – 1260	C=C stretching	1219.06	√
8	1050 – 1020	Symmetric C-OH stretching of lignin	1440.89	√
9	800 – 600	C-S stretching	670.29	√
10	545 - 240	S-S stretching	548.77	√

Table 7: FT-IR peak values of *Ampelocissus latifolia* fruit extract

S NO	STANDARD	BOND	WAVE NUMBER	FUNCTIONAL GROUP ASSIGNMENT
1	3500 – 4000	O-H stretching of α -cellulose	3716.02	✓
2	3700 – 3500	N-H stretching(amine)	3301.31	✓
3	2960 – 2850	C-H stretching	2929.03	✓
4	1740 – 1720	C=O stretching of hemicellulose	1730.22	✓
5	1600	C=O stretching(amide)	6006.77	✓
6	1600, 1580, 1500,1450	C=C stretching	1531.55	✓
7	1450 – 1400	CH ₂ symmetric bending	1437.02	✓
8	1350 – 1260	C=C stretching	1219.06	✓
9	1050 – 1020	Symmetric C-OH stretching of lignin	1034.85	✓

Table 8: FT-IR peak values of *Ampelocissus latifolia* leaf extract

S.NO	Standard	Bond	Wave number	Functional Group Assignment
1	3500-4000	O-H stretching of α -cellulose	3854.90,3733.38,3613.79	✓
2	3500-3300(m)	N-H stretching(amine)	3338.92	✓
3	2960-2850	C-H stretching	2926.14	✓
4	1740-1720	C=O stretching of hemicellulose	1731.19	✓
5	1600,1580,1500, 1450	C=C stretching	1614.49	✓
6	Above-1500	C=O,NH ₂ ,NH,C=C,C=N functional group stretching	1519.01	✓
7	1350-1260	C= O stretching	1225.82	✓
8	1050-1020	Symmetric C-OH stretching of lignin	1036.78	✓
9	800-600	C-S stretching	638.47	✓
10	545-240	S-S stretching	457.15	✓

Table 9: FT-IR peak values of *Ampelocissus latifolia* root extract

S.NO	Standard	Bond	Wave number	Functional Group Assignment
1	3500-4000	O-H stretching of α -cellulose	3715.06,3584.86	✓
2	3500-3300(m)	N-H stretching(amine)	3338.92	✓
3	2960-2850	C-H stretching	2934.82	✓
4	1740-1720	C=O stretching of hemicellulose	1739.87	✓
5	1600,1580,1500, 1450	C=C stretching	1603.88	✓
6	1050-1020	Symmetric C-OH stretching of lignin	1014.60	✓
7	800-600	C-S stretching	666.43	✓

CONCLUSION

From the above investigation fruit and stem extract of *Ampelocissus latifolia* was evaluated against fungi such as *Aspergillus niger* and *Penicillium candidum*. *Ampelocissus latifolia* have poor antifungal activity against the tested fungi. The same extracts were also tested for phytochemical study indicates the alkaloids, reducing sugar and flavonoids. The stem and fruit powder of *Ampelocissus latifolia* was tested to quantify the protein by ELISA method. The FT-IR analysis of stem and fruit powder showed the alkanes, alkenes, alcohols, esters, amines, ketones, aldehydes. Despite its well-recognised medicinal and economic potential, there are no commercial plantations worldwide. Wild plants have continuously been used to meet the growing commercial demand in terms of their socio-economic value. *Ampelocissus latifolia* is the plant which may not be freely available in future due to over exploitation, habitat destruction or lack of domestication and cultivation. Since this plant species is an important ingredient of several medicines due to its usefulness, phytochemical investigation for isolation of important active ingredients through cell culture will be helpful.

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