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RESEARCH ARTICLE

An investigation on the phytoconstituents of *Clausena lansium* (Rutaceae) – an underutilized fruit of Assam, India

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Abstract

Clausena lansium (Lour.) Skeels, commonly known as wampee (in China) belongs to the family Rutaceae. It is popularly known for its edible fruits and medicinal properties. The present study evaluated the proximate, minerals, total phenol content, total flavonoid content and GC-MS analysis of fruits of *C. lansium* occurring in Assam. Proximate analysis revealed the highest moisture content (78.25±0.32%) and low fat content (0.30±0.32%) which serve as an excellent source of low fat diet. Due to low fat and carbohydrate content, the total energy content was found to be comparatively low (63.18±0.74 kcal/100 gm). The fruit also contains notable amount of minerals helpful in maintaining homeostasis of the body. The total phenolic content and total flavonoid content was evaluated using two solvents viz. methanol and water revealed that methanolic extract showed the high total phenol content (27.72±1.03 mg GAE/g extract) and total flavonoid content (21.67±1.54 mg QE/g extract). The phytoconstituents evaluated through GC-MS revealed that the fruits have several important phytoconstituents which have proven antimicrobial, anti-inflammatory, antibacterial, anticancer, antioxidant, antitumor, anti-HIV and anti-viral activities. These findings assist that wampee can be a great source of low fat and low calorie diet enriched with significant amount of minerals and bioactive compounds that have numerous health promoting effects.

Keywords: *Clausena lansium*; Total Phenolic Content; Total Flavonoid Content; Proximate Composition; Mineral; Bioactivity

1. Introduction

Assam is a biodiversity rich region comprising a large number of fruit plants popularly used by many ethnic groups in traditional recipe and medicines for ages. These fruits are excellent source of nutrients and minerals bearing numerous health benefits. However due to lack of proper knowledge and practical utility of these edible fruits, most of them are wasted or harvested inappropriately. To overcome these problems, researchers from all over the world growing their interest in evaluating the nutritional value and therapeutic potentialities of the edible fruits (Nazarudeen, 2010; Deshmukh, 2011).

C. lansium (Lour.) Skeels, an evergreen tree belonging to the family Rutaceae, is native to China and cultivated in India (Lim, 2012). It is commonly known as 'wampee' in China. The fruits are oval in shape and 20-27 mm in size. It generally matures during May - July depending on various geographic and climatic conditions. The fruits are sweet sour in taste and eaten in fresh or preserved form such as jam, jelly and beverages. Due to its high nutritional value and demand, the fruit is cultivated in different parts of the world like China, Vietnam, Thailand, Malaysia, Philippines and India (Huang et al., 2023).

The species holds a significant place in medicinal and pharmaceutical point of view. Traditionally, the leaves, fruits and roots are used to treat various gastrointestinal disorders including ulcers. Additionally, in India and China, the peel of *C. lansium* has many uses in treating bronchitis and as a vermifuge. The decoction of leaves can be used as a shampoo to remove dandruff from hair (Musa et al., 2022). Leaf and seed extracts have hepato protective, antiplatelet, hypoglycemic, antifungal and antiviral activities (Shen et al., 1989; Liu et al., 1996; Fan et al., 2001; Ng et al., 2003). Wampee fruits can be consumed as processed items like jams, jelly, beverages and wine in addition to being eaten as raw fruits (Lim, 2012). According to reports, the pulp and seeds help to reduce the symptoms of some gastrointestinal diseases, cough and dyspepsia (Wen-Shyong, 1991; Adebayo, 2009). Accordingly, wampee is a great source of several nutrients and phytochemicals with various promising health benefits including antioxidant, anti-inflammatory and antibacterial activities (Peng et al., 2019). It was reported that wampee fruits contain high amount of phenols and flavonoids (Prasad et al., 2009; Huang et al., 2010).

Various reports shows the use of the fruits in therapeutic purposes but it remains as underutilized in many areas of Assam due to lack of proper knowledge (Prasad et al., 2010; Musa et al., 2022; Huang et al., 2023; Deka et al., 2024). There are no scientific records available on nutritional content, phytochemicals and bioactive compounds present in the fruits of *C. lansium* growing in Assam. Therefore, the study investigated the proximate and mineral composition, phytochemical composition and GC-MS analysis of *C. lansium* occurring in Assam.

2.1. Material and method

2.1. Sample collection and authentication

Fruits of *C. lansium* were collected from Baihata Chariali, Kamrup district (26°21′08″ N 91°45′23″ E) of Assam, India. The species was identified by comparing various literatures (Hooker, 1875, Hajra et al., 1997, Wu et al., 2008) and online databases (POWO, GBIF). The herbarium specimen was deposited at Gauhati University Botanical Herbarium (GUBH).

2.2. Sample preparation

Fruits were collected, washed and shade dried until it completely moisture free. The dried samples were ground and stored in an

Table 1. Proximate composition of *Clausena lansium* fruit.

Components	Percentage composition (%)
Moisture	78.25±0.32
Ash	1.27±0.11
Crude Fibre	5.06±0.72
Crude Fat	0.30±0.32
Crude Protein	2.59±0.62
Total Carbohydrate	12.53±0.17

Values are given in mean ± standard deviation of three independent determinations

Table 2. Mineral analysis of *C. lansium* fruit.

Elements	Concentration (ppm)
Na	8.64±0.21
K	40.81±0.32
Ca	28.07±0.61
Mg	3.08±0.26
Fe	16.40±0.05
Cu	0.06±0.17
Mn	0.09±0.07
Zn	0.16±0.05

Values are given in mean ± standard deviation of three independent determinations

Table 3. Quantitative phytochemical analysis of *C. lansium* fruit (total phenolic and flavonoid).

Phytoconstituents	fruit extract	
	Methanol	Aqueous
Phenol (mg GAE/g extract)	27.72±1.03	21.71±0.86
Flavonoid (mg QE/g extract)	21.67±1.54	11.10±2.01

Values are given in mean ± standard deviation of three independent determinations

airtight containers. Thereafter extracts were prepared using different solvents and the filtrates were evaporated according to the boiling point of the solvent. The concentrated residues were stored at 4°C for further analysis.

2.3. Proximate analysis

According to the standard methodology given by AOAC (2000), the moisture, ash, crude fibre, crude fat, protein and carbohydrate content of the fruits were analysed. Fresh fruits were used for moisture analysis and dried powder samples were used for rest of the analysis. Total energy or calorific value was calculated using the equation given by FAO 2003.
Total Energy Content (kcal/100g) = 4 × Protein (%) + 9 × Fat (%) + 4 × Carbohydrate (%)

2.4. Mineral analysis

The mineral content of the fruit was analysed following the methodology of Mallikarjuna et al., 2013 with slight modification. 2 grams of dried samples were taken and ash was prepared in a muffle furnace at temperature 450°C for 5-6 hours. The ash was mixed with 2 mL of concentrated nitric acid and heated on low flame for 1 minute. Thereafter, it was cooled, filtered and the filtrate was diluted to 50 ml with distilled water. The filtrate was further used for mineral analysis and it was done using Atomic Absorption Spectrophotometer (AA7000, Shimadzu).

2.5. Phytochemical analysis

2.5.1. Determination of total phenol and flavonoid content

Total phenolic content (TPC) was determined by Folin-Ciocalteu's method as described by Sahu and Saxena (2013). Sample solution was mixed with diluted Folin-Ciocalteu reagent (1:9), then after 3 minutes Na_2CO_3 solution (1%) was added and the sample absorbance was read at 760 nm after 90min incubation in dark at room temperature. Results were expressed as mg of Gallic acid equivalents (mg GAE/g extract).

Total flavonoid content (TFC) was determined by Aluminium trichloride ($AlCl_3$) method as described by Sahu and Saxena 2013. 1ml of Sample solution was mixed with an equal volume of Aluminium trichloride (2%) in solvent. A blank sample was also prepared in the same way by adding sample solution to solvent without $AlCl_3$. The absorbance was measured at 415 nm, after 30 min incubation at room temperature. Results were expressed as mg of Quercetin equivalents (mg QE/g extract).

2.6. Statistical analysis

To maintain the reliability and consistency of the results, all the experiments were conducted in triplicates. The results were expressed as mean ± standard deviation.

2.7. GC-MS analysis

The Gas Chromatography Mass Spectrometry (GC-MS) analysis of the methanolic fruit extract was done to know the bioactive compounds. The experiment was performed in Clarus 680 GC & Clarus 600 C MS PerkinElmer, USA. Interpretation on GC-MS data was done by using the database software of National Institute Standard and Technology- 2008 (NIST 2008). The mass spectra of unknown components were compared with the spectrum of known components of NIST library and the compounds were identified with name, molecular weight and empirical formula.

3. Result

3.1. Proximate analysis

The proximate compositions of the fruits were analysed and results were presented in Table 1. The results revealed that the fruits contain a high percentage of moisture content (78.25±0.32%). Fat content was very low (0.30±0.32%). With 5.06±0.72% of fibre content, the fruit is an exceptionally rich source of dietary fibre. The carbohydrate and protein content were found to be 12.53±0.17% and 2.59±0.62% respectively.

Based on the above values, total energy content or nutritive value was calculated and found to be 63.18±0.74 kcal/100 gm.

3.2. Mineral analysis

The mineral composition of the fruits of *C. lansium* was represented in Table 2. The wampee fruit could be an important source of vital minerals. *C. lansium* contain significant amount of minerals such as 40.81±0.32 ppm potassium followed by calcium 28.07±0.61 ppm, iron 16.40±0.05 ppm. Sodium content was found to be 8.64±0.21 ppm. The amount of copper, manganese and zinc in the fruit were 0.06±0.17 ppm, 0.09±0.07 ppm and 0.16±0.05 ppm respectively.

3.3. Total phenol and flavonoid content

The total phenolic content and total flavonoid content of the fruits were evaluated in both methanolic and aqueous extract. The results were summarized in Table 3. Methanolic extract has higher total phenolic content (27.72±1.03 mg GAE/g extract) and flavonoid content (21.67±1.54 mg QE/g extract) compared to aqueous extract.

3.4. GC-MS analysis

GC-MS analysis of methanolic extracts of *C. lansium* has been done and identified various major and minor bioactive compounds. The GC-MS chromatogram was presented in Figure 1. The phytocompound 5 Hydroxymethylfurfural content was detected highest (26.53%) in the fruit extracts of *C. lansium* (Table 4). Ten major compounds were detected in each species based on their retention time and peak area, along with their molecular weight, molecular formula, and peak area (Table 4). Reports shown that tetrahydrocyclopenta[1,3]dioxin-4-one possesses antimicrobial and anti-inflammatory properties (Vijayan, 2017), trans-2,4-dimethylthiane,s,s-dioxide have antibacterial, anticancer, antidote, antimicrobial, antioxidant, antitumor, anti-HIV, anti-viral properties (Thanigaivel et al., 2014). Similarly, beta.-l-arabinopyranoside, methyl have been reported for anticancer (liver, lung, breast, and prostate) and antioxidant activities (Rao et al., 2019). However, furyl hydroxymethyl ketone have antiapoptotic, cytoprotective as well as antioxidant properties (Ravi et al., 2015) and 2,6,10,14-tetramethyl-7-(3-methylpent-4-enylidene) pentadecane possesses anti-inflammatory and antioxidant properties (Marchioni et al., 2020).

4. Discussion

The proximate analysis of the present study revealed that the fruits contain highest amount of moisture which was quite similar with the previous investigation (Huang et al., 2023). The fibre content was found to be 5.06% which could be an excellent source of dietary

fibre. Furthermore, high fibre content may be beneficial for preventing many digestive issues such as constipation, piles and gastrointestinal disorders as well as lowering blood cholesterol level and maintain healthy weight to promote the food as a well-balanced diet (Asaolu et al., 2012; Waddell and Orfila, 2023; Li and Sen, 2024). The fat content was found to be low in our study which is an exceptional source of low fat diet. Due to low fat and carbohydrate content, the total energy content or nutritive value was found to be comparatively low.

The most abundant mineral present in the fruit was potassium followed by calcium. A high content of potassium combined with sodium helps in maintain ionic balance of the body. The dietary interaction between potassium and sodium is also crucial for preventing hypertension (Seal 2011, Saupi et al., 2009). The other important minerals reported significant amount in the fruit was magnesium, copper, iron, manganese and zinc which play an important role in maintaining the equilibrium in the body.

Phenolic compounds are common secondary metabolites in plants, known for their free radical scavenging activity due to the presence of hydroxyl groups (Jyoti et al., 2019). In the present study, methanolic fruit extract of *C. lansium* exhibited the highest phenolic as well as flavonoid content. High phenolic content suggests potential anti-inflammatory properties and a role in wound healing (Akhtar et al., 2015). GC-MS analysis evaluated 10 compounds which showed various bioactivities corroborated through literature studies. These phytocompounds possesses proven antimicrobial and anti-inflammatory antibacterial, anticancer, antioxidant, antitumor, anti-HIV and anti-viral activities.

5. Conclusion

The present study reveals that the fruit of *C. lansium* could be used as a source of high fibre diet consisting of low fat and low energy content. The methanolic extract of fruit also demonstrated an excellent source of macronutrients such as potassium, calcium, magnesium and sodium while micronutrients profile constituting copper, manganese and zinc. Additionally, the compounds quantified through GC-MS analysis with reported anti-inflammatory, antioxidant, antibacterial activities might be due to high phenolic and flavonoid content that signifies the pharmaceutical potentialities of this underutilized fruit of *C. lansium*.

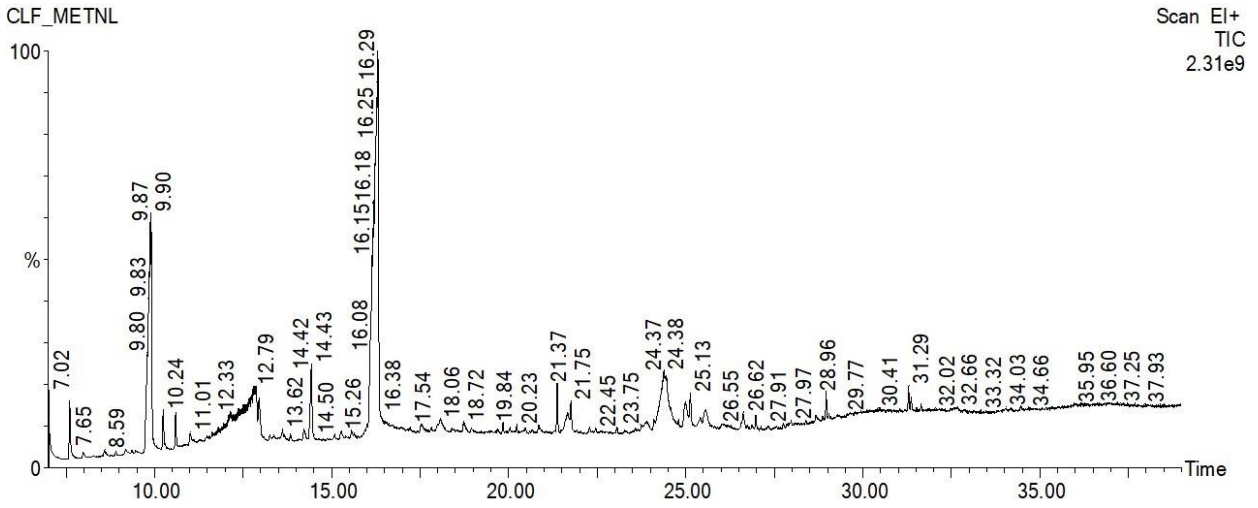

Figure 1. GC-MS chromatogram of methanolic extract of *C. lansium* fruit.

Table 4. List of compounds quantified from methanol extract of *C. lansium* using GC-MS and their bioactivity.

SN	Name of the compound	Retention time (min)	Molecular formula	Molecular weight (g/mol)	Peak area (%)	Bioactivity
1	**TETRAHYDROCYCLOPENTA[1,3]DIOXIN-4-ONE**	**9.9**	**C7H10O3**	**142**	**11.263**	Antimicrobial, Anti-inflamatory (Vijayan, 2017)
2	TRANS-2,4-DIMETHYLTHIANE, S,S-DIOXIDE	12.14	C10H20O2S	162	1.717	Antibacterial, anticancer, antidote, antimicrobial, antioxidant, antitumor,anti-HIV, anti-viral (Thanigaivel et al., 2014)
3	BETA.-L-ARABINOPYRANOSIDE, METHYL	12.381	C6H12O5	164	1.083	Anticancer (liver, lung, breast, and prostate), Antioxidant (Rao et al., 2019)
4	3,4-ALTROSAN	12.566	C6H10O5	162	1.186	Bacteriostatic, Fungicide (Jadav et al., 2014)
5	PROPANE, 2-FLUORO-2-METHYL	12.862	C4H9F	76	5.117	No activity reported
6	FURYL HYDROXYMETHYL KETONE	12.956	C6H6O3	126	1.348	Antiapoptotic, Cyto protective. Work against oxidative stress (Ravi et al., 2015)
7	MESO-2,5-DIMETHYL-3,4-HEXANEDIOL	14.431	C8H18O2	146	1.762	No activity reported
8	**5 HYDROXYMETHYLFURFURAL**	**16.302**	**C6H6O3**	**126**	**26.53**	No activity reported
9	CARBONIC ACID, 2-ETHYLHEXYL HEXADECYL ESTER	24.38	C27H54O3	398	6.51	No activity reported
10	2,6,10,14-TETRAMETHYL-7-(3-METHYLPENT-4-ENYLIDENE) PENTADECANE	24.991	C25H48	348	1.108	Anti-inflammatory , antioxidant (Marchioni et al., 2020)

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Conflict of interest

Authors declare that there is no conflict of interest.

Author's Contribution

Nilakshee Devi designed the experiment and prepared the manuscript. Bhumasri Kalita performed all the experiments. Both the authors analysed the data, read and approved the final manuscript before submission this journal.

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