

PHARMACOGNOSTICAL AND PHYTOCHEMICAL SCREENING OF FRUIT AND LEAVES OF *CISSUS ARNOTTIANA*

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ABSTRACT

Cissus arnottiana is erect woody shrub belongs to Vitaceae family distributed throughout India. The plant bruised roots are applied for rheumatic swellings. Since the review literature has not been reported more information on the leaves and fruits of this plant, the present attempt was undertaken to investigate the Phytochemical studies and pharmacognostical studies, physiochemical parameters in preliminary level. The Pharmacognostical results showed the total ash 6.2%, 7.8%, acid insoluble ash 1.3%, 1.0%, water soluble ash 2.0%, 2.6% in leaf and fruit respectively. Phytochemical screening of fruit and leaves of this plant revealed presence of bioactive constituents such as tannins, phenols, terpenoids, flavonoids, glycosides, carbohydrates and saponins.

Keywords: *Cissus arnottiana*, Vitaceae, Phytochemical constituents, secondary metabolites

INTRODUCTION

Medicinal plants have played a significant role in ancient traditional systems of medication in many countries. Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world's population, especially in the developing world (World Health Organization 2002). In India, thousands of plant species are known to have medicinal values and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient time (Parekh *et al.*, 2005). The medicinal value of the plants lies in some active chemical substances called phytochemicals that produce a definite physiological action on the human body. Phytochemicals are divided into two groups, which are primary and secondary constituents according to their functions in plant metabolism. Primary constituents comprise common sugars, aminoacids, proteins and chlorophyll while secondary constituents consists of alkaloids, terpenoids, flavonoids, tannins, phenolic compounds etc. (Krishnaiah *et al.*, 2007).

Cissus arnottiana is an erect woody shrub distributed throughout India commonly known as Nanaminukki in tamil and Nelagummadi in telugu. The plant reported as bruised roots are applied for rheumatic swellings (Shetty *et al.*, 2000). Since the review literature has not been reported more information on the leaves and fruits of this plant, the present study focus on the pharmacognostical studies and phytochemical screening of *Cissus arnottiana*. The plants of *Cissus* genus are often used as medicinal plants because they contain some bioactive compounds such as vitamins, proteins, carbohydrates and polyphenols (Singh SP *et al.*, 1984).

MATERIALS AND METHODS

Collection of plant material

The plant parts were collected and authenticated by Botanical Survey of India Coimbatore. The plant parts were later shade dried, powdered and stored in an air-tight container for further use. The shade dried plant powder was used for pharmacological investigation, while for Phytochemical screening the powder was extracted with different solvents in their increasing order of polarity such as hexane, chloroform, acetone, ethanol and water on orbital shaker. All the extracts were concentrated by distilling the solvent in a rotary flash evaporator and stored at 4°C.

PHARMACOGNOSTICAL STUDIES

Physicochemical parameters

Ash Values

The determination of various physicochemical parameters such total ash, water-soluble ash, alkalinity of water soluble ash and acid-

insoluble ash values of the leaf and fruit powder was determined as per the Indian Pharmacopoeia (Anonymous 1996).

Extractive Values

Extract of the powdered leaf and fruit were prepared with different solvents for the study of extractive value (Kokashi 1958).

Fluorescence Analysis

A small quantity of dried and finely powdered leaf and fruit was placed on grease free

clean microscopic slide and added 1-2 drops of the freshly prepared reagent solution, mixed gently by tilting the slide and waited for 1-2 minutes. Then the slide was viewed in day light and (365 nm) ultraviolet radiations. The colors observed by application of different reagents in different radiations were recorded. (Pratt *et al.*, 1949).

PHYTOCHEMICAL SCREENING

Phytochemical screenings of all extracts were carried out by following standard procedures (Raaman 2008, Harborne 1999, Trease *et al.*, 1983).

Test for Alkaloids

Mayer's Test

To a fraction, each extracts were treated with Mayer's test reagent, observed for the formation of cream colored precipitate.

Wagner's Test

About 5 ml of each extract few drops of Wagner's reagent was added to observe the formation of reddish brown colour precipitate.

Hager's Test

To 1 ml of each extract 3 ml of Hager's reagent was added for the formation of prominent yellow precipitate.

Test for flavonoids

NaOH test

To 1 ml of the extract few drops of aqueous NaOH and HCl were added along the sides of the test tube to observe the formation of yellow orange colour.

Sulfuric Acid Test

A fraction of the extract was treated with concentrated H₂SO₄ for the formation of orange colour.

Lead Acetate Test

A volume of 3 ml of extract were mixed with 5 drops of lead acetate was added to observe the formation of white or cream precipitate.

Test for Glycosides

Each extracts were dissolved (0.1g) in pyridine, added sodium nitro prusside reagent and made alkaline with NaOH solution. Pink to red colour solution indicates the presence of glycosides.

Test for phenols**Ferric chloride test**

A fraction of each extracts were treated with 5% ferric chloride and observed for the formation of deep blue or black colour.

Test for Tannin

The extracts were dissolved in water and then it was then subjected to water bath at 37°C for 1 hour and the filtrate was treated with ferric chloride and observed for the formation of dark green colour.

Test for saponin**Foam test**

To a small amount of each extract few drops of distilled water were added and shaken vigorously until a persistent foam forms.

Test for Quinone

To 1 g of the extract 5 ml of concentrated HCl was added for observing the formation of yellow colour precipitate.

Test for carbohydrates**Molisch's test for carbohydrates**

A few drops of Molisch's reagent were added to each of the portion dissolved in distilled water; this was then followed by addition of 1 ml of conc. H₂SO₄ by the side of the test tube. The mixture was then allowed to stand for two minutes and then diluted with 5 ml of distilled water. Formation of a red or dull violet colour at the inter phase of the two layers was a positive test.

Test for terpenoids**Chloroform test**

A volume of 5 ml of the plant extract was taken in a test tube with few ml of chloroform and added concentrated sulfuric acid carefully along the sides of the test tube to form a layer and observed for presence of reddish brown color.

RESULTS AND DISCUSSION**Ash value**

The powdered drug was evaluated for its physico-chemical parameters like Ash values, Acid Insoluble ash, Water soluble ash and all the results are tabulated in Table 1.

Extractive values

Extractive values are given in the Table 2.

Fluorescence Analysis

The powder was subject to fluorescence analysis as per the standard procedure and shown in Table 3.

Table 1: Physico-Chemical Studies of Powdered Leaf and Fruit of *Cissus Arnottiana*

Types of Ash value	Observation (% w/w)	
	Leaf	fruit
Total ash	6.2	7.8
Acid insoluble ash	1.3	1.0
Water soluble ash	2.0	2.6

Table 2: Percent Extractives and Colors of Successive Extracts of Leaf and Fruit of *Cissus Arnottiana*

Solvents	Extract values (% w/w)		Colors of extracts	
	Leaf	Fruit	Leaf	Fruit
Hexane	1.58	1.0	Green	Pale grey
Chloroform	2.45	1.8	Dull green	Pale grey
Acetone	2.8	2.2	Dull green	Dull grey
Ethanol	9.0	7.4	Green	Grey
Water	7.5	6.5	Pale green	Grey

Table 3: Fluorescence Analysis of *Cissus arnottiana* Leaf and Fruit

Plant sample	Leaf		Fruit	
	Day light	Uv light	Day light	Uv light
Powder	Green	Dark greenish	Pale grey	Grayish brown
Powder + NaOH	Dark brown	Fluorescence green	Light brown	Brown
Powder + acetic acid	Dull green	Dark green	Light brown	Brown
Powder + HNO ₃	Brown	Greenish brown	Dark brown	Black
Powder + H ₂ SO ₄	Dull green	Dull green	Black	Black
Powder + HCl	Greenish black	Brown	Reddish brown	Dark brown
Powder + water	Pale green	Green	Light brown	Brown
Powder + FeCl ₃	Bluish black	Brownish black	Bluish brown	Dark brown

Phytochemical screening

Preliminary phytochemical screening of fruits and leaves of *Cissus arnottiana* revealed the presence of bioactive compounds such as tannins, phenols, terpenoids, flavonoids, glycosides, carbohydrates and saponins in different extracts (Table 4 and 5). Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. For example, glycosides, saponins, flavonoids, tannins and alkaloids have hypoglycemic activities, anti-inflammatory activities (Augusti et al.,

2008) The terpenoids have been shown to decrease blood sugar level in animal studies (Mandal et al., 2009). Flavonoids serve as health promoting compounds as a result of its anion radicals (Hausteen 1983). Free radicals are known as major contributors to several clinical disorders such as diabetes mellitus, cancer, liver diseases, renal failure and degenerative diseases as a result of deficient natural antioxidant defence mechanism (Parr et al., 2000). The observation of flavonoids and phenols in this plant supports to may have free radical scavenging activity.

Table 4: Preliminary Phytochemical tests for extracts of *Cissus arnottiana* leaves

Phytoconstituents	Hexane	Chloroform	Acetone	Ethanol	Water
Alkaloids	-	-	-	+	+
Flavonoids	-	-	-	+	-
Phenols	-	-	+	+	+
Tannins	-	-	+	+	+
Terpenoids	-	-	-	+	-
Quinines	-	-	-	-	-
Saponins	+	+	+	-	-
Glycosides	-	+	+	-	-
Carbohydrates	+	-	-	-	-

Table 5: Preliminary Phytochemical tests for extracts of *Cissus arnottiana* fruit

Phytoconstituents	Hexane	Chloroform	Acetone	Ethanol	Water
Alkaloids	-	-	-	+	+
Flavonoids	-	-	+	+	-
Phenols	-	-	+	+	+
Tannins	-	-	+	+	+
Terpenoids	-	-	+	+	+
Quinines	-	-	-	-	-
Saponins	+	+	+	-	-
Glycosides	-	+	+	+	+
Carbohydrates	+	-	-	-	-

CONCLUSION

Cissus arnottiana leaf is thick green in colour; fruit is purple grape in colour and bitter in taste. The physical constants such as total ash value, (Table 1) acid insoluble ash, water soluble ash and extractive values (Table 2) are evaluated. The behavior of the leaf powder upon treatment with different chemical reagents was also observed and reported (Table 3). Phytochemical screening tests found that most of the biologically active phytochemicals were present in the ethanolic extract of the *Cissus arnottiana* leaves and fruit (Table 4, 5). Further studies are in progress in our laboratory to isolate the active components which are responsible for antimicrobial activity and antioxidant activity.

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