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Preliminary phytochemical, physicochemical and antimicrobial studies of *Loranthus elasticus* of *Loranthaceae* family

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Abstract
Siddha system of medicine is a traditional system which constitutes medicinal plants and bhasmas (metal oxides) found mostly in south India especially in Tamil Nadu. Siddhars (Priests with traditional knowledge) very enlightened souls, had reputation to treat several diseases. This system is almost identified with the Tamil literature and has its own distinct tradition and cultural heritage. The term AYUSH means Ayurvedha, Unani, Siddha and Homeopathy (AYUSH) which is the name of the Union Government Health department and incidentally AYUSH also means longevity of life. In the traditional system as well as in the modern systems of medicine the plant *Azadirachta indica* (Neem tree) used as a medicine for various ailments prepared from different parts of the plant tree. Parasitic plants or Mistletoes are common in various kinds of plants or trees which are belonging to the family *Loranthaceae*. Many parasitic plants are used as medicine in various parts of the world. In India also the plants grown in the mango tree and lime tree also used as medicine. Based on the available literature, the *Loranthus* plant grown on the neem tree (in Tamil called as Veppa Maram) was chosen for the study. The *Loranthus elasticus* plant of *Loranthaceae* grown on the neem tree (*Azadirachta indica* Linn) was collected, dried and powdered and was subjected to successive Soxhlet extraction with different solvents and finally macerated with water so as to get respective extracts. The preliminary phytochemical screening was done and physicochemical analysis was also carried out. The plant extract is subjected to preliminary antimicrobial studies such as antibacterial and antifungal studies were carried out and their results are discussed.

Keywords: Siddha sytstems of medicine, *Loranthus elasticus*, Phytochemistry, antibacterial activity, fluorescence, mistletoe.

1. Introduction
With the advent of the development of science, especially chemistry, pharmacology, random clinical developments in medicinal practices, the awareness to understand the active chemical principle responsible to exert medicinal activity, its quantification are necessary to make popular the plant or herbal medicine of traditional origin. There are considerable advancements in this direction in the recent years. In fact, for most of the development of allopathic drugs, natural products used in traditional medicines provides leads or they themselves in their own right became drugs, during the latter half of 1940's. Examples are quinine, reserpine, atropine, etc. Thus the importance of natural products for the discovery of new drugs is not neglected.
Loranthaceae are largest flowering parasitic plant occurs mainly in tropical regions, it comprises about 70 genera and 1000 species (Calvin and Wilson, 2006) [1]. This family consists of epiphytic and hemi parasitic plants which adhere to branch twigs of the tree by means of haustoria, which penetrate into the host in order to absorb water and nutrients popularly known as mistletoe (Loranzei, 2000) [2]. *Loranthaceae* species play an important and complex rule in the biological system where they live by interacting with insects, birds and mammals (Watson, 2001) [3]. Mistletoe plants attached to lime trees have been reported to be used as medicinal herbs for the treatment of tonsillitis and otitis.
The aim of the study was to evaluate the phytochemical and antimicrobial evaluation of *L. elasticus* plant grown in neem tree.

Materials and Methods
Collection of plant material and authentication
Fresh whole plants of *L. elasticus* plant grown in the Neem tree (*Azadirchta indica* Linn) were collected from the local fields of Salem district of Tamil Nadu, India. The plant specimen was identified and authenticated in the department of Botany, Annamalai University.

The whole plant material was subjected to shade drying. The dried plant material was further crushed to powder and the powder was passed through the mesh 22 and stored in airtight container for further analysis.

Extraction of powdered plant material

The shade dried, powdered plant material was subjected to sequential Soxhlet extraction using the solvents of different polarity such as petroleum ether (40-60°), chloroform, ethanol and finally macerated with water so as to get respective extracts. Cold maceration was also done using ethanol and water. The extracts were filtered individually, evaporated to dryness and the percent yields of all the extracts were determined. All the extracts were then stored in a refrigerator till further analysis.

Preliminary phytochemical analysis

Preliminary qualitative phytochemical analysis of all the extracts was carried out by employing standard conventional methods. (Trease and Evans, 2009) [4].

Determination of Physicochemical parameters

The dried plant material was subjected for determination of physicochemical parameters such as total ash value, acid insoluble ash value water soluble ash value, moisture content, foreign organic matter, crude fibre, alcohol soluble extractive and water soluble extractive (Usha *et al.,* 1984) [5]

Determination of fluorescence character

Fluorescence character of powdered plant material with different chemical reagents was determined under ordinary and ultraviolet light (Chause and Pratt, 1949) [6].

Antibacterial and antifungal studies

The preliminary antimicrobial properties of *Loranthus elasticus* were investigated against one fungal species *Aspergillus niger*, three Gram negative bacteria *Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa* and two Gram positive organisms *Bacillus subtilis* and *Staphylococcus aureus.* The antimicrobial activities of the compounds are carried out the Department of Microbiology, Rajah Muthiah Medical College, Annamalai University.

Inoculation of microorganisms

The pure cultures of microorganisms were streaked onto Nutrient agar plate (Hi Media) and incubated at 37 °C for 24 h. The well-isolated colonies were aseptically transferred to Nutrient broth (Hi Media) and again incubated at 37 °C for 24 h.

Sample preparation

Antimicrobial activity of the extracts was tested at various concentrations ranging from 25-200µg/ml. The plant extracts of five selected solvents were weighed and dissolved in acetone to prepare stock solution of 200µg/ml concentration. The same stock solution has been utilized to get desired concentrations of 100, 50 and 25 µg/ml by the serial dilution method using equation $c_1v_1 = c_2v_2$ where c = concentration and v = volume.

Disc diffusion method

The petroleum ether, chloroform, ethyl acetate, methanol and ethanol extractions of L. elasticus were preliminarily screened for antimicrobial activity by using disc diffusion method (Zaidan *et al.,* 2005) [7]. In the assay each inoculum suspension (10^3 CFU/ml) was spread evenly over the entire Nutrient agar surface by sterile collection swab. Then, discs of diameter 6mm were sterilized at 121 °C for 15 min and loaded with prepared positive control Ciprofloxacin, 20µg/ml) and extract solutions of *L. elasticus* at various concentrations. The impregnated discs were dried for 3-5 min and dispensed onto the surface of the inoculated plates with flamed forceps. Each disc was pressed down firmly to ensure complete contact with nutrient agar surface. The discs were placed suitably apart and not relocated once contacted with the agar surface. The plates were then labelled and incubated at 37 °C for 24 h for both bacteria and fungi (Espinel-Ingroff *et al.,* 2007, Zaidan *et al.,* 2005) [8, 7]. The results were measured and expressed in terms of zone of inhibition (ZI) of bacterial and fungal growth around each disc in millimetres as low activity (inactive 1-6mm), moderate 7-10mm) and active >12mm and no activity (-) (Parveen *et al.,* 2010) [9]. The antimicrobial index (AI) was calculated by using equation (Bhat and Abdul Khalil, 2010) [10].

$$\text{Antimicrobial index} = (1 - D_a/D_b) \times 100$$

Where D_a is the diameter of growth zone in the test plate and D_b is the diameter of growth zone in the control plate.

Results and Discussion

Qualitative Phytochemical analysis

Successive solvent extracts of leaves of the plant *L. elasticus* – Neem Tree was subjected to qualitative phytochemical screening and the values are given in Table - 1.

Table 1: Qualitative phytochemical screening of leaf powder of *L. elasticus* in Neem Tree

S. No.	Test applied	*L. elasticus* – Neem Tree					
		Reagents used	Pet ether	Benzene	Chloroform	Alcohol	Water
1	Alkaloids	Mayer's	-	-	-	-	+
		Wagner's	-	-	-	+	+
		Hager's	-	-	-	+	+
		Dragondroff's	-	-	-	+	+
2	Amino acids	Ninhydrin's	+	+	+	+	+
3	Anthocyanins		+	+	+	+	+
4	Carbohydrates	Fehling's	-	-	-	+	+
		Benedict's	-	-	-	+	+
5	Cardial glycoside		-	+	-	+	+
6	Coumarins		+	+	+	+	+
7	Diterpenes	Copper acetate	+	+	+	+	-
8	Emodins		-	-	-	+	-
9	Fatty acids		+	+	+	+	+

10	Flavonoids		-	-	-	-	+
11	Fixed oils and fats	Spot test	+	+	-	-	-
12	Glycosides		+	+	+	+	+
13	Gum and mucilage	Alcoholic precipitation	-	-	-	+	+
14	Leucoanthocyanin		-	-	-	-	-
15	Phenolic	$FeCl_3$	+	+	+	+	+
16	Phlobatannin		-	-	-	-	-
17	Phytosterols	L.B. Test	+	+	+	+	+
18	Proteins	Xanthophoretic	+	+	+	+	-
19	Saponins	Foam test	-	-	-	+	+
20	Steroids		+	+	+	+	-
21	Tannins and Phenols	10% lead acetate	-	-	-	+	+
22	Terpenoids		+	+	+	+	-

'+' presence of compounds; '-'absence of compounds

The phytochemical analysis of the petroleum ether, benzene, chloroform, methanol and water extract of the plant *Loranthus elasticus* present in the Neem Tree was presented in the table 1. The result indicated that the phytochemical constituents, namely alkaloids, amino acids, anthocyanin, carbohydrates, cardiac glycosides, coumarins, diterpenes, emodins, fatty acids, phlobatannin, phenols, saponin and terpenoids were present in methanol extract, whereas the flavonoids, glycosides, leucoanthocyanin, phytosterol, proteins, steroids and tannin were absent in methanol extract. The other phytochemical constituents present in the rest of the solvents are given. Small amounts of Saponins were noticed in alcohol and water extracts. In water extracts of the leaves shows moderate amounts of gums and mucilage.

Preliminary physicochemical analysis

The results of physicochemical parameters are given in Table – 2. The total ash value, acid insoluble ash value, water soluble ash value and sulphate ash values was found to be 8.72, 0.92, 5.80 and 3.20% respectively. The total ash value was relatively high which may be due to high content of carbonates, phosphates, silicates and silica. Ash value is useful in determining authenticity and purity of plant material and also these values are important quantitative standards (Kokate *et al.*, 2006). Percent weight has on drying or moisture content could prevent bacterial, fungal or yeast growth (African Pharmacophores, 1986) [11].

Table 2: Physicochemical analysis and extractive values of leaf powder *Loranthus elasticus* present in Neem Tree

S. No.	Parameter	*L. elasticus*
	Organoleptic characteristics	**Powder**
1	Appearance	Powder
2	Loss on drying at 105 °C	6.0 – 8.2
3	Total Ash value	12.10
	Water soluble Ash	6.20
`	Acid insoluble ash	1.20
	Sulphated ash	3.21
4	Moisture content	8.20
5	Foreign organic matter	7.50
6	Crude fibre content	2.75
7	Solubility values	
	Alcohol	7.20
	Water	6.52

Foreign organic matter in the powdered plant material was 8.20%, this may be contributing to the wildness of the plant, leading to the contamination in the course of its collection. The crude fibre content of the plant material was found to be 2.80%. Determination of crude fibre is useful in distinguishing between similar drugs or in the deduction of adulteration. It also helps to remove the more resistant parts of plant organs which can be used for microscopic examination. Alcohol soluble and water soluble extractive values were found to be 6.50% and 10.12% respectively. The percent yields of different extracts are given in Table -2. The percent yields of petroleum ether, chloroform, ethanol and aqueous extracts were found to be 3.0, 2.0, 2.2 and 11.8% respectively.

Table 3: Percentage extractive values of *L. elasticus* in Neem Tree

S. No.	Extractive values in	L. elasticus in Neem Tree
1	Petroleum ether	10.216
2	Benzene	2.130
3	Chloroform	2.110
4	Ethanol	14.220
5	Water	9.500

The percent yield of alcohol and aqueous extracts by cold extraction were found to be 2.4 and 9.2% respectively. The results of preliminary phytochemical analysis of different extracts are given in Table – 3. Secondary metabolites were found in good proportion in ethanolic and aqueous extracts when compared with petroleum ether and chloroform extracts.

These secondary metabolites may be responsible for various pharmacological effects of ethanolic and aqueous extracts of the plant material.

Table 4: Colour, nature and per cent yields of extracts of L. elasticus – Neem Tree

S. No.	Extract	Solvent	Colour	Nature	Yield (%) (w/w)
1	Sequential	Pet. Ether	Yellowish green	Semisolid	7.52
2		Chloroform	Green	Solid	5.20
3		Ethanol	Light green	Solid	7.70
4		Water	Dark green	Solid	6.00
5	Cold	Ethanol	Dark green	Solid	5.00
6		Water	Brown	Solid	5.00

Study of powdered plant material

The behaviour of powder of the leaf of the plant sample of Vembu on treatment with different chemical reagents and their fluorescence behaviour is given in Table – 4. The leaf powder of Vembu treated with sulphuric acid to give a black colour in both UV and visible light. The leaves treated with Hydrochloric acid (HCl), Picric acid, Iodine (I_2), Ferric chloride ($FeCl_3$), and ammonium hydroxide (NH_4OH) gives almost green colour in both UV and visible light. The leaf powder Odhiyan gives brown colour in nitric acid (HNO_3) in visible light and brownish green in UV light whereas in CH_3OH produce dark green in daylight and orange red colour in UV light.

Table 5: Fluorescence analysis of leaf powder of L. elasticus – Vembu

S. No.	Powder + Reagent used	L. elasticus in Vembu	
		Daylight	UV light
1	Powdered plant material	Pale green	Light green
2	Powder + 1M H_2SO_4	Black	Dark black
3	Powder + dil. HNO_3	Brown	Yellowish brown
4	Powder + 50% HNO_3	Brown	Brownish green
5	Powder + Acetic acid	Dark brown	Brown
6	Powder + 1M HCl	Light green	Greenish yellow
7	Powder + picric acid	Light green	Greenish yellow
8	Powder + Iodine solution	Green	Green
9	Powder + $FeCl_3$ (5% solution)	Dark green	Light green
10	Powder + 1M NaOH	Green	Dark green
11	Powder + 25% NH_4OH	Green	Greenish yellow
12	Powder + CH_3OH	Green	Orange red

The fluorescent studies of the plant material using different chemical reagents are given in Table- 5. Fluorescence is an important phenomenon exhibited by various chemical constituents present in plant material. Since constituents show fluorescence in the visible range to many natural products (e.g., alkaloids like beriberine, which do not visibly fluoresce in day light. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives by applying different reagents hence some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation (Ansari, 2006) [12].

Antimicrobial studies

Antibacterial and antifungal screening of L. elasticus of Neem: Preliminary antibacterial and antifungal activities of the *L. elasticus* of Neem powder was carried out against three Gram positive and three Gram negative bacteria and two fungal organisms.

The antimicrobial activity of *L. elasticus* extracts are given in Table-6. All the organisms are inactive in the organic solvents up to a level of 200 μg/ml. Both Gram positive and negative organisms show activity in the methanol and ethanol extracts at a concentration of 25μg/ml. The fungal *A. niger* shows activity at a concentration of 25μg/ml in the ethanol extracts. All other solvents shows inactive at a concentration of 200μg/ml. The ethanol and methanol extracts show positive for all the organisms tested.

Table 6: Antimicrobial activity of *L. elasticus* extracts of Neem

Microorganism	Petroleum ether	Chloroform	Ethyl acetate	Methanol	Ethanol
Escherichia coli	7	7	7	12	14
Klebsiella pneumoniae	7	7	7	14	12
Pseudomonas aeruginosa	7	6	7	14	12
Bacillus subtilis	7	6	7	12	14
Staphylococcus aureus	7	6	7	12	12
Enterococcus faecalis	7	6	7	12	12
Aspergillus niger	7	7	7	7	7
Aspergillus flavus	7	7	7	7	7

< 8 mm – inactive; 8-12mm – moderate 12 mm active.

Standard drug for bacteria: Ciprofloxacin. Fungi: Griseofulvin. Control: acetone.

The antimicrobial activity of *L. elasticus* extracts are given in Table-6. All the organisms are inactive in the organic solvents up to a level of 200 μg/ml. Both Gram positive and negative

organisms shows activity in the methanol and ethanol extracts at a concentration of 25µg/ml. The fungal *A. niger* shows activity at a concentration of 25µg/ml in the ethanol extracts. All other solvents shows inactive at a concentration of 200µg/ml. The ethanol and methanol extracts shows positive for all the organisms tested.

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Conclusion
The *Loranthus elasticus* plant of *Loranthaceae* family grown in the neem tree was subjected to preliminary phytochemical screening and pharmacognastic analysis. The present investigation adds to the knowledge of the plant material and will be quite useful for development of a formulation for treating various ailments.

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