

Chapter 6

Analysis of primary and secondary metabolites of *Coccinia grandis* (L.) Voigt leaf: a nutraceutical of tribals

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Abstract

As per ethnobotanical claims (Santhal tribe of Odisha and Gujar tribe of Himachal Pradesh) on the leaf of *Coccinia grandis*, qualitative analysis of primary and secondary metabolites was carried out and results were presented here to validate the claims. The results showed that the leaf has food and medicinal value and can serve during present era of climate change. Results showed that leaf could be used as nutraceutical in urban areas where there is a lack of wild food plants.

Keywords: tribal food, climate resilient, organic food, nutraceutical

Introduction

Humans have disturbed the ecological balance in every aspect, which has created uncountable problems on the earth (Rosenblatt, 2005). This situation has put a question mark on the existence of living organisms and the sustenance of life on earth (Hunter, 2007). Due to the imbalance in the ecosystem, the weather pattern has turned erratic. Such weather and climatic changes created havoc in agricultural practices and resulted in a lack of adequate food, pest resistance, low crop yield, and many more (Dimri *et al.*, 2024). Scientific anthropogenic causes, like the development of GMO crops, are also responsible. Now, researchers are developing polycultures, cover crops, and many more, but they are not thinking about what we had or what we ignored. In this aspect, Indigenous Traditional Knowledge (ITK) on our wild food and crops could be the solution to frequent climatic changes and related problems (Kumar *et al.*, 2021). During the different floristic surveys in Odisha and Himachal Pradesh, the authors observed that the local tribal communities (Santhal in Odisha and Gujar in Himachal Pradesh) collect different wild plant parts

and consume them as food. During the observations, the authors observed in both states that locals were collecting the leaf of *Coccinia grandis*. After discussion with them, it was found that they consume it as medicinal food. *C. grandis* (Figures 1–2) has a very good population in both study areas and can grow in different climatic and soil conditions. It is known as Kanoori in Himachal Pradesh and Ban Kundri in Odisha (Nayak and Kumar, 2023). Keeping in mind the above-mentioned significance of *C. grandis*, an attempt has been made to identify the primary and secondary metabolites of the leaves of *C. grandis*. The present study highlights the nutraceutical values of *C. grandis* in contemporary global situations.

Methodology

Collection of food and medicinal values

Food and medicinal values are documented from Santhal community of Odisha and Gujjar community of Himachal Pradesh by the authors (Figures 3-5) through a set of questionnaires (Das *et al.*, 2022).

Collection of plant sample and preparation of extracts

The leaves of *C. grandis* were collected from CDA-13 nearby Mahanadi River area of Cuttack district of Odisha state in June 2024 (Figure 6). The plant was identified by the senior author. The Soxhlet method of extraction was adopted using different solvents for phytochemical analysis (Kumar *et al.*, 2013; Devi *et al.*, 2023). Detection of six primary and nine secondary metabolites were carried out using standard methods (Jena *et al.*, 2024; Soni *et al.*, 2024).

Qualitative phytochemical analysis

Primary metabolites (Mohanty *et al.*, 2021)

Test for Protein

One ml of the filtrate of leaf extract was mixed with 2-5 drops of Millon's reagent and kept it in water bath for 30 minutes. The pink coloration indicates the presence of protein.

Test for carbohydrate

One ml of the filtrate of leaf extract was mixed with 2-5 drops of Fehling's solution A and B and kept it in the water bath for 30 minutes. A reducing sugar reacts with Fehling's reagent in an alkaline medium to form orange-to-red precipitates. The appearance of this orange or red precipitate indicates the presence of carbohydrates.

Test for starch

To 1 ml of the filtrate of leaf extract, 2-3 drops of concentrated HCL were added and heated for 1-2 minutes. NaOH following Benedict's reagent was added to this

solution and kept in the water bath. The change in coloration from bluish-green to red-orange indicates the presence of starch.

Test for sucrose

About 1 ml of the filtrate of leaf extract was mixed with 2-3 drops of iodine. The appearance of a blue-black colour indicates the presence of sucrose.

Test for lipid

About 1 ml of the filtrate of leaf extract was mixed with 2-5 drops of Sudan-III. The appearance of pink droplets indicates the presence of lipids.

Test for amino acid

One ml of the filtrate of leaf extract was mixed with 5 drops of Ninhydrin reagent and kept it in water bath for 30 minutes. Ninhydrin is a powerful oxidizing agent to give a purple-coloured product termed Rhuemann's purple.

Secondary metabolites (Kumar and Jena, 2014)

Test for tannin

About 1 ml of the filtrate of leaf extract was taken. Added about 3-5 drops of 0.1% lead acetate solution into it. The gelatinous precipitate formation confirmed the presence of tannin (Mahendru *et al.*, 2024).

Test for saponin

About 1 ml of the filtrate of leaf extract was taken and mixed with 1 ml of distilled water and allowed to shake it vigorously. The persistent froth formation after shaking confirmed the presence of saponin (Rani *et al.*, 2024).

Test for flavonoids

About 1 ml of the filtrate of leaf extract was taken and added 2 ml of 2 % NaOH solution, followed by H₂SO₄ into it. The colour initially turned to an intense yellow colour with the addition of NaOH solution and later became colourless with sulphuric acid. This change in colour confirmed the presence of flavonoids (Swain *et al.*, 2020).

Test for terpenoids

About 2 ml of the filtrate of leaf extract was added with 6 drops of chloroform and placed it in the water bath for few minutes. Then 6 drops of concentrated H₂SO₄ were added. The appearance of reddish-brown interface confirmed the presence of terpenoids (Chandra *et al.*, 2024).



Figure 1: Whole plant of *Coccinia grandis* (L.) Voigt



Figure 2: Fruits of *Coccinia grandis* (L.) Voigt



Figure 3: Fruits of *Coccinia grandis* (L.) Voigt



Figure 4: Collection of food values by discussing with a Santhal child

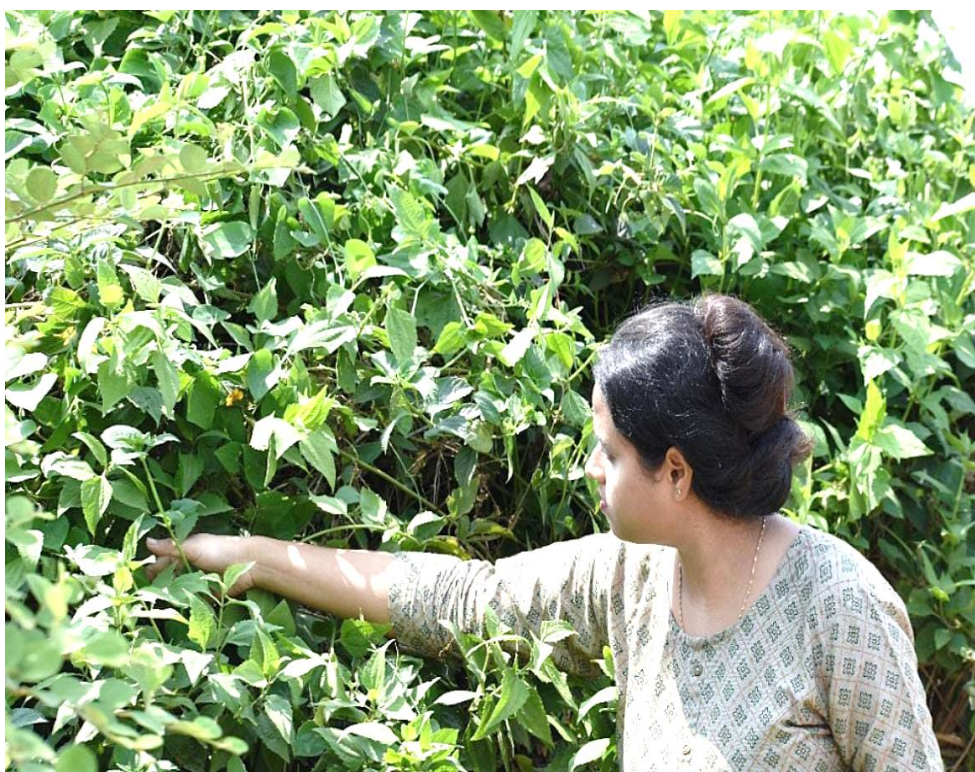


Figure 5: Collection of *C. grandis* leaves for experimental works



Figure 6: Collected leaves of *C. grandis* for experimental work

Test for phenolic compounds

About 1 ml of the filtrate of leaf extract was taken. Few drops of 5% Ferric chloride solution were added into the filtrate extract. The appearance of bluish black colour provides the positive result of the phenolic compounds (Vajha *et al.*, 2024).

Test for reducing sugar

About 1 ml of the filtrate of leaf extract was taken and 2 drops of Fehling's solution A followed by Fehling's solution B were added and kept it in water bath for few

minutes. The presence of red orange precipitate confirmed the positive result of the test for reducing sugar (Snehalatha *et al.*, 2022).

Test for steroids

About 1 ml of the filtrate of leaf extract was taken. 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 for the presence of steroids (Valigatla *et al.*, 2024).

Test for alkaloids

About 1 ml of the filtrate of leaf extract was taken and added 3 to 4 drops of Dragendroff's reagent. The formation of reddish-brown precipitate confirmed the presence of alkaloids (Hebbar *et al.*, 2024).

Test for carbonyl compounds

About 1 ml of the filtrate of leaf extract was taken and added 3 to 4 drops of 2,4- Di-nitrophenylhydrazine reagent. The formation of yellow crystal confirmed the presence of carbonyl compounds (Kumar *et al.*, 2012).

Results and discussion

Food and medicinal survey revealed that leaves and tender parts are consumed by Santhal and Gujjar communities. In Hamirpur, Himachal Pradesh, young green fruits are also cooked as vegetable and consumed in diabetes and to regulate the blood pressure. Keeping in mind their food and medicinal uses, biochemical studies were carried out to identify qualitative primary and secondary metabolites of leaves. It was observed that protein, carbohydrate, and amino acids are detected in the leaf extracts of *C. grandis* (Table 1; Figures 7-9).

Table 1: Phytochemical screening (Primary metabolites) using different extracts of *C. grandis* leaves

Primary metabolites	Aqueous	Methanol	Ethanol
Protein	+ve	+ve	+ve
Carbohydrate	+ve	+ve	+ve
Starch	-ve	-ve	-ve
Sucrose	-ve	-ve	-ve
Lipid	-ve	-ve	-ve
Amino acid	+ve	+ve	+ve

(+ve: positive; -ve: negative)



Figure 7: Screening of primary metabolites using aqueous extract of *C. grandis* leaves



Figure 8: Screening of primary metabolites using methanolic extract of *C. grandis* leaves

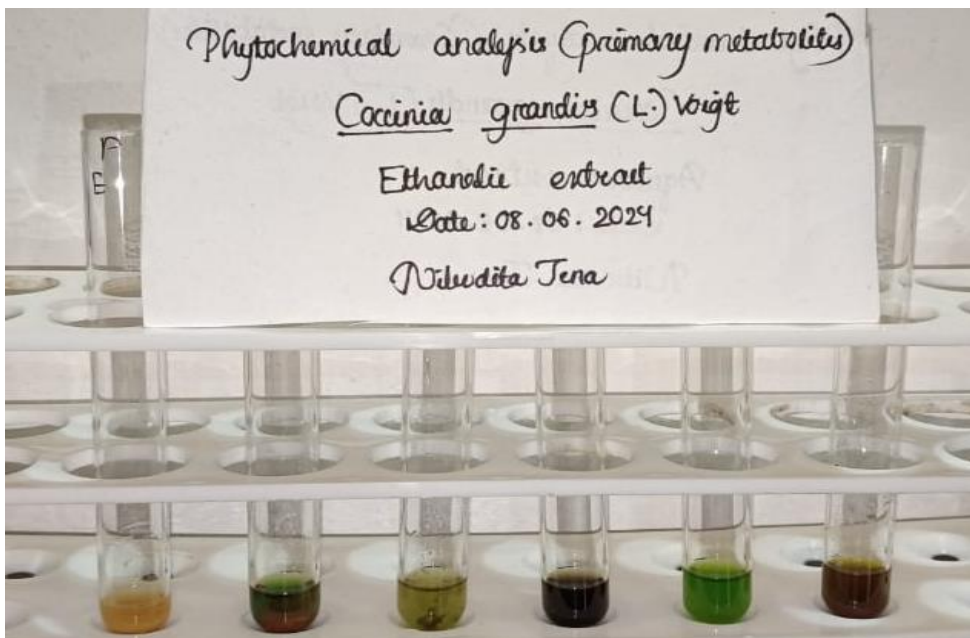


Figure 9: Screening of primary metabolites using ethanolic extract of *C. grandis* leaves

The phytochemical screening revealed that tannin, saponin, phenolic compounds, reducing sugar and steroids are detected in aqueous, methanol and ethanol extracts (Table 2; Figures 10-12). The presence of phenolic compounds may be responsible to control the complication of diabetes and regulate the blood sugar (Shahwan *et al.*, 2022).

Table 2: Phytochemical screening (Secondary metabolites) using different extracts of *C. grandis* leaves

Secondary metabolites	Aqueous	Methanol	Ethanol
Tannin	+ve	+ve	+ve
Saponin	+ve	+ve	+ve
Flavonoids	-ve	-ve	-ve
Terpenoids	-ve	-ve	-ve
Phenolic compounds	+ve	+ve	+ve
Reducing sugar	+ve	+ve	+ve
Steroids	+ve	+ve	+ve
Alkaloids	-ve	-ve	-ve
Carbonyl compounds	-ve	-ve	-ve

(+ve: Positive; -ve:negative)



Figure 10: Screening of secondary metabolites using aqueous extract of *C. grandis* leaves

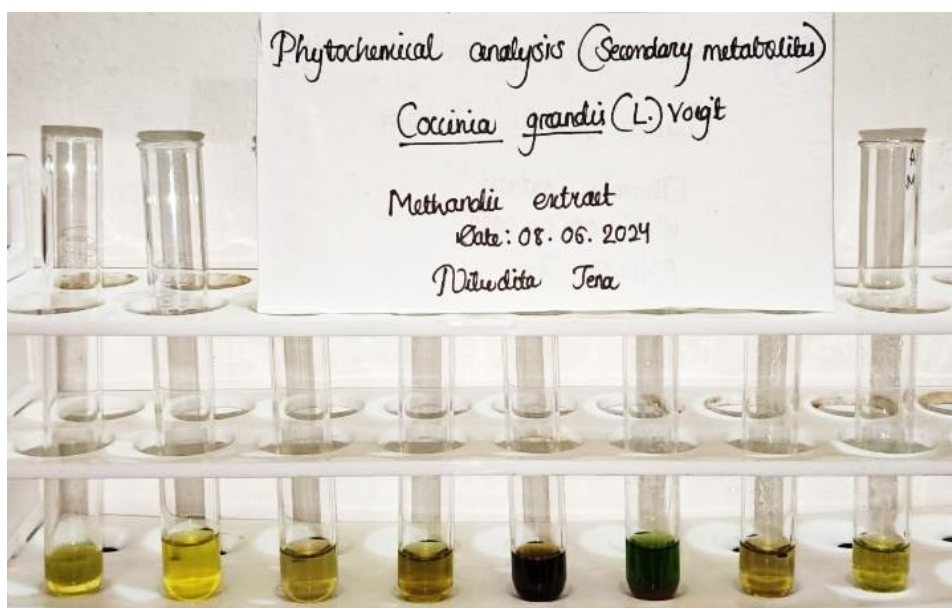


Figure 11: Screening of secondary metabolites using methanolic extract of *C. grandis* leaves



Figure 12: Screening of secondary metabolites using ethanolic extract of *C. grandis* leaves

A lot of wild leafy vegetables are documented from India (Misra *et al.*, 2008; Gupta and Prakash, 2009; Gupta *et al.*, 2013; Konsam *et al.*, 2016; Devidas *et al.*, 2023), but now it is time to do value addition.

Conclusion

Empirical and field research revealed that *C. grandis* grows in a range of climates and are utilized as medicinal foods. Therefore, it might be a nutraceutical in urban areas and places have low crops and high scarcity of foods. These food plants are common in rural and tribal areas, which need to be appropriately addressed going forward to combat climate change.

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