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Evaluation of Phytochemical Constituents of the Extracts of *Cassytha Filiformis. L*

Vijisara Elizabeth D¹, Arumugam S^{1*}

1. PG & Research Department of chemistry, Nehru Memorial College (Autonomous)
Puthanampatti- 621 007, Tamil Nadu, India

ABSTRACT

Cassytha filiformis (Common name: love-vine, Vernacular name: Kothan) is a creeper plant in the family Lauraceae, traditionally used in treatment of several diseases (cancers, human birthing issues, hypertension, pains and fever). The present study was carried out to investigate the phytochemical profile of whole plant (except root) of *Cassytha filiformis.L*. The powder of the *cassytha filiformis.L* was successively extracted with acetone, aqueous, benzene, chloroform, cyclohexane, ethanol, ethyl acetate and methanol. Phytochemical analysis shows the presence of carbohydrates, glycosides, proteins, amino acids, phenolic compounds, tannins, steroids, anthraquinone, anthocyanin, flavonoid, and alkaloid. The result of the study could be useful to description and foundation of monograph of the plant.

Keywords: *Cassytha filiformis*, Flavonoid, Phytochemical, Anthocyanin, Alkaloids and Glycosides.

*Corresponding Author Email: nmcaaru@gmail.com

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INTRODUCTION

Plant materials have provided the models for 25-50% of synthetic drugs^{1,2}. Many commercially proven drugs used in modern medicine were initially used in crude form in traditional or folk healing practices, or for other purposes that suggested potentially useful biological activity. Plant derived medicines are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment³. Modern clinicians are now inclined towards the use of herbal medicines due to efficiency of a number of phytopharmaceuticals and herbal drugs which is often enhanced by the presence of many minor constituents producing synergistic action, which results in lower side effects in comparison to many allopathic modern drugs⁴. Biomolecules of plant origin appear to be one of the alternatives for the control of many antibiotic resistant human pathogens⁵. Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because the medicinal value of plant lies in the chemical substances that produce a definite therapeutic action on the human body. Some of these important bioactive compounds are alkaloids, flavonoids, tannins and phenolic compounds. In addition, the knowledge of the chemical constituents of plants would further be valuable in the discovery of the actual value of folkloric remedies.

Cassytha filiformis.L is a leafless, climbing, twining, vine-like, autoparasitic and plant-hyperparasitic phanerogam (seed-bearing plant) in the plant family Lauraceae. It infests a wide variety of coastal plants throughout Hawai'i, the Pacific, and the tropics worldwide. Indigenous to Hawai'i, it is one of many higher flowering plant species that have, through evolutionary divergence, become parasitic on various organs of other higher plants^{6,7,8}. *Cassytha filiformis*.L climbs to other, mainly woody, plants for physical support, nutrition, and water. Its common name is love vine. This species is widely used as a medicinal plant^{9,10}. For example, it is used to treat jellyfish stings in Fiji. There are other reports of *C. filiformis*.L being used in traditional medicines in the Bahamas and the West Indies. *C. filiformis*.L is purported to be used by several different Polynesian cultures for treatment of cancers. The plant is also used traditionally for treatment of some human birthing issues^{9,10}. Thus the present study was carried out to evaluate the phytochemical constituents present in the medicinal herb of *Cassytha filiformis*.L.

MATERIALS AND METHODS

Collection of Plant Material

Cassytha filiformis.L were collected from Kumarai Village in cuddalore District of Tamilnadu, India during January to December 2012 and authenticated by the Director of the Rapinat

Herbarium and Centre for Molecular Systematic, St. Joseph's college (campus), Trichirappalli, Tamilnadu, India. Fresh whole plant were cleaned with running tap water and dried under the shade. Then the dried plant were ground to fine powder mechanically and preserved in airtight containers for further analysis.



Figure 1: Image of *Cassytha filiformis.L*

PHYTOCHEMICAL ANALYSIS

The preliminary phytochemical evaluation of *Cassytha filiformis.L* was carried on extract prepared by successive extraction method in Soxhlet. The previously dried powdered whole plant (100g) was extracted in a Soxhlet apparatus with acetone, chloroform, cyclohexane, ethanol, ethyl acetate, aqueous and methanol successively. The resultant extracts were subjected to chemical test for different phytoconstituents viz. alkaloids, carbohydrates, phenolics, flavonoids, proteins, amino acids, saponins and steroids etc.

The percentage extractive yield was calculated by formula as mentioned below:

$$\% \text{ Extractive yield (w/w)} = \frac{\text{Weight of dried extract}}{\text{Weight of dried plant powder}} \times 100$$

Test for flavonoids

Shinoda test (Magnesium Hydrochloride reduction test):-

To *Cassytha filiformis.L* extract, 5ml. 95% ethanol was added. The mixture was treated with 0.5g magnesium turnings and few drops of conc. HCL. Pink color, if produced, may confirm the presence of flavonoids¹¹.

Alkaline reagent test: -

Small quantity of each extract sample was taken and added with lead acetate solution. After few minutes appearance of yellow color precipitates which indicated the presence of flavonoids¹¹.

Test for Phenolics and Tannins

Ferric chloride test:-

Small amount of *Cassiytha filiformis*.L extract were shaken with water separately and warmed. Then about 2 ml of 5% ferric chloride solution was added and observed for the formation of green or blue color which may indicate the presence of phenols¹¹.

Gelatin test:-

1% gelatin solution containing 10% sodium chloride was added to each extract. Formation of precipitate indicated the presence of tannins and phenolic compounds¹¹.

Iodine test:-

Cassiytha filiformis.L extract were treated with diluted iodine solution separately. Appearance of transient red color indicated the presence of tannins and phenolic compounds¹¹.

Nitric acid test:-

Cassiytha filiformis.L extract were treated with dilute nitric acid separately. Formation of reddish to yellowish color indicated the presence of tannins and phenolic compounds¹¹.

Test for Alkaloids**Dragendroff's test:-**

Few drops of Dragendroff's reagent (solution of potassium bismuth iodide) were added to each extract and observed for the formation of orange yellow precipitate which may indicate the presence of alkaloids¹¹.

Mayer's test:-

Few drops of Mayer's reagent (Potassium mercuric iodide solution) were added to each extract and observed for the formation of white or cream color precipitate which may indicate the presence of alkaloids¹¹.

Hager's test:-

Few drops of Hager's reagent (saturated aqueous solution of picric acid) were added to each extract and observed for the formation of yellow precipitate which may indicate the presence of alkaloids¹¹.

Wagner's test:-

Few drops of Wagner's reagent (solution of iodine in potassium iodide) were added to each extract and observed for the formation of reddish brown precipitate which may indicate the presence of alkaloids¹¹.

Test for Amino acid and Proteins**Biuret test:-**

To each of the extract were treated with 1 ml 10% sodium hydroxide solution separately and heated. A drop of 0.7% copper sulphate solution to the above mixtures was added. The formation

of purplish violet color may indicate the presence of proteins¹¹.

Million's test:-

3 ml test solutions were mixed with 5 ml Million's reagent separately. White precipitate was formed which on heating turned to brick red. It may indicate the presence of amino acids¹¹.

Test for Glycosides**Keller kelliiani test:-**

To each of the extract were treated with chloroform and evaporate it to dryness. Separately 0.4 ml of glacial acetic acid containing a trace amount of ferric chloride was added and transferred to a small test tube added with carefully 0.5 ml of conc.H₂SO₄ acid by the side of the test tube; blue color appears in the acetic acid layer indicating the presence of glycosides¹¹.

Borntrager's test:-

To each of the extract were boiled with 1 ml of dilute H₂SO₄ in a test tube separately for 5 min, filtered while hot, pipette out the supernatant or filtrate, cooled and shaken with an equal volume of dichloromethane. The lower levels of dichloromethane separated and shaken with half its volume with dilute ammonia. A rose pink to red color appeared in the ammonical layer, indicating the presence of glycosides¹¹.

Froth test:-

To each of the extracts were treated with water in a semi-micro tube separately shaken well. The froth appeared thus indicating the presence of glycosides¹¹.

Test for Carbohydrates**Molish's test:-**

To each of the extract were treated with 2 drops of alcoholic α -naphthol solution in a test tube separately and 2 ml of conc.H₂SO₄ was added carefully along the sides of the test tubes. Formation of violet ring at the junction may indicate the presence of carbohydrates¹¹.

Fehiling's test:-

To each of the extract were treated in equal volumes with 1ml Fehling A and 1ml Fehling B solutions, boiled for one minute separately. The mixtures were boiled for 5-10 minutes on water bath. Reddish brown color was obtained due to formation of cuprous oxide which indicated the presence of reducing sugar¹¹.

Benedict's test: -

To each of the extract were treated with equal volumes of Benedict's reagent in test tubes separately. The mixtures were boiled for 5-10 minutes on water bath. Solution appeared green, yellow or red depending on amount of reducing sugar present in each filtrate¹¹.

Test for Steroids

Salkowsti's Test:-

To each of the extract was dissolved in 2ml of chloroform. Conc.H₂SO₄ was carefully added to form a low layer. A reddish brown color in the interface is indicative of steroidal ring¹¹.

Test for Anthraquinone

Borntrager's test

To each of the extract was put in a test tube and 5ml of chloroform added and shaken for 5 minutes. This was filtered and shaken with equal volume of 10% Ammonium solution. A pink violet or red color in the ammonical layer (lower layer) is indicative of the presence of anthraquinone¹¹.

Test for Anthocyanin

Sodium Hydroxide Test:-

Weigh about 0.2gm of plant each extract in separate test tube, 1ml of 2N Sodium hydroxide was added, and heated for 5 minutes at $100^{\circ} \pm 2^{\circ}C$. A bluish green color is indicative of the presence of anthocyanin¹¹.

RESULTS AND DISCUSSION

The present investigation was carried out to study the presence of medicinally active phytochemicals in the plant of *Cassytha filiformis*.L from caddalur District, India. The results are summarized in table 2. Carbohydrates, glycosides, proteins, amino acids, phenolic compounds, tannins, steroids, anthraquinone, anthocyanin, flavonoids and alkaloids are present in the plant investigated. Most of the phytochemical constituents were present in the extracts of ethanol, methanol, aqueous, ethylacetate and cyclohexane, but in the least number of phytochemical constituents were present in benzene extract. Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities¹¹. The result reveals that the plant extracts owing to their phytochemical constituents, are very important and beneficial in industrial and medicinal sciences. Anthraquinones are used as dyes and antibacterial agents. Tannins are anti- viral, anti-bacterial, and anti-tumor in effectiveness. Certain tannins are also able to inhibit HIV replication, selectivity, and diuretic¹². Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity¹³. Glycosides are known to lower the blood pressure according to many reports¹⁴. Glycosides are naturally cardio active drugs used in the treatment of congestive heart failure and cardiac arrhythmia¹⁵. Flavonoids are effective antioxidant

and show strong anticancer activities^{16, 17& 18}. Phenolic and tannins composition of medicinal plants is mainly responsible for antioxidant activity and contributes to their biofunctionalities such as reduction of chronic and degenerative diseases including cancer, cardiac and infectious diseases etc¹⁹.

Table 1: Physical Characteristics and % Yield of Various Extracts of *Cassytha filiformis.L*

Solvent	Color of the extract	Odour	Consistency	Sense of touch	Amount of extract (gm)	% of yield (w/w)
Acetone	Yellowish black	Characteristic	Semisolid	Sticky	1.00	1
Benzene	Greenish yellow	Characteristic	Semisolid	Sticky	1.50	1.5
Chloroform	Brownish green	Characteristic	Semisolid	Sticky	2.00	2
Cyclohexane	Yellowish black	Characteristic	Semisolid	Sticky	2.30	2.3
Ethanol	Reddish brown	Characteristic	Semisolid	Sticky	4.09	4.09
Ethylacetate	Greenish yellow	Characteristic	Semisolid	Sticky	2.15	2.15
Methanol	Reddish brown	Characteristic	Semisolid	Sticky	4.00	4
Aqueous	Brown	Characteristic	Semisolid	Sticky	3.50	3.5

The % yield was maximum (4.09%) obtained with ethanol and least (1%) with acetone.

Table 2: Phytochemical Screening of Various Extracts of *Cassytha filiformis.L*

Chemical test	Acetone extract	Benzene extract	Chloroform extract	Cyclohexane extract	Ethanol extract	Ethylacetate extract	Methanol extract	Aqueous extract
Flavonoids								
Shinoda test	+	-	-	+	++	+	++	+
Alkaline reagent	+	-	+	+	++	+	++	+
Phenolic compounds & Tannins								
Ferric chloride test	-	-	+	+	++	+	++	++
Gelatin test	-	-	++	-	++	-	++	+
Iodine test	-	+	-	+	+	+	+	++
Nitric acid test	+	-	-	+	+	-	+	++
Alkaloids								
Dragendroff's reagent	+	-	-	+	++	-	++	+
Mayer's reagent	-	+	+	++	-	+	-	+
Hager's reagent	+	+	+	++	+	+	++	-
Wagner's reagent	+	+	+	++	+	+	++	+
Amino acids & Proteins								
Biuret test	+	-	+	+	++	+	++	+
Million's reagent	+	-	+	+	++	+	++	+
Glycosides								
Molish's test	-	-	+	+	++	++	++	+
Fehiling's test	+	-	-	+	+	++	+	++
Benedict's test	+	-	+	-	+	+	+	++
Steroids								
Salkowsti's Test	-	-	-	+	-	+	-	-
Anthraquinone								
Borntrager's test	-	-	-	-	+	-	+	+
Anthocyanin								
Sodium Hydroxide Test	-	-	-	+	-	+	+	-

++ : Present in high concentration;
+ : Present in trace concentration;
- : Constituents not detectable using the specified assay method

CONCLUSION

The results revealed the presence of medicinally important constituents in the plants studied. The phytochemical constituents present in the plant act as potential source of useful drugs to improve the health status of humans. Many evidences gathered in earlier studies which confirmed the identified phytochemicals to be bioactive. The traditional medicine practice is recommended strongly for these plants as well as it is suggested that further work should be carried out to isolate, purify, and characterize the active constituents responsible for the activity of these plants.

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