



Pharmacognostical standardization, preliminary phytochemical investigation of root stocks of *Ardisia solanacea* roxb.

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ABSTRACT

Ardisia solanacea Roxb. belongs to family Myrsinaceae. It is commonly known as adavi mayuri, is used traditionally in the treatment of many diseases. Traditionally, the root stocks of *A. solanacea* is reported to be used in cases of fever and pain whereas the leaf has hepatoprotective and the other parts of this plant can be used in fits, snake bite, dropsy, internal injuries, febrifuge, diarrhoea, rheumatic pneumonia, eye pain, stimulant, carminative, stomachache after child birth. But yet, the plant root stock has not been explored scientifically for its pharmacognostical, phytochemical details. Therefore, the study of morpho-anatomical characters, physicochemical analysis and phytochemical investigation was undertaken to establish the pharmacopoeial standards. The organoleptic, powder and histological studies of root stocks showed specific diagnostical characteristics. The plant root stocks were subjected to determination of ash value, extractive value, moisture content and fluorescence analysis. The powdered drug was extracted successively with various solvents by increasing polarity and extracts were screened for various phytochemical compounds. The results of these examinations delineate the presence of tannins, alkaloids, flavanoids, saponin glycosides. The outcome obtained from preliminary evaluation on the plant can be utilized as a basis for anatomical identification and preparation of monograph of the plant.

Key words: *Ardisia solanacea*, morpho-anatomical, phytochemical compounds.

INTRODUCTION

The use of herbs, herbal extracts or plant derived therapeutically active compounds is a new pharmacological approach to treat various ailments. Mostly in Asian countries especially in India, plants have been traditionally used for human health care. At present all over the world, there is an increased interest in plant drug extracts and this is due to several reasons, specifically synthetic medicine can be abusive and/or incorrect use of these drugs results in deleterious side effects where as drugs obtained from natural origin are having fewer side effects and are also cost effective [1]. There is an increased demand for herbal drugs and/or their active phytochemical compounds in pharmaceutical, phytochemical and perfumery industries. Hence these medicinal plants are often adulterated with inferior quality crude drugs. Therefore, it is essential to standardize the valuable plants medicinally and pharmaceutically.

The synonyms of *Ardisia solanacea* are *Ardisia polycephala* Wall., *Ardisia humilis* Vahl., *Ardisia elliptica*. *Ardisia* group is the famous herbs in China, which have been used as medicinal plants for more than 900 years. *Ardisia* species are distributed in India, Indonesia, Malaysia, Thailand, Vietnam, and Taiwan [1]. *Ardisia solanacea* Roxb. has credited with different local names in various regions of India. In the regions of Tamilnadu, where it is being called kohlikottai, the seed paste applied externally used for fungal infections [2], and lidi kutti, kitti gocho, Nbong thithi and goli is widely used in the treatment of fits, eye pain, and also leaf used as vegetable and analgesic [3]. In North western Maharashtra, it was popularly known as

kodna, kantapengu, and is administered twice a day for 15 days against asthma [4]. In Andhra Pradesh, *A. solanacea* commonly known as spear flower, elliptic leaved tree, adavi mayuri, chavvalakura and is traditionally gained importance in the treatment of fever, diarrhoea, rheumatism, pains, bacterial infections, liver disorders since it possess stimulant, carminative. [5-7].

Ecological significance, botanical description, distribution of *Ardisia solanacea* [8]:

A. solanacea classified as a common weed in Hawaii. Naturalized in Jamaica, forming secondary thicket in moderately wet place. Introduced to Florida for ornament by 1900. Noted as escaping cultivation in South Florida. In Miami – Dade country, now abundant in hammocks, old fields, disturbed wet lands, and tree islands in marshes, forming dense single-species stands in forest and crowding out to invading native plants.

Ever green, glabrous shrub 5-6m tall, with smooth stems and new foliage often reddish. The leaves are arranged opposite to each other. They are simple and elliptic with entire margin. Leaves are 7.5 to 17cm long and 2.5 to 7cm wide, towards the front, they are pointed or blunt short, they are narrowed down to a pointed base. The flowers are pink with five petals and blooms from January to September. The berries average around 8mm long and 11mm wide. They are pink at first gradually ripening to red and then black in colour. The flesh is white and the juice is purple. The plant fruits in the months of January, April and August.

Common in East Indies, naturalized in Hawaii, the Caribbean, and Florida. Herbarium specimens now recorded for naturalized populations in Dade, Monroe, and St. Lucie counties. *A. solanacea* is native of Pakistan, India, Sri Lanka, Southeast Asia and China.

EXPERIMENTAL SECTION

Plant material: The root stocks of *Ardisia solanacea* Roxb. was collected from Tirumala hills, Tirupati, India. Plant material was identified and authenticated by Dr. K. Madhavachetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India.

Chemicals and reagents: All the chemicals and solvents used for the study were of analytical grade and procedures were taken from official methods.

Pharmacognostical Studies

Macro morphology: Macroscopical evaluation can be done by means of sense organs, which provide the simplest as well as quickest means to establish the identity and purity to ensure quality of a drug. Here, the macroscopic evaluation of root stocks was carried out by using dissecting microscope. The shape, apex, base, margin, taste and odour of root stocks were determined as per the reported methods[9].

Preparation of specimen for anatomical study of root stocks of *A. solanacea*: The anatomical study which is done by taking appropriate sections of the plant parts under study. The fresh root stocks parts were cut into small pieces and fixed in FAA solution (formalin 5ml + glacial acetic acid 5ml + 70% ethanol 90ml). After fixing, the specimens were dehydrated with graded series of tertiary butyl alcohol (TBA) as per the standard procedure[9]. After complete dehydration, the specimens were embedded in paraffin wax.

Sectioning: The paraffin embedded specimens sectioned by free hands sections were taken of the fresh root stocks. The resulting sections were boiled in chloral hydrate for 10 minutes to clear of interfering pigments in the tissues. The sections were then treated with phloroglucinol and concentrated hydrochloric acid (1:1) and mounted with glycerin for 10 minutes and were observed under binocular microscope. Photography was done by using Sony camera.

Powder microscopical studies: Powder study is similar to histological study except here dried powder is taken instead of section. To study the powder characteristics of root stocks, powdered drug was separately treated with equal ratios of phloroglucinol and concentrated hydrochloric acid, glycerin, to determine the presence of various cell contents.

Quantitative study for determination of crude fibre content: 2gm of powder drug was mixed with 50ml of 10% nitric acid in a casserole. It was boiled and maintained at boiling point for 30 seconds diluted with water and strained through a fine cloth held over the mouth of filter funnel. The washed residue was

transferred to casserole and boiled further with 50ml of 2.5% sodium hydroxide for 30seconds and filtered. The residue was washed successively with hot water and dried at 105⁰C in hot air oven. The powder was weighed and the % crude fibre was calculated using the formula [9.10].

% crude fibre content = Wt. of crude fibre/Wt. of powder taken X 100

Fluorescence analysis: Some phytochemical compounds shows fluorescence in the visible range in daylight. The ultra violet light produces fluorescence in many natural products which do not visibly fluoresce in daylight. If substance themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents. Hence crude drugs are often assessed qualitatively in this way and it is an important parameter for pharamcognostical evaluation of crude drugs when physical and chemical methods produce inadequate results plant materials may be identified from their adulterants on the basis of fluorescence nature. The fluorescence nature of root stocks powder of *A. solanacea* was studied by, a small quantity of dry root stocks powder was placed on grease free clean microscopic slide and 1-2 drops of freshly prepared different reagents added, mixed by gentle tilting the slide and placed inside the UV chamber and observed the colour changes [11-12].

Physicochemical standardization: Physicochemical standardization of powdered drug for various parameters such as total ash, acid insoluble ash, water soluble ash, extractive values of petroleum ether, methanol and water solvents and moisture content were carried out and calculated as per the reported official methods and recommended procedures [13]. Ash values are used to determine quality and purity of crude drug. It indicates presence of various impurities like carbonate, oxalate and silicate. The water soluble ash is used to estimate the amount of inorganic compound present in drug. The acid insoluble ash consist mainly silica and indicate contamination with earthy material. Estimation of extractive values determines the chemical nature and amount of active constituents present in crude drug. It also gives an indication whether the crude drug is exhausted or not. Moisture content of drug should be at minimal level to discourage the growth of bacteria, yeast or fungi during storage.

Total ash: Accurately weighed 2 gm of the powdered drug was taken in a tarred silica crucible dish and it was incinerated at a temperature not exceeding 450⁰C until free from carbon. The sample was cooled and weighed. If a carbon free ash cannot be obtained in this way, the mass was exhausted with hot water. The residue was collected on ash less filter paper was incinerated and then filtrate was evaporated to dryness, and ignited at a temperature not exceeding 450⁰C. The percentage of ash was calculated with reference to the air dried drug.

Acid insoluble ash: The ash obtained described as total ash was boiled for 5 min. with 25 ml of dilute hydrochloric acid. The insoluble matter was collected on ash less filter paper and washed with hot water and ignited to constant weight. The percentage of acid insoluble ash was calculated with reference to the air dried drug.

Water soluble ash: To the ash obtained as total ash 25 ml water was added and boiled for 5 min. The insoluble matter was collected on ash less filter paper, washed with hot water and ignited in a crucible for 15 min. at a temperature not exceeding 450⁰C. The weight of this residue was subtracted from the weight of total ash. The content of water soluble ash with reference to dried drug was calculated.

Extractive values:

Water soluble extractives: 5 gm of powdered drug was taken in conical flask. Then added 100 ml of water was added in the flask containing powdered drug. 5% solution in water was made. Then the flask was closed with the help of the cotton plug. The mixture was shaken after regular interval of time without touching the solution on to the cotton plug. The mixture was kept for 24 hr, after the period of 24 hr the solution was filtered out with the help of the Whatman filter paper. Discarded the upper solid content and collected the filtrate. Empty evaporating dish was taken and weighed the dish and noted down. Then 25 ml of 5% solution of each of drug was taken in evaporating dish. Then the evaporating dish was heated until the damp mass was formed. Then cooled the evaporating dish and weighed it. Difference of evaporating dish containing damp mass and empty evaporating dish was taken and directly calculated the water soluble extractive value.

Alcohol soluble extractives: 5 gm of powdered drug was taken in conical flask. Then 100 ml of methanol was added to each of the flasks powdered drug. 5% of solution in methanol was made. Then the flasks were closed with the help of the cotton plug. The mixture was shaken after regular interval of time

without touching the solution on to the cotton plug. The mixture was kept for 24 hr. After the period of 24 hr the solution was filtered out with the help of Whatman filter paper. Discarded the upper solid content and collected the filtrate. Empty evaporating dish was taken and weighed all dishes and noted down. Then 25 ml of 5% solution of each drug was taken in evaporating dish. Now all evaporating dishes were heated until the damp mass is formed. Then cooled the evaporating dish and weighed it. Difference of evaporating dish containing damp mass and empty evaporating dish was taken and directly calculated the extractive value.

Petroleum ether soluble extractives: 5 gm of powdered drug was taken in conical flask. Then 100 ml of petroleum ether was added to each of the flasks powdered drug. 5% of solution in methanol was made. Then the flasks were closed with the help of the cotton plug. The mixture was shaken after regular interval of time without touching the solution on to the cotton plug. The mixture was kept for 24 hr. After the period of 24 hr the solution was filtered out with the help of Whatman filter paper. Discarded the upper solid content and collected the filtrate. Empty evaporating dish was taken and weighed all dishes and noted down. Then 25 ml of 5% solution of each drug was taken in evaporating dish. Now all evaporating dishes were heated until the damp mass is formed. Then cooled the evaporating dish and weighed it. Difference of evaporating dish containing damp mass and empty evaporating dish was taken and directly calculated the extractive value.

Moisture content: Powdered root stocks of *A. solanacea* (W_2 , 2gm) was placed in a weighed petridish (W_1). The petridish was kept in a hot air oven at 60°C till constant weight (W_3) was achieved. The sample was placed in a desiccators after it had achieved constant weight and then weighed to determine the moisture content.

$$\text{Moisture content \%} = (W_1 + W_2) - W_3 / W_2 \times 100$$

Preliminary phytochemical investigation

The shade dried powder drug (50gm) subjected to successive solvent extraction with petroleum ether, chloroform, methanol in soxhlet apparatus and macerated with distilled water. The extracts were collected and subjected to investigation for various phytoconstituents such as carbohydrates, steroids, triterpenoids, alkaloids, glycosides, tannins and flavanoids [13].

RESULTS AND DISCUSSION

Macroscopic characteristics of root stocks: The root stocks were cylindrical, slightly tortuous; with 2-3cm in diameter; had rough surface and at places rugged due to transverse wrinkles; had slight odor; bitter in taste and light brown in colour and root stock shown in Fig 1.

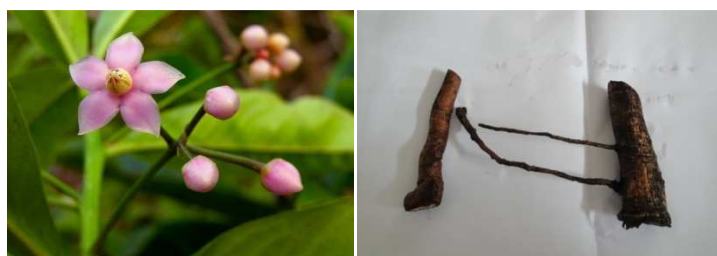


Fig 1: Morphological Features of *Ardisia solanacea* Roxb.

Anatomy of root stocks: The transverse section of root stocks showed non-lignified cork composed of 6 to 10 layers of tangentially elongated, rectangular cells, outermost layer was obliterated, cortex parenchymatous 5 to 8 cells wide; pericycle characterized by 2 to 5 celled thick continuous ring of stone cells embedded with group of lignified fibres; vascular zone was composed of radially arranged discrete vascular stands with 5 to 6 narrow streaks of xylem, some reaching upto the centre. Xylem was composed of vessels, parenchyma, phloem; medullary rays were 2 to 3 cells wide, parenchymatous; simple starch grains were present in parenchymatous cells and given away in Fig 2.

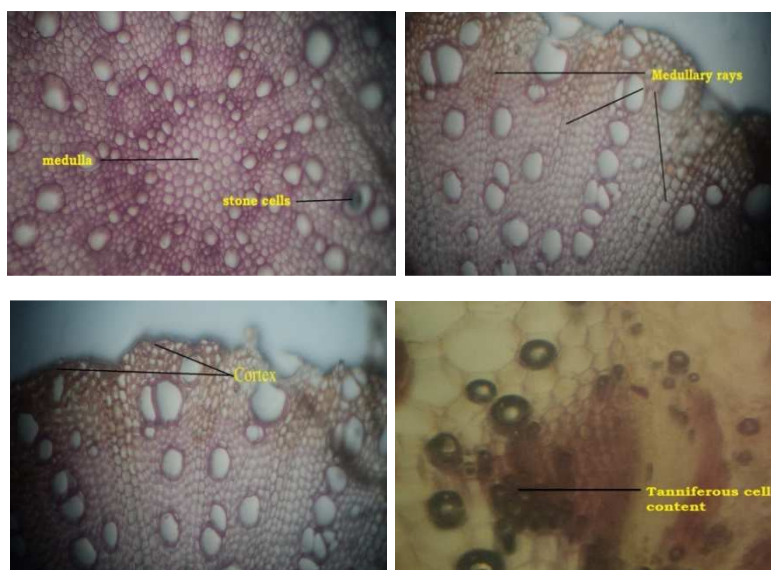


Fig 2: Anatomical Features of Root Stocks of *Ardisia solanacea* Roxb.

Microscopic examination of powdered root stocks:

The powdered root stocks were fibrous and light brown in colour with characteristic odour and bitter in taste. On microscopic examination, the powdered root stocks showed the presence of simple starch grains; unicellular, uniseriate trichomes; parenchyma cells were in rectangular or squarish in shape, had thin walls and wide lumen. Most of the parenchyma cells possess some kind of cell inclusions. Vessel elements were mostly elongated and cylindrical. They had wide, simple, horizontal perforations at the end walls. Their lateral walls had either close spiral thickenings or dense elliptical bordered pits and shown in Fig 3.



Fig 3: Powder Characteristics of *Ardisia solanacea* Roxb. Root Stocks

Quantitative study for determination of crude fibre content: The crude fibre content of the root stocks was found to be 27.65%

Fluorescence analysis: The fluorescence analysis of the powdered root stocks of *A. solanacea* with various reagents was performed under normal and UV light. The results were summarized as in Table 1.

Table 1- Fluorescence Analysis of the Ardisia solanacea Root Stocks Powder

Reagent	Day light	UV 254nm	UV 365nm
Power as such	Grey	mahogany	Dark green
1M sodium hydroxide	Brownish green	Brown	Green
Acetic acid	Brown	yellowish	Brown
1M hydrochloric acid	Brown	Golden brown	Light green
Dilute nitric acid	Lemon yellow	Pale brown	Green
Methanol	Brown	Brown	Heavy brown
50% nitric acid	Brown	Green	Brown
1M sulphuric acid	Green	Brownish green	Dark green
Dil.ammonia	Pale brown	Brown	Brown
Sodium hydroxide in methanol	Green	Brown	Light green

Physicochemical analysis: The results of different standardization parameters such as total ash, water soluble ash, acid insoluble ash, moisture content were as indicated in the Table 2.

Table 2: Physicochemical Analysis of Ardisia solanacea Root Stocks

Physicochemical Parameter	%W/W
<i>Ash Values</i>	
Total ash	12.45
Acid insoluble ash	7.82
Water soluble ash	10.24
<i>Extractive Values</i>	
Water soluble	5.21
Methanol	10.46
Petroleum ether (60 – 80°C)	2.13
<i>Moisture content</i>	6.78

Phytochemical screening: Powdered root stocks were separately extracted with petroleum ether, ethyl acetate, chloroform, methanol and water. Highest yield of extract was found in methanol extract followed by water indicating presence of polar constituents. Preliminary phytochemical investigation was performed on the different extracts which showed that the root stocks of *A. solanacea* was credited with alkaloids, flavanoids, saponin glycosides, tannins. The results were summarized as in Table 3.

Table 3 – phytochemical analysis of various extracts of Ardisia solanaceae root stocks powder

Phytoconstituent	Petroleum ether	Ethyl acetate	Chloroform	Methanol	Water
Carbohydrates	-	-	-	+	-
Alkaloids	-	+	-	+	-
Tannins	-	-	-	+	+
Flavanoids	-	-	-	+	-
Steroids	+	-	-	-	-
Triterpenoids	-	-	-	-	-
Anthracene glycosides	-	-	-	-	-
Saponins	-	+	+	+	-
Cardiac glycosides	-	-	-	-	-

Note : + = present, - = absent

The scientists from the past few decades are keen to evaluate traditionally used medicinal plants due to their desirable therapeutical actions. The root stocks of *Ardisia solanacea* Roxb. are widely used in treatment of various disorders in traditional systems of medicine and this is the first report on pharamcognostical studies and phytochemical investigation of plant root stocks. The study was undertaken to develop the standardization parameters of the root stocks and various morpho-anatomical, physicochemical studies were reported which may serve as diagnostic tool for identification. Morphological observations of root stocks possess characteristic organoleptic characteristics such as erect cylindrical in shape with deep brown colour, characteristic odour with bitter taste. The root stock surface had rough texture. Microscopical study of root stocks showed non lignified cork composed of 6 to 10 layers of tangentially elongated cells, aerenchymatous cortex; vessel elements were elongated and cylindrical. Stone cells were embedded with group of lignified fibers, xylem composed of vessels, medullary rays were 2 to 3 cells wide; had simple

starch grains; pitted parenchyma cells; bizarre shaped sclereids; oil globules; unicellular trichomes. The crude fibre content of root stocks was found to be 26.75% .

Physicochemical parameters of root stocks showed total ash 12.45 %, acid insoluble ash 7.82 % and water soluble ash 10.24 %. This percentage clearly indicates that the root stocks is for the drug action and its effects. The extractive values play an important role in evaluation, especially when the constituents of a drug cannot be readily estimated by any other means of crude drugs. Less extractive value indicates addition of exhausted material, adulteration or incorrect processing during drying or storage. Further, these values indicate the nature of the constituents present in a crude drug. The methanol soluble extractive value proved to be higher than water and petroleum ether soluble extractive values. It was found to be 10.46 %. This shows that the constituents of the *Ardisia solanace* root stocks are more extracted and soluble in methanol (medium polar) as compared to water (polar) and petroleum ether (non polar). Moisture is one of the major factor responsible for the deterioration of the drugs and formulations. Minimal moisture content is always desirable for higher stability of drugs. The moisture content of the crude drug was found to be 6.78%. Preliminary phytochemical screening is one of the initial and necessary step to find out the type of phytochemical compounds present in different extracts of plant which further leads to the isolation of compounds. Qualitative analysis carried on root stocks extract and its various fractions confirmed the presence of various pharmacologically important phytoconstituents like alkaloids, saponin glycosides, tannins, flavanoids.

CONCLUSION

The diagnostic features have been established to identify root stocks of *Ardisia solanacea* Roxb. The root stock has shown significant active constituents like alkaloids, tannins, flavonoids and have tremendous potential for the prevention and treatment of various ailments which are yet to be explored. Various morpho – anatomical, physicochemical studies, reported for the earliest time in this study may serve as a diagnostic tool for the identification, authentication and could be used in the preparation of a monograph on this plant .

Acknowledgements

The authors wish to thank to Management and Principal of Sri Padmavathi School of Pharmacy, Tiruchanoor, Tirupati, for providing the necessary facilities for the research work.

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