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A preliminary phytochemical analysis of a medicinal plant *Litsea cubeba* found in Kohima district, Nagaland India

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Abstract
Litsea cubeba is a predominant plant of the Himalayas region. From the ancient times, the plant parts were used for curing gastro-intestinal ailments, burns, sprains, indigestion, asthma, paralysis, and even mental disorders like hysteria. In recent times, it is used to aromatherapy as a stimulant and anti-inflammatory activities. This study was aimed to screen preliminary phytochemicals presence in various parts of plant parts like leaves, steam bark and fruits extracted from polar and non-polar solvent like ethanol, methanol and hexane. The analysis revealed the significance presence of alkaloids, terpenoids and flavonoids which are of important medicinal value and therefore need the plant conservation strategies for the benefits of the local people and the society in large.

Keywords: *Litsea cubeba*, phytochemicals, plants parts, soxhlet extraction, conservation

Introduction
Litsea cubeba is an evergreen aromatic tree with dieocious flowers and smell pepper-like fruits. It reaches a height of 8-10 metres, belongs to the laurel family (Lauraceae Juss.). It grows wild in Southeast Asian countries including India, China, Bhutan, Nepal, Myanmar, Vietnam, Korea, Taiwan and Indonesia. The plant is cultivated for a variety of purposes from feeding muga silk worms to producing fast growing timber, apart from its uses in aromatherapy and treatment of many diseases. In India, the species grows abundantly in the Himalayas and reported from Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim, Uttaranchal, Uttar Pradesh and West Bengal. It is cultivated in North eastern states of India for rearing muga silk worms [1]. Antibacterial activity of *Litsea cubeba* and its effects on the biological response of common carp Cyprinus carpio challenged with Aeromonas hydrophilla was reported in North. Vietnam. *Litsea cubeba* leaf essential oil was investigated for the biological effects induced by the leaf powder on the growth, nonspecific immunity and survival of common carp (Cyprinus carpio) challenged with Aeromonas hydrophilic [2]. The antifungal activity of the volatile *Litsea cubeba* essential oil and its component (citral & limonene) on brown rice snack bars by applying He-Ne lasser treatment has been reported. A count of total phenolic content (TPC) and Fourier transform infrared spectroscopy (FTIR) on the properties of essential oil, citral and limonene before and after the laser treatment was studied for possible modes of action. It was found that the laser treatment improved the antifungal activity of the examined volatile L. cubeba and citral with Aspergillus flavus inhibition by 80% in comparision with those of the control not treated with the laser. L. cubeba vapour [3]**.** The study of extraction of citral from *Litsea cubeba* oil has also been reported in the literature. It showed that molecular distillation technique would be a certain feasibility and a certain industrialized foreground for isolating natural citral from *Litsea cubeba* oil [4]. The compositions and *in vitro* anticancer activities of the leaf and fruit oils of *Litsea cubeba* has also been reported. The main component in the leaf oil was 1,8-cineol, and in the fruit, citral. The fruit oil, but not that of the leaf, exhibited auto toxic activity against human lung, liver and oral cancer cells [5]**.** Study on the designer of *Litsea cubeba* and its main compounds against two stored product insects is also reported in the literature. The essential oils of *Litsea cubeba* fruits was found to possess strong contact toxicity against the cigarette beetle Lasioderma serricorne adults and the booklouse Liposcelis bostrychophila. It also showed strong fumigant toxicity against the two stored product insects [6].

The chemical composition of *Litsea cubeba* essential oils and the bioactivity of its major constituent's citral and limonene has also been reported in the literature. *Litsea cubeba* essential oils exhibit broad antimicrobial activity against bacteria and fungi with minimal inhibitory concentrations [7]. Study on the ethno pharmacological properties, essential oils, medicinal uses and health benefits of an indegineous plant of North east India, emphasizing the profound research to uplift the core and immense potential present in the conventional medicine of the country has been reported [8]. Study of the composition of the stem, flower and fruit oils of *Litsea cubeba* pers from two locations of Assam, India were reported. The essential oils from the stems, flower and fruits of two populations of *Litsea cubeba* pers, growing spontenously in Assam India, were examined. The major constituents of the stem oils appeared to be citronellol (11.9-20.4%) and citronellal (7.7-10.0%). The fruit oils were characterised by high concentrations of these compounds, but in reversed order: citronellal (44.8-77.2%) and citronellol (10.9-14.0%). The flower oils were rich in Sabinene (41.8-42.3%), citronellal (14.3-17.3%) [9]. The study on the medicinal plants used for skin diseases and related problems in Northeastern India and the treatments of skin diseases and related problems in Northeastern India were reviewed based on the enthnobotanic report Of the 275 plant species examined, 224 species including *Litsea cubeba* are used among a range of ethnic groups for disease treatment and have been used for treatment of specific human ailments such as allergies, burns, cuts, and wounds, inflammation, leprosy, leucoderma, scabies, smallpox and sexually transmitted diseases [10]. Study reported the examination to formulate and evaluate controlled release mosquito repellent gel containing encapsulated essentials oils (EO) obtained from natural resources indigenous to Northeast India. Essential oils were extracted from fruits of *Zanthoxylum acanthopodium* (ZA) and *Litsea cubeba* (LC). The study resulted in successful development of a novel herbal controlled release mosquito repellent gel using essential oils from North East India [11]. The traditional uses of different species of the genus Litsea as traditional herbal medicines and as sources of important secondary metabolites has been reported [12]. Most of Litsea species produces odour active compounds while the fruits contain biologically active components and are utilised in various foods as a source of natural ingredients and flavor [13].

Fig 1: *Litsea cubeba* tree in Jotsoma, Kohima Nagaland

Fig 2: *Litsea cubeba* fruits

Material and Method

Plant Material

Extensive collection of *Litsea cubeba* plants sample was done from the early season of plants flowering till the formation of fruits stages from Jotsoma and some other adjoining areas in the year 2019-20 under Kohima district Nagaland which lies at an altitude of about 1444 metres above sea level [14]. The collected specimens were processed into mounted herbarium sheets. The processed specimen was identified by taxonomist from the botany department of the college.

Preparation of plant extract

The collected plants material was properly washed and separated from foreign material such as topsoil, pebbles or rocks, weeds, and materials non-suitable for extraction. The cleaned, healthy plant material were cut into small sections and dried under shade for few days. In the process, it was ensured to preserve the active biomolecules in the plants prior to extraction. The dried material was ground in porcelain dish. About 10g of the crushed plant material was used and exhaustively extracted with 500ml of ethanol, methanol and hexane respectively using simple maceration process continuously for a week and as well as soxhlet extraction continuously for a numerous cycle respectively. It was then followed by reflux condensation to separate plant extract from the solvent. The used solvent was recovered and plant extract containing essential oil was separated. The solvent used for maceration and soxhlet extraction were both polar and non-polar solvent like ethanol, methanol and hexane respectively.

Identification Test

Phytochemical analysis were carried out with the extracted oil following standard procedure to identify the active phytochemical constituents [15, 16, 17].

Qualitative technique for the determination of Phytochemicals

1. Alkaloids

a. Mayer's Test: Two drops of Mayer's reagent were added along the sides of test tube into a 1ml of plant extract. The presence of alkaloids is indicated by the presence of white creamy precipitate.

b. Wagner's Test: A few drops of Wagner's reagents were added to the 1ml of plant extract. Formation of reddish brown precipitate depicts the presence of alkaloids.

c. Dragendroff's Test: The addition of Dragendroff's reagent into the plant extract gives the red precipitate indicate the presence of alkaloids.

d. Hager's Test: A small amount of Hager's reagent is added to the plant extract. The formation of yellow precipitate indicates the presence of alkaloids.

2. Carbohydrates

a. Molish's Test: Two drops of alcoholic solution of α-napthol were added to 2ml of filtrate and 1ml of concentrated sulphuric acid is added slowly along the sides of the test tube. A violet ring indicates the presence of carbohydrates.

b. Fehling's Test: An equal volume of Fehling solution A and B were added to an equal volume of filtrate and it should boil in a water bath. The formation of red precipitate indicates the presence of sugar.

c. Barfoed's Test: An equal volume of filtrate and Barfoeds reagent (1ml each) were mixed and heat in a water bath. A red precipitate confirms the presence of sugar.

d. Benedict's test: A mixture of plant extract and the Benedicts Reagent is heated gently on water bath for two minutes. Formation of orange red precipitate or coloured indicates the presence of sugar. For detection of glycosides, the plant extract is hydrolysed with concentrated hydrochloric acid and the filtrate was subjected to the following test,

e. Borntrager's Test: A 2ml of filtrate is mixed with 3ml of chloroform and 10% ammonia is added to that. A pink colour solution indicates the presence of glycosides.

f. Legal's Test: The plant extract is dissolved in pyridine and sodium nitroprusside is added to that. Then the solution is made alkaline using 10% sodium hydroxide and pink colour solution proves the presence of glycosides.

3. Saponins

A small quantity of plant extract is diluted with 10ml of distilled water and is shaken for 15 minutes in a graduated cylinder. The formation of foam indicates the presence of saponins.

4. Protiens and amino acids

a**. Million's test:** A few drops of Million's reagent is added to 2ml of filtrate. The white precipitate proves the presence of proteins.

b. Biuret Test: One drop of 25% copper sulphate solution is added to 2ml of filtrate. Then 1ml of 95% ethanol is added following by excess of potassium hydroxide pellets. Pink colour in ethanolic layer indicates the presence of proteins.

c. Ninhydrin Test: Two drops of ninhydrin solution are added to 2ml of the filtrate and purple colour proves the presence of amino acids.

d. Xanthoproteic test: The plant extract is treated with few drops of concentrated Nitric acid. The formation of yellow colour indicates the presence of proteins.

5. Flavonoids

Sodium hydroxide test: A small amount of plant extract is treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid indicate the presence of flavonoids.

Lead acetate test: 1ml of the extract was treated with few drops of lead acetate solution, the formation of yellow colour indicate the presence of flavonoids.

6. Steroids

1ml of plant extract was added with 2ml of chloroform and 2ml of conc. sulphuric acid. The appearance of red colour in the chloroform layer indicate the presence of steroids

7. Terpenoids Test

To the plant extract, 2ml of chloroform and 3ml of conc. sulphuric acid is carefully added. Formation of a reddish brown ring at the interphase of the test tube indicated the positive results of terpenoids.

Result and Discussion

Table 1: Data for phytochemical analysis of fresh leaves of *Litsea cubeba*

Sr. No.	Phytochemicals	Fresh Leaves *Litsea cubeba*		
		Ethanol Extract	Methanol Extract	Hexane Extract
1	Alkaloids	+	+	+
2	Saponnins	-	-	-
3	Carbohydrates	-	-	-
4	Protien and amino acids	-	-	-
5	Flavonoids	+	+	+
6	Steroids	-	-	-
7	Tannins	-	-	-
8	Fatty acids	-	-	-
9	Glycosides	-	-	-
10	Terpenoids	+	+	+

Table 2: Data for phytochemical analysis of fresh steam barks of *Litsea cubeba*

Sr. No.	Phytochemicals	Ethanol Extract	Steam bark of *Litsea cubeba*	Hexane Extract
		Methanol Extract		
1	Alkaloids	+	+	+
2	Saponnins	-	+	-
3	Carbohydrates	-	-	-
4	Protien And amino acids	-	-	-
5	Flavonoids	+	+	+
6	Steroids	-	-	-
7	Tannins	-	-	-
8	Fatty acids	-	-	-
9	Glycosides	-	-	-
10	Terpenoids	+	+	+

Table 3: Data for phytochemical analysis of fresh fruits of *Litsea cubeba*

Sr. No.	Phytochemicals	Ethanol Extract	Fruits of *Litsea cubeba*	Hexane Extract
Methanol Extract				
1	Alkaloids	+	+	+
2	Saponnins	-	-	-
3	Carbohydrates	-	-	-
4	Protien And amino acids	-	-	-
5	Flavonoids	+	+	+
6	Steroids	-	-	-
7	Tannins	-	-	-
8	Fatty acids	-	-	-
9	Glycosides	-	-	-
10	Terpenoids	+	+	+

Note: [(+)= Positive, (-)=Negative

Phytochemical analysis of plant parts like leaf, steam bark and flower of *Litsea cubeba* in different solvent; polar and non-polar like ethanol, methanol and hexane indicate the presences and absences of different phytochemical as shown in table1,2 and 3 respectively. It was found that the plant species *Litsea cubeba* have considerable proportion of important phytochemicals which are convienently detected by qualitative analysis test. In our analysis, it was clear that all plant parts of *Litsea cubeba* are rich in alkaloids, flavonoids, terpenoids and minor presence of saponins which was detected from the steam bark in methanol solvent. It was also observed that, of the solvents used i.e hexane, methanol, ethanol; the extracted compound was most soluble in non-polar hexane solvent.

Conclusion

Litsea cubeba is found to be rich in phytochemicals and have the potential to produce bioactive compounds which have a significance pharmacological and medicinal value and therefore the plant under study has the potential to cure various ailments. The in-depth exploration of *Litsea cubeba* for its various withstanding pharmacological properties can potentially be employed as an initiative to discover new drugs to treat serious diseases. It is essential to conserve the plant for its various medicinal uses which bring good health to individual and benefit the society in large. In conclusion, research involving clinical evaluation along with conservation strategies is imperative for long term benefits to society.

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