

## Chapter 4

# Qualitative phytochemical screening of *Limonia acidissima* L.

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### Abstract

*Limonia acidissima* has nutritious fruits that are used in a variety of food preparations. The fruit as well as different parts of *L. acidissima* are also used in traditional systems of medicine to treat numerous ailments and reportedly possess different pharmacological activities. Keeping this in mind, an attempt has been made to do a qualitative analysis of phytochemical screening of fruit pulp. The results revealed that the aqueous, methanolic, and ethanolic extracts of fruit pulp have diverse secondary metabolites like tannin, saponin, flavonoids, phenolic compounds, reducing sugars, alkaloids, etc. The present study brings attention towards its value addition at the regional level. The detected phytochemicals, along with photographs, are presented here.

**Keywords:** Nutritional fruit, pharmacological activities, secondary metabolites, traditional medicine

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### Introduction

India is blessed by different agro-climatic conditions that have smoothed the path to growing a variety of wild fruits in different seasons throughout the year. The state of Odisha is enriched with wild trees bearing edible fruits (Tamang et al. 2021). They play an important role in the lives of rural and tribal populations in every aspect, such as supplemented food, nutritionally balanced diets, medicine, and fodder, along with their daily income potential (Mahadkar and Patil 2023). Among different parts of plant species, fruits are more consumed by the tribals. They usually grow in

monsoon climates and dry tropical plains, and seasonal fruit is reaped from March to May (Murakonda et al. 2022). Among them, *Limonia acidissima*, commonly known as wood apple, comes under the wild edible fruits of Odisha belonging to the Rutaceae family (Rodrigues et al. 2018). *L. acidissima* is a deciduous, slow-growing, erect tree with a few upward-reaching branches bending outwards near the summit, where they are subdivided into slender branchlets drooping at the tips (Murrinie et al. 2017). The fruits are spherical in shape (Plate 1). It has a woody and extremely hard outer shell that is very difficult to crack open. This is commonly found in India, Pakistan, Bangladesh, Sri Lanka, and Southeast Asian countries (Rodrigues et al. 2018). The peculiar wood apple looks like a hardy cover of coconut on the outer shell, while on the inside it has soft pulp. The fruit smells like a mixed stench of rotten blue cheese with overripe bananas. Wood apple pulp has a sour and funky taste, which is delicious with a touch of sugar in desserts or with warm spices for savory dishes (Murakonda et al. 2022). This plant has a wide array of medicinal properties. All parts have tremendous therapeutic and functional properties, including leaves, which have diuretic properties and prevent children's stomach disorders (Pandey et al. 2014); roots; and bark, which has insecticidal activity and is used against snakebite (Rodrigues et al. 2018). The spines used for liver disorders are gum, which is used for diarrhea (Vijayvargiya and Vijayvargiya 2014). The fruit pulp possesses polysaccharides, amino acids, tannins, flavonoids, saponins, phenolic compounds, alkaloids, etc., which are effective in preventing many ailments like skin cancer (Pradhan et al. 2012), dysentery, sore throat, jaundice, and gastropathy (Pandey et al. 2014). It is also a remedy for diabetes, ulcers, and high blood pressure. The present study aims to highlight medicinal importance of *L. acidissima* with the detection of secondary metabolites. In this regard, a qualitative phytochemical analysis of *L. acidissima* fruit pulp from different extracts was carried out and presented here.

## Methodology

### Collection of fruits and preparation of extracts

The fruits of *L. acidissima* were collected from South Chirebeda of Rourkela district, Odisha in March 2024. The plant was identified by correspondence author. The Soxhlet method of extraction using three different solvents (aqueous, methanol and ethanol) was carried out (Kumar et al. 2013; Kumar et al. 2017; Swain et al. 2020; Devi et al. 2023) for phytochemical analysis (Das et al. 2019). Detection of nine secondary metabolites were carried out using standard methods (Misra et al. 2012; Kumar et al. 2013;

Plate 2). A voucher specimen (APRFH 082) is deposited to the herbarium unit, Biodiversity and Conservation Lab., APRF, Odisha, India (Figure 1).

### **Qualitative phytochemical analysis**

#### **Test for tannin**

1 ml of the filtrate of fruit pulp extract was taken, three drops of 0.1% lead acetate solution was added to it. The creamy gelatinous precipitate provides the positive result of the presence of tannins.

#### **Test for saponin**

1 ml of the filtrate of fruit pulp extract of *L. acidissima* was added with 1 ml of distilled water and shaken it vigorously. The persistent froth formation of shaking provides the positive evidence of saponin presence.

#### **Test for flavonoids**

1 ml of the filtrate of fruit pulp extract was taken and added 2 ml of 2% NaOH solution and 3 drops of dilute HCL. The colour initially turned an intense yellow colour with NaOH solution and later became colourless. This change in colour confirmed the positive result for the presence of flavonoids.

#### **Test for terpenoids**

2 ml of the filtrate of fruit pulp extract was used and 6 drops of chloroform was added and placed it in the water bath for few minutes. Then, 6 drops of concentrated sulphuric acid were added. The reddish-brown interface confirmed the positive result of the presence of terpenoids.

#### **Test for phenolic compounds**

1 ml of the filtrate of fruit pulp extract was taken. Few drops of 5% Ferric chloride solution were added. The dark bluish black colour provides the positive result of the test of phenolic compounds.

#### **Test for reducing sugars**

1 ml of the filtrate of fruit pulp extract was taken and 2 drops of Fehling's solution A followed by Fehling's solution B were added and put it in the water bath for some time. The formation of red orange precipitate confirmed the presence of reducing sugars.

#### **Test for steroids**

1 ml of the filtrate of fruit pulp extract was used. 1 ml of chloroform and 1 ml of concentrated sulphuric acid was added into it. The appearance of upper red and lower yellow with green fluorescence provide the positive result of the test of steroids.

#### Test for alkaloids

1 ml of the filtrate of fruit pulp extract was taken and added 3 to 4 drops of Dragendorff's reagent. The formation of reddish-brown precipitate confirmed the presence of alkaloids.

#### Test for carbonyl compounds

1 ml of the filtrate of fruit pulp extract was taken and added 3 to 4 drops of 2,4- dinitrophenylhydrazine reagent. The formation of yellow crystal confirmed the presence of carbonyl compounds.



**Plate 1:** Different plant parts of *L. acidissima*; (a) Whole plant, (b) Leaves, (c) Fruit and (d) flowers

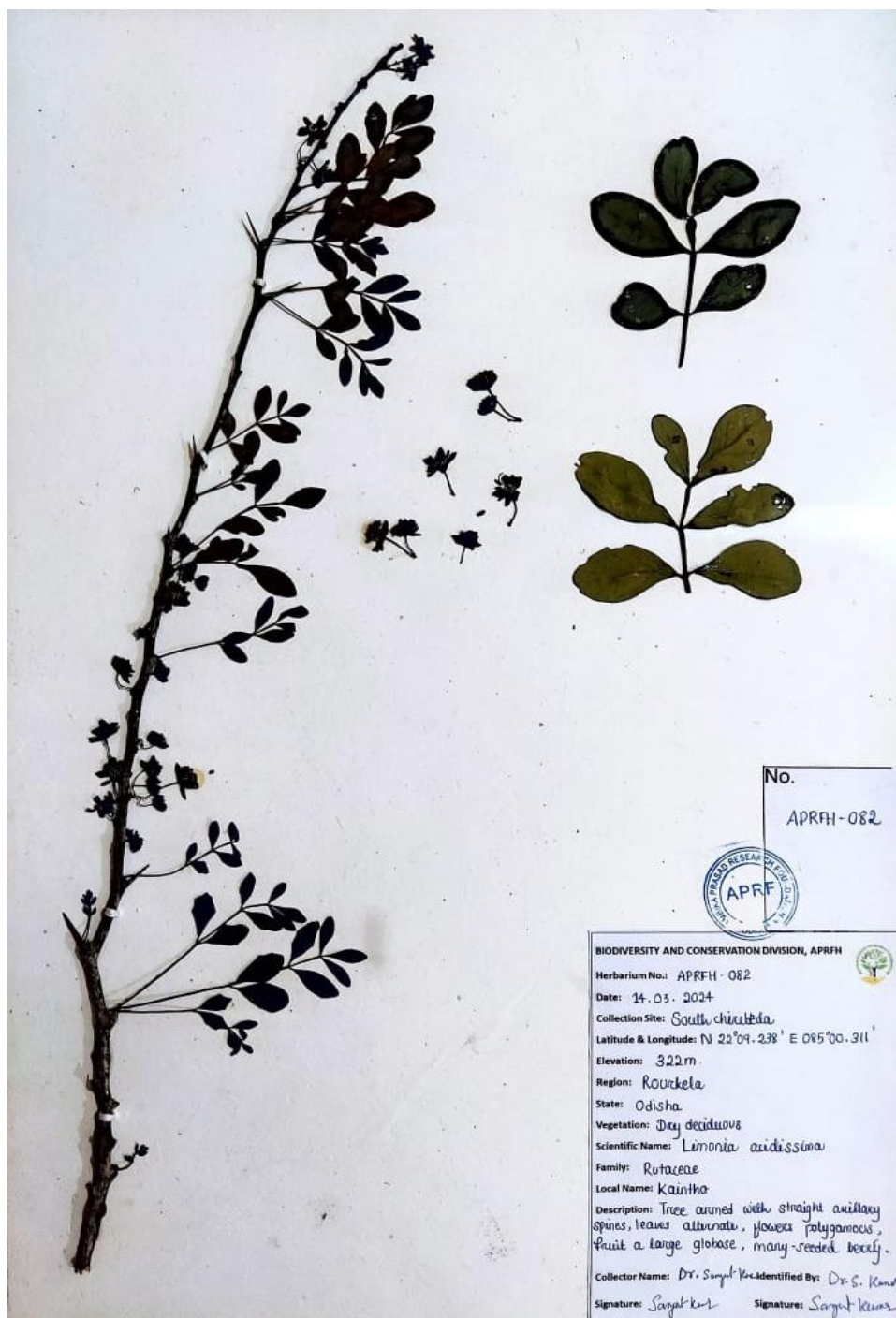


Figure 1: Herbarium specimen of *L. acidissima*

### **Literature survey**

The literature survey was carried out in the year 2023-2024. The scientific information on the enumerated medicinal plant species from the survey is presented, which was collected from scientific databases such as Google Scholar, Science Open, SpringerLink, Mendeley, Indian Journals online, Wikipedia, PubMed Central, and offline publications, along with books (Mahanta et al. 2024).

### **Results and discussion**

The field survey in South chirebeda of Rourkela district, Odisha state during November 2023-March 2024 revealed that the fruits of *L. acidissima* are mainly consumed by the rural and tribal communities. It comes under wild edible fruits of Odisha and needed for further research studies on its nutritional values for the value addition using its existing ethnomedicinal information to produce novel therapeutics in the sector of healthcare solutions. The present investigation on qualitative phytochemical analysis resulted with the presence of several secondary metabolites (Table 1) like tannins, saponins, flavonoids, phenolic compounds, and alkaloids in the aqueous and ethanolic fruit extracts of *L. acidissima*.

### **Food values**

*L. acidissima* is a nutritious fruit plant species that contains a surprisingly high amount of essential minerals like protein, carbohydrates, calcium, magnesium, iron, phosphorus, and zinc (Rao et al. 2011). The fruit may be eaten raw but it has a sour taste and requires sweetening. The fruit pulp is suitable for making food products such as jam, fruit bar, wine, chutney, sherbet, pulp powder etc. (Poongodi et al. 2013). Pulp has low amount of fat which makes it an ideal diet for overweight people (Kerkar et al. 2020). In 2004, Sakate and his co-workers reported that *L. acidissima* burfi is prepared using cow milk khoa (Sakate et al. 2004). In 2010, Lande and his team reported that ready to serve beverage prepared from *L. acidissima* pulp using fully ripened fruit (Kerkar et al. 2020).

### **Ethnobotanical uses**

Various parts of wood apple have been used in traditional medicines since long by local communities. Both ripe and unripe fruits have recognition for its medicinal properties. The phytoconstituents are responsible for possessing therapeutic roles. In traditional system of medicine, it is used to cure asthma, diarrhoea, dysentery, wounds, tumors etc. It has also a sound role on lowering cholesterol levels (Kerkar et al. 2020; Sakate et al. 2004).

## Pharmacological properties

*L. acidissima* has pharmacological potential. In 2008, Ahamed and his team reported that the fruit pulp of *L. acidissima* has anti-inflammatory, analgesic and antipyretic activities (Ahamed et al. 2008). The seed flours of *L. acidissima* composed of good source of essential amino acid (Sonawane et al. 2016). In 2017, Sonawane and Arya reported that the peptides isolated from the *L. acidissima* seeds possess antioxidant activity and the protein hydrolysate of the seeds shows antimicrobial activity against *E. coli* and *Salmonella typhi* (Sonawane and Arya 2017).

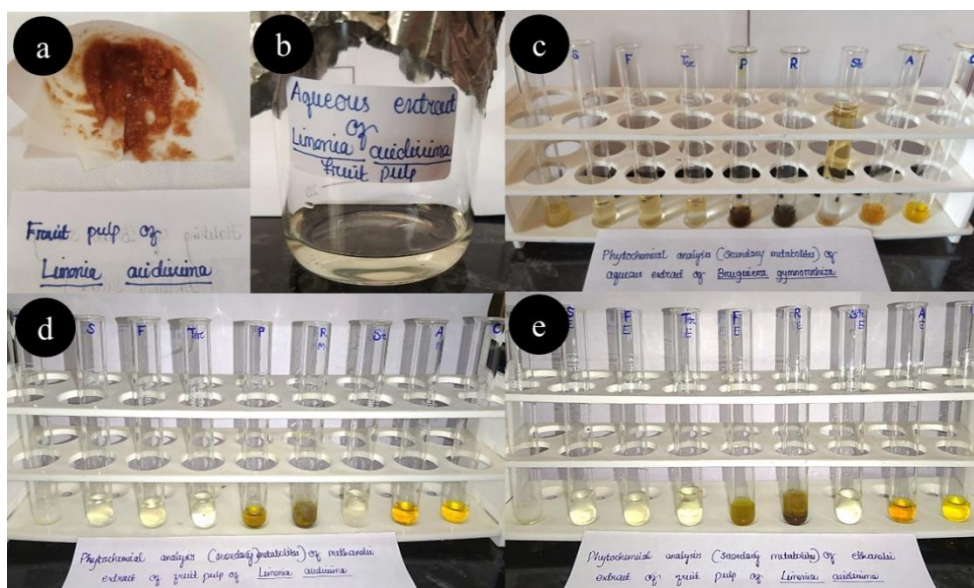


**Plate 2:** Phytochemical analysis of different extracts of (a) fruit pulp of *L. acidissima*, (c) aqueous, (d) methanolic, (e) ethanolic

**Table 1:** Qualitative phytochemical analysis of different fruit extracts of *L. acidissima*

| Bioactive compounds | Solvents |            |           |
|---------------------|----------|------------|-----------|
|                     | Aqueous  | Methanolic | Ethanolic |
| Tannin              | +ve      | +ve        | +ve       |
| Saponin             | +ve      | -ve        | -ve       |
| Flavonoids          | +ve      | +ve        | +ve       |
| Terpenoids          | -ve      | -ve        | -ve       |

|                    |     |     |     |
|--------------------|-----|-----|-----|
| Phenolic compounds | +ve | +ve | +ve |
| Reducing sugars    | +ve | +ve | +ve |
| Steroids           | -ve | -ve | -ve |
| Alkaloids          | +ve | +ve | +ve |
| Carbonyl compounds | -ve | +ve | +ve |

## Conclusion

Wild edible fruits are distributed globally, but they are still unexplored. They received more demand during the COVID-19 situation for enhancing immunity. The present study concludes that the fruits of *L. acidissima* have diverse secondary metabolites and possess various pharmacological potentials. Therefore, the fruits can be good nutraceuticals, and further research studies are necessary for value addition using the existing ethnomedicinal information for sustainable utilization in Odisha. Moreover, conservation of such plants will be helpful for sustainable food, medicine, livelihood, and a balanced ecosystem.

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