

Original Article

PHYSICO CHEMICAL AND PHYTOCHEMICAL EVALUATION OF AMPELOCISSUS LATIFOLIA (ROXB) PLANCH LEAVES

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ABSTRACT

Objective: *Ampelocissus latifolia* (Roxb.) Planch. belongs to family Vitaceae. Literature survey shows that very less amount of work has been done on the leaf of this plant, hence it was selected for the study of physico chemical and phytochemical properties.

Methods: It includes preparation of 14 different polar and nonpolar extracts by soxhlet extraction for detailed analysis.

Results: Physico chemical studies showed constant and definite values which will help in the correct identification of the plant. The preliminary phytochemical studies showed the presence of alkaloids, saponins, carbohydrates, glycosides, tannins, flavonoids, steroids, phenols, proteins, monosaccharides, hexose sugars, starch, mucilages & gums. Further fluorescent analysis of different soxhlet extracts and leaf powder provides additional support for the qualitative chemical analysis findings.

Conclusion: The results suggest that the leaf of the plant has many phytochemical properties and may be used for curing various ailments.

Keywords: Fluorescent analysis, Physico chemical, Phytochemical, Soxhlet extraction, *Ampelocissus latifolia* (Roxb.) Planch.

INTRODUCTION

The use of natural plant remedies around the world has created the need for information about the properties and uses of the medicinal plants. Standardization of plants and herbal materials is the activity of assigning a set of standards or inherent characteristics, definitive qualitative and quantitative values which gives the assurance of quality, efficacy, safety and reproducibility. Proximate analysis for plants gives added information and help to access the quality of the plant raw material sample. It gives information on moisture content, ash content, fixed carbon etc. Ash is the inorganic residue remaining after water and organic matter have been removed by heating, which provides a measure of total amount of minerals within the drug. Ashes give us an idea of the mineral matter contained in a plant. Measuring it is important, because mineral matter may be the cause of a pharmacological effect [1].

Ampelocissus latifolia belongs to family Vitaceae (Grape family). It is a large herbaceous climber, with a tuberous root stock. Stem and branches are hollow, more or less smooth. Leaves circular or broadly heart-shaped with lobes acute and toothed [2]. The roots have been used for the treatment of snake bite and for its astringent effect. The decoction of the root is also used in chronic dysentery. The Sandals of Bihar use this plant for muscular pains, sores and

fractured bones [3]. The common name of the plant in English language is "Jungli angoor" or the other common name is "Panibel" in India. Juice of tender leaves is used in dental problems and as a detergent for indolent ulcers [4]. The main aim of our present study is to investigate the proximate analysis and phytochemical properties of *Ampelocissus latifolia* leaves.

MATERIALS AND METHODS

Collection and authentication of plant

The leaves of *Ampelocissus latifolia* were collected from Mumbai, Maharashtra. The identification of the plant was done at the Blatter Herbarium, St. Xavier's College, Mumbai. The *Ampelocissus latifolia* (Roxb.) Planch specimen matches with the Blatter Herbarium specimen no. Shah-I of G. L. Shah. The leaves were thoroughly washed with distilled water, dried in an oven at 40°C and grounded into fine powder by using a mechanical grinder.

Physicochemical analysis

The physicochemical parameters like total ash, water soluble ash, acid insoluble ash, sulphated ash, loss on drying, water soluble and alcohol soluble extractive values, pH, foaming index, crude fibre content were determined [5-8], the results of which are given in Table no.1.

Table 1: Physicochemical test of powdered leaves of *Ampelocissus latifolia*

S. No.	Parameters	Mean values (% w/w) ± SEM
1.	Total ash	15.703 ± 0.178
2.	Water soluble ash	3.557 ± 0.286
3.	Acid insoluble ash	9.259 ± 0.144
4.	Sulphated ash	0.556 ± 0.043
5.	Water soluble extractive value	10.640 ± 0.08
6.	Alcohol soluble extractive value	16.933 ± 0.218
7.	Moisture content (Loss on Drying)	6.712 ± 0.256
8.	Crude fibre	23.983 ± 1.516
9.	pH	6.00
10.	Foaming index	Less than 100

*SEM = Standard error of the mean (n=3).

Table 2: Fluorescence Analysis of Powdered Leaves of *Ampelocissus latifolia*

S. No.	Test	Visible Light	Under U.V.light (254 nm)	Under U.V.light (366 nm)
1.	Powder as such	Dark Brown	Dark Brown	Black
2.	Powder + 5% Aqueous NaOH	Dark Brown	Black	Black
3.	Powder + Aqueous 60% H ₂ SO ₄	Dark Brown	Black	Black
4.	Powder + conc. H ₂ SO ₄	Dark Brown	Black	Black
5.	Powder + conc.HNO ₃	Reddish Brown	Light Green	Light Pink
6.	Powder + conc. HCl	Yellow Brown	Dark Green	Black
7.	Powder + Glacial Acetic acid	Yellowish Brown	Light Green	Purple Violet
8.	Powder + 1N NaOH in Methanol	Yellowish Brown	Dark Green	Purple
9.	Powder + Ethanol	Black	Dark Green	Light Red
10.	Powder + HNO ₃ + NH ₃ Solution	Orange	Dark Brown	Light Purple
11.	Powder + 50% HNO ₃	Yellowish Brown	Dark Green	Purple
12.	Powder + Alcoholic KOH	Dark Brown	Dark Green	Black
13.	Powder + 5% KOH	Yellowish Brown	Dark Green	Black
14.	Powder + Ammonia solution 25% v/v	Dark Brown	Dark Green	Black
15.	Powder + 5% Ferric chloride	Light Brown	Light Green	Black
16.	Powder + Picric acid	Yellowish Brown	Light Green	Dark Brown
17.	Powder + Iodine Solution	Black	Light Green	Black

Table 3: Fluorescence characteristic of leaf extract of *Ampelocissus latifolia*.

S. No.	Leaf Extract	Under ordinary light	Under UV light (254 nm)	Under UV light (366 nm)
I	Water	Dark Yellowish Brown	Dark Yellow	Dark Brown
II	Chloroform	Light Green	Light Green	Dark Red
III	Toluene	Yellow Brown	Dark Green	Light Purple
IV	Carbon tetra chloride	Yellow	Black	Red
V	Ethyl acetate	Dark Green	Dark Green	Dark Red
VI	Hexane	Yellow	Light Green	Light Pink
VII	Ethyl alcohol	Light Green	Dark Green	Dark Red
VIII	Methanol	Light Green	Light Green	Light Red
IX	Acetone	Light Green	Light Green	Light Pink
X	2- Propanol	Yellowish Green	Dark Green	Dark Pink
XI	Petroleum ether (60-80°C)	Yellow	Light Green	Light Red
XII	2- butanone	Dark Green	Black	Dark Pink
XIII	Dichloromethane	Yellow	Light Green	Light Pink
XIV	Ethyl ether	Light Green	Light Green	Light Pink

Preparation of plant extract

The leaf powder of *Ampelocissus latifolia* (20 gm) was extracted with 250 ml each of polar and nonpolar solvents by soxhlet extraction for 8 hrs. The extracts obtained were later kept for evaporation to remove the excessive solvents. These extracts were then stored in plastic bottle in refrigerator for preliminary phytochemical analysis. Powdered leaf material was extracted using water, chloroform, toluene, carbon tetra chloride, ethyl acetate, hexane, ethyl alcohol, methanol, acetone, 2-propanol, petroleum ether (60-80°C), 2-butanone, dichloromethane, ethyl ether and was subjected for identification of various plant constituents [9].

Fluorescence Analysis

Fluorescence characteristics of powdered leaf and leaf extract were examined [10]. The observed results are given in the Table no.2 & 3 respectively.

Phytochemical screening

Extracts of *Ampelocissus latifolia* leaves were subjected to phytochemical screening. The results obtained in the present investigation are shown in Table no.4. It showed the presence of alkaloids, saponins, carbohydrates, glycosides, tannins, flavonoids, steroids, phenols, proteins, monosaccharides, hexose sugars, starch, mucilages & gums. [11-14].

RESULTS

The mean values (n=3) of % w/w \pm SEM (Standard error of the mean) of total ash, acid insoluble ash, sulphated ash, water soluble ash etc. are tabulated in the Table no.1. The loss on drying at 105°C in leaf was found to be 6.712 \pm 0.256. The analytical results showed that total ash value content was 15.703 \pm 0.178. Similarly, the amount of acid insoluble matter present in the plant was 9.259 \pm 0.144. & water soluble ash was 3.557 \pm 0.286. The water soluble and

alcohol soluble extractive values were 10.640 \pm 0.08 and 16.933 \pm 0.218. The crude fibre content was 23.983 \pm 1.516. The fluorescence characteristics was also studied under U.V. light (254 and 366 nm), wherein the powdered leaf sample and leaf extracts showed the visibility of varying colors which are as tabulated in the Table no. 2 & 3. The phytochemical results are tabulated in Table no. 4. Water extracts showed maximum positive tests for alkaloids, saponins, carbohydrates, glycosides, tannins, flavonoids, steroids, phenols, proteins, monosaccharides, hexose sugars, starch, mucilages & gums. Chloroform extracts showed maximum positive tests for alkaloids, flavonoids, steroids, diterpenes, starch. Toluene extracts showed maximum positive tests for alkaloids, glycosides, flavonoids, steroids, phenols, hexose sugars, starch. Carbon tetrachloride extracts showed maximum positive tests for alkaloids, glycosides, tannins, flavonoids, steroids, hexose sugars, starch. Ethyl acetate extracts showed maximum positive tests for alkaloids, steroids, hexose sugars, diterpenes. Hexane extracts showed maximum positive tests for glycosides, flavonoids, steroids, hexose sugars, diterpenes. Ethyl alcohol extracts showed maximum positive tests for alkaloids, tannins, flavonoids, steroids, phenols, hexose sugars, diterpenes, starch, mucilages & gums. Methanol extracts alkaloids, glycosides, tannins, flavonoids, steroids, phenols, hexose sugars, diterpenes, starch, mucilages & gums. Acetone extracts showed maximum positive tests for alkaloids, flavonoids, hexose sugars, diterpenes, starch. 2-propanol extracts showed maximum positive tests for alkaloids, saponins, glycosides, flavonoids, steroids, phenols, hexose sugars, starch. Petroleum ether extracts showed maximum positive tests for glycosides, steroids, hexose sugars, diterpenes. 2-butanone extracts showed maximum positive tests for alkaloids, glucosides, flavonoids, steroids, hexose sugars, diterpenes, starch. Dichloromethane extracts showed maximum positive tests for alkaloids & steroids only. Ethyl ether extracts showed maximum positive tests for alkaloids, saponins, glycosides, flavonoids, steroids, diterpenes.

Table 4: Qualitative Phytochemical analysis of various extracts of *Ampelocissus latifolia* leaves.

S. No.	Name of the Test Procedure	Observation	Soxhlet Extracts of Leaves													
			I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
1.	ALKALOIDS		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
i	Dragendorff's Test	Orange Red ppt.	+	+	+	+	-	-	+	+	+	+	-	+	+	+
ii	Mayer's Test	Whitish Yellow or Cream coloured ppt.	+	+	+	+	+	-	+	+	+	+	-	+	+	+
iii	Hager's Test	Yellow coloured ppt.	+	+	+	+	+	-	+	+	+	+	-	+	+	+
iv	Wagner's Test	Reddish Brown ppt.	+	+	+	+	+	-	+	+	+	+	-	+	+	+
2.	SAPONINS		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
i	Foam Test	Foam persists for 10mins.	+	-	-	-	-	-	-	-	-	-	+	-	-	+
3.	CARBOHYDRATES		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
i	Molisch Test	Purple or reddish violet color.	-	-	+	+	+	+	-	-	-	-	+	+	+	+
ii	Fehling's Test	Brick Red ppt.	+	-	-	-	-	-	-	-	-	-	-	-	-	-
iii	Benedict's Test	Red ppt.	+	-	-	-	-	-	-	-	-	-	-	-	-	-
4.	GLYCOSIDES		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
i	Legal's Test	Pink to Red color.	+	-	+	+	-	+	-	+	-	+	+	+	-	+
ii	Baljet Test	Yellow to Orange color.	+	+	+	+	+	+	+	+	-	+	+	+	-	+
5.	TANNINS		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
i	Lead acetate Test	White ppt.	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ii	Ferric chloride Test	Dark Blue or Greenish Black.	+	+	-	+	-	+	+	+	-	-	+	+	-	-
iii	Potassium dichromate Test	Yellow color ppt.	+	+	+	+	-	-	+	+	+	+	-	+	-	-
iv	Gelatin Test	White ppt.	+	-	-	-	-	-	-	+	+	-	-	-	-	-
v	Potassium ferric cyanide Test	Deep red color.	+	-	+	+	-	-	+	+	-	+	-	-	-	-
6.	FLAVONIDS		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
i	Shinoda's Test	Cherry Red color.	+	+	+	+	-	-	-	+	+	-	-	+	-	-
ii	Alkaline Reagent NaOH Test	Intense Yellow color.	+	+	+	+	-	-	+	+	-	+	-	+	-	+
iii	H ₂ SO ₄ Test	Yellow or Orange color.	+	-	-	-	+	+	+	+	+	-	-	-	-	-
iv	Lead acetate Test	Yellow color ppt.	+	-	+	+	-	+	+	+	+	+	+	+	+	+
7.	STEROIDS		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
i	Salkowski Test	Bluish red to cherry color in Chloroform layer & Green in acid layer.	+	-	+	+	-	-	-	+	-	+	+	+	-	-
ii	Libermann burchard Test	Brown ring at junction & green or deep red upper layer.	+	+	+	+	+	+	+	+	-	+	-	-	+	+
8.	PHENOLS		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
i	Ferric chloride Test	Bluish Black color.	+	-	+	-	-	-	+	+	-	+	-	-	-	-
9.	PROTEINS		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
i	Biuret Test	Pinkish or Purple violet color.	+	-	-	-	-	-	-	-	-	-	-	-	-	-
ii	Ninhydrin Test	Blue color.	-	-	-	-	-	-	-	-	-	-	-	-	-	-
iii	Xanthoproteic Test	Orange color.	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10.	MONOSACCHARIDE		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
i	Barfoed's Test	Red ppt.	+	-	-	-	-	-	-	-	-	-	-	-	-	-
11.	HEXOSE SUGARS		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
i	Selwinoff's Test for ketohexose like fructose	Red color.	+	-	-	-	-	-	-	+	-	+	-	-	-	-
ii	Tollen's phloroglucinol Test for galactose	Yellow to Red color.	+	-	+	+	+	+	+	+	+	+	+	+	-	-
iii	Cobalt chloride Test	Upper layer Greenish blue & Lower Purplish.	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12.	DITERPENES		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
i	Copper acetate Test	Emerald Green color.	-	+	-	-	+	+	+	+	+	-	+	+	-	+
13.	NONREDUCING POLSACCHARIDES [STARCH]		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
i	Iodine Test	Blue color.	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ii	Tannic acid Test	ppt formation.	+	+	+	+	-	-	+	+	+	+	-	+	-	-
14.	Mucilages & Gums		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
i	Ruthenium Red Test	Pink color.	+	-	-	-	-	-	+	+	-	-	-	-	-	-

(+) = indicates presence, (-) = indicates absence.

I= water, II= chloroform, III= toluene, IV= carbon tetrachloride, V= ethyl acetate, VI= hexane, VII= ethyl alcohol, VIII= methanol, IX= acetone, X= 2-propanol, XI= petroleum ether 60-80° C, XII= 2-butanone, XIII= dichloromethane, XIV= ethyl ether.

DISCUSSION

Standardization distinguishes the authentic plant material from its adulterants and substitutes. Leaves of *Ampelocissus latifolia* were subjected to systematic physico chemical and phytochemical screening by extracting with various organic solvents to determine the amount of soluble phytoconstituents in a given amount of medicinal plant material. The data generated helps in determining the quality and purity of a crude raw plant drug in powdered form. In this study the parameters used for the evaluation were Total ash, Water soluble ash, Acid insoluble ash, Sulphated ash, Water soluble extractive value, Alcohol soluble extractive value, Moisture content (Loss on Drying), Crude fibre, pH, Foaming index. Determination of ash is useful for detecting low-grade products, exhausted drugs and excess of sandy or earthy matter; it is more applicable to powdered drugs [15]. Phytochemical analysis was performed on 14 extracts and it was found that maximum phytoconstituents were present in water, methanol & ethyl alcohol extracts. Dichloromethane extracts showed presence of only two phytoconstituents i.e. alkaloids & steroids.

CONCLUSION

In the present study, an effort was made to highlight the potential of these Ayurvedic plant as a valuable resource of biologically active molecules. We have identified the biologically active phytochemicals like alkaloids, saponins, carbohydrates, glycosides, tannins, flavonoids, steroids, phenols, proteins, monosaccharides, hexose sugars, starch, mucilages & gums present in the 14 Soxhlet polar and nonpolar extracts of the leaves of *Ampelocissus latifolia*. The present study can be used as the diagnostic tool to substantiate and authenticate the drug.

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