

Comparative Qualitative Phytochemical Profiling of Common Medicinal Plant Leaves Found in Kerala

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Abstract

The present study provides a comparative qualitative phytochemical screening of aqueous leaf extracts of four medicinal plants commonly available in Kerala, India: guava (*Psidium guajava*), karinochi (*Vitex negundo*), bottlebrush (*Callistemon* sp.), and vathakodi (*Justicia gendarussa*). Leaf extracts were prepared under two extraction conditions using 5 g of leaf material in 50 mL of deionised water: reflux extraction at 100 °C for 1 h and mild heating at 80 °C for 30 min. The resulting extracts were subjected to qualitative colour and precipitation tests to detect alkaloids, flavonoids, glycosides, saponins, steroids, tannins, phenols, terpenoids, carbohydrates, proteins, coumarins, quinones, anthraquinones, phlobatannins, and unsaturated compounds. All four plant extracts exhibited a diverse phytochemical profile, with flavonoids, glycosides, saponins, steroids, and unsaturated compounds consistently detected under both extraction conditions. Anthraquinones were not detected in any of the investigated extracts. Differences were observed in the phytochemical profiles depending on plant species and extraction conditions. Alkaloids were detected following extraction at 80 °C but were not detected after prolonged reflux at 100 °C, suggesting possible thermal degradation or alteration of alkaloid constituents. Karinochi exhibited a distinctive profile, showing positive reactions for quinones and phlobatannins under both extraction conditions. Vathakodi consistently showed negative results for terpenoids, carbohydrates, proteins, and coumarins. Overall, the findings demonstrate that both plant source and extraction temperature and duration can influence the qualitative phytochemical composition of aqueous leaf extracts. The study provides a comparative baseline for selecting appropriate extraction conditions for subsequent phytochemical characterization and bioactivity-guided investigations of these medicinal.

Keywords: Medicinal plants; Phytochemical screening; Aqueous extracts; Extraction temperature; Qualitative analysis.

1. Introduction

Medicinal plants are important sources of naturally occurring bioactive compounds and continue to attract scientific interest because of their chemical diversity and traditional therapeutic applications. They produce diverse secondary metabolites, including alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids, steroids and glycosides, many of which are associated with biological activities [1–4]. Phytochemical evaluation therefore provides an important preliminary basis for investigating medicinal plants and their traditional

uses [5&6]. Qualitative phytochemical screening is a simple, rapid and relatively inexpensive approach for obtaining an initial profile of major phytochemical classes. Conventional colour and precipitation reactions provide preliminary evidence for alkaloids, flavonoids, phenols, tannins, saponins, steroids, terpenoids, glycosides and other groups [5–8]. Although such tests do not establish the identity or concentration of individual compounds, they are useful before confirmatory techniques such as thin-layer chromatography, high-performance liquid

chromatography, gas chromatography–mass spectrometry and liquid chromatography–mass spectrometry [6&8]. Classical qualitative methods therefore remain useful preliminary tools when complemented by instrumental analysis [7]. *Psidium guajava* L. (guava; Myrtaceae) is a widely distributed medicinal and food plant in tropical and subtropical regions, including India. Its leaves have traditionally been used, particularly for gastrointestinal and inflammatory conditions [9,10]. Guava leaves contain diverse secondary metabolites, especially flavonoids and phenolic compounds. Other investigations have reported flavonoids, triterpenoids, lignans and additional phenolic constituents, supporting studies of guava leaves for antioxidant, antimicrobial, antidiabetic and antidiarrhoeal activities [9–12]. *Vitex negundo* L., commonly known as karinochi or nirgundi, is an important medicinal plant used traditionally in India. Its leaves and other parts have been associated with preparations for inflammatory and painful conditions [13&14]. Phytochemical studies have revealed flavonoids, lignans, iridoids, diterpenoids, triterpenoids, steroids and phenolic compounds [13–16]. The genus *Callistemon* (bottlebrush; Myrtaceae) includes ornamental and medicinal species with diverse phytochemical constituents, including flavonoids, terpenoids, triterpenoids, phloroglucinol derivatives, steroids, saponins and essential-oil components [17–20]. Studies of *Callistemon viminalis* leaves have identified acylphloroglucinols, flavonoids and other secondary metabolites [18&19], while preliminary screening of *Callistemon citrinus* has demonstrated several phytochemical classes, including alkaloids, steroids, triterpenoids and quinones [20]. *Justicia gendarussa* Burm. f., known as vathamkolli or gandarusa, belongs to the Acanthaceae and is distributed in India and other parts of South and Southeast Asia. Investigations of its leaves have reported alkaloids, flavonoids, phenolic compounds, saponins, steroids and other constituents [21&22]. UPLC-QTOF-MS profiling has shown diverse metabolites in its leaves and differences among plant organs [23]. These findings demonstrate the chemical complexity of this medicinal plant. The phytochemical profile of plant

material depends not only on species and plant part but also on extraction conditions. Solvent, temperature, extraction time, solid-to-liquid ratio and other parameters influence the release and recovery of phytochemicals from plant tissues [24&25]. Temperature and extraction duration are particularly important in aqueous extraction. Higher temperatures can enhance mass transfer and release some constituents, but prolonged exposure may affect the stability or detectability of heat-sensitive compounds [24–26]. The present study, comparatively evaluated aqueous leaf extracts of *P. guajava*, *V. negundo*, *Callistemon* sp. and *J. gendarussa* commonly found in Kerala, India. The study aimed to compare the qualitative phytochemical profiles of the four plants and assess the influence of extraction temperature on the qualitative recovery of the investigated phytochemical groups.

2. Method

2.1. Plant Material and Extraction

Fresh, healthy and mature leaves of guava (*Psidium guajava* L.), karinochi (*Vitex negundo* L.), bottlebrush (*Callistemon* sp.) and vathamkolli (*Justicia gendarussa* Burm. f.) were collected from Kerala, India [1]. The plant materials were cleaned thoroughly with distilled water to remove adhering dust and other extraneous matter. The leaves were dried under shadow and subsequently used for the preparation of aqueous extracts. The selected plant leaves were shown in figure 1.

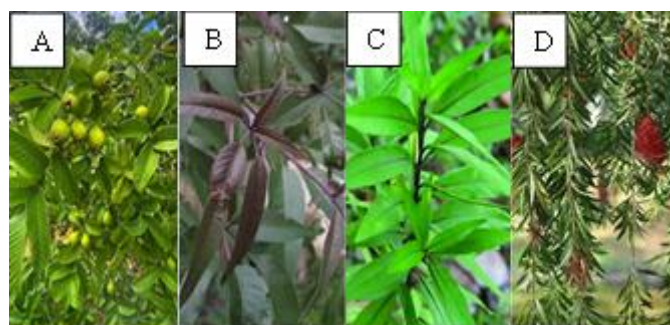


Figure 1 The medicinal plant leaves: A) *Psidium guajava* L., B) *Vitex negundo* L., C) *Justicia gendarussa* Burm. f., and D) *Callistemon* sp

The aqueous extracts were prepared under two different temperature conditions using deionised water as the extraction solvent. In both cases, a fixed

plant material to solvent ratio of 1:10 (w/v) was maintained by extracting 5 g of fresh leaf material with 50 mL of deionised water[2].

- Extract A: The leaf material was subjected to reflux extraction at 100 °C for 30 min.
- Extract B: The leaf material was extracted at 80 °C for 30 min.

Both the extracts were allowed to cool to room temperature and were filtered through Whatman No. 1 filter paper to remove suspended plant material. The resulting aqueous filtrates were used directly for qualitative phytochemical screening[3].

2.2. Qualitative Phytochemical Screening

The aqueous extracts obtained under both extraction conditions were subjected to standard qualitative phytochemical tests for the preliminary detection of fifteen major classes of phytoconstituents. The tests included alkaloids, flavonoids, glycosides, saponins, steroids, tannins, phenols, terpenoids, carbohydrates, proteins, coumarins, quinones, anthraquinones, phlobatannins and unsaturated compounds. The observations for all the tests were recorded based on the characteristic colour change, and precipitate formation.

2.2.1. Test for Alkaloids–Wagner's Test

A few drops of Wagner's reagent were added to a measured volume of the aqueous extract. The presence of alkaloids were confirmed by the development of a reddish-brown precipitate.

2.2.2. Test for Flavonoids–Alkaline Reagent Test

A few drops of dilute sodium hydroxide solution were added to the extract, followed by dilute hydrochloric acid. Development of an intense yellow colour that disappeared or diminished upon acidification was confirmed the presence of flavonoids.

2.2.3. Test for Glycosides–Keller-Killiani Test

The extract was treated with glacial acetic acid containing a trace amount of ferric chloride, followed by careful addition of concentrated sulfuric acid along the wall of the test tube. The formation of a reddish-brown ring at the interface confirmed presence of cardiac-type glycosides.

2.2.4. Test for Saponins–Froth Test

Formation of persistent foam was confirmed the presence of saponins when the extract was diluted with distilled water and vigorously shaken for several minutes[4].

2.2.5. Test for Steroids–Salkowski Test

The extract was treated with chloroform, followed by careful addition of concentrated sulfuric acid along the side of the test tube. Development of a characteristic reddish-brown colour at the interface was confirmed the presence of steroidal constituents.

2.2.6. Test for Tannins–Ferric Chloride Test

Tannins were confirmed when a blue-black, greenish-black or related dark colouration developed when a few drops of 5% ferric chloride solution were added to the aqueous extract[5].

2.2.7. Test for Phenols – Ferric Chloride Test

Development of blue, green, violet or other characteristic colouration indicated the presence of phenolic compounds when a few drops of freshly prepared neutral 5% ferric chloride solution were added to the extract.

2.2.8. Test for Terpenoids–Salkowski Test

The aqueous extract was mixed with chloroform and concentrated sulfuric acid was carefully added to form a separate acid layer. The development of a reddish-brown colour at the interface was confirmed terpenoid constituents.

2.2.9. Test for Carbohydrates–Molisch's Test

Two drops of alcoholic α -naphthol solution were added to the extract, followed by careful addition of concentrated sulfuric acid along the side of the test tube. The formation of a violet or purple ring at the interface was confirmed the presence of carbohydrates[6].

2.2.10. Test for Proteins–Biuret Test

A few drops of dilute copper sulfate solution were added to the extract, followed by addition of dilute sodium hydroxide solution. Development of a violet or purple colour was indicated the presence of proteins or peptide-containing compounds.

2.2.11. Test for Coumarins

The extract was treated with 10% sodium hydroxide solution and examined for a characteristic colour change. Development of yellow colouration was confirmed the presence of coumarin-type compounds [7].

2.2.12. Test for Quinones

A measured quantity of the extract was treated with concentrated hydrochloric acid. Development of a characteristic colour change was recorded as a positive indication for quinone-type compounds [8].

2.2.13. Test for Anthraquinones–Bornträger's Test

The extract was boiled with dilute hydrochloric acid and subsequently filtered. The filtrate was extracted with an organic solvent, and the organic layer was treated with dilute ammonia solution. Development of pink, red or violet colouration in the alkaline layer was considered indicative of anthraquinone-type constituents. This test was negative for all the four leaf extracts at 80 °C and 100 °C [9].

2.2.14. Test for Phlobatannins

The aqueous extract was boiled with 1% hydrochloric acid. Formation of a red-coloured precipitate was confirmed the presence of phlobatannins [10].

2.2.15. Test for Unsaturated Compounds – Baeyer's Test

A few drops of dilute potassium permanganate solution were added to the extract. Decolourisation of the purple colour of potassium permanganate, with possible formation of brown manganese dioxide, was indicated the presence of oxidizable unsaturated compounds[11 – 20].

2.3. Recording and Interpretation of Results

Each qualitative test was performed under identical conditions for all four plant extracts. The observations were recorded based on the presence or absence of the characteristic reaction. The results were expressed qualitatively as positive (+) or negative (–).

3. Results and Discussion

3.1. Results

The results of the qualitative phytochemical screening of the aqueous leaf extracts of guava, karinochi, vathamkolli, and bottlebrush at 80 °C and 100 °C were presented in Tables 1 and 2, respectively. Representative photographic observations of the qualitative tests are shown in Figures 2–5.

3.1.1. Comparative qualitative phytochemical screening

At 80 °C for 30 min, flavonoids, glycosides, saponins, steroids, tannins, phenols and unsaturated compounds were detected in all four plant extracts. Alkaloids were detected in guava, karinochi and vathakodi, but not in bottlebrush. Terpenoids, carbohydrates, proteins and coumarins were detected in guava, karinochi and bottlebrush, whereas these four groups were not detected in vathakodi. Quinones and phlobatannins were detected only in karinochi. Anthraquinones were not detected in any of the four extracts. As shown in Table 1 Phytochemical screening at 80 °C/30 min.

Table 1 Phytochemical screening at 80 °C/30 min

Phytochemical Test	Guava	Nirgundi	Bottlebrush	Vathamkolli
Alkaloids	–	–	–	–
Flavonoids	+	+	+	+
Glycosides	+	+	+	+
Saponins	+	+	+	+
Steroids	+	+	+	+
Tannins	–	+	+	+
Phenols	–	+	+	+
Terpenoids	+	+	+	–
Carbohydrates	+	+	+	–
Proteins	+	+	+	–
Coumarins	+	+	+	–
Quinones	–	+	–	–
Anthraquinone s	–	–	–	–
Phlobatannins	–	–	–	–
Unsaturation	+	+	+	+

3.2. Phytochemical profile after extraction at 100 °C

Following extraction at 100 °C for 30 min, flavonoids, glycosides, saponins, steroids and unsaturated compounds were detected in all four plants. Alkaloids were not detected in any of the four extracts. Tannins and phenols were detected in karinochi, bottlebrush and vathakodi, but were not detected in guava. Terpenoids, carbohydrates, proteins and coumarins were detected in guava, karinochi and bottlebrush but not in vathakodi. Quinones were detected only in karinochi. Phlobatannins were not detected in any extract, and anthraquinones remained undetected in all four plants. As shown in Table 2. Phytochemical screening at 100 °C/30 min.

3.3. Photographic observations

The photographic observations provide visual support for several of the recorded qualitative reactions. In the guava extract, Wagner's test, the foam test, ferric[27 – 31] chloride test for tannins and Shinoda test for flavonoids produced the characteristic positive reactions, whereas the Bornträger's test showed a negative reaction for anthraquinones (Figure 2). Nirgundi exhibited a distinctive phytochemical profile. In addition to the common positive reactions, phlobatannins. The photographic record includes positive Wagner's, hydrochloric-acid quinone, foam, ferric chloride tannin and ferric chloride phenol reactions (Figure 3).

Table 2 Phytochemical screening at 100 °C/30 min

Phytochemical Test	Guava	Nirgundi	Bottle brush	Vathamkoll i
Alkaloids	+	+	-	+
Flavonoids	+	+	+	+
Glycosides	+	+	+	+
Saponins	+	+	+	+
Steroids	+	+	+	+
Tannins	+	+	+	+
Phenols	+	+	+	+
Terpenoids	+	+	+	-
Carbohydrates	+	+	+	-
Proteins	+	+	+	-
Coumarins	+	+	+	-
Quinones	-	+	-	-
Anthraquinone s	-	-	-	-
Phlobatannins	-	+	-	-
Unsaturation	+	+	+	+



Figure 2 Representative qualitative phytochemical test photographs of guava leaf extract

The bottlebrush extract showed positive reactions for flavonoids, glycosides, saponins, steroids, tannins, phenols, terpenoids, carbohydrates, proteins, coumarins and unsaturated compounds, while alkaloids, quinones, anthraquinones and phlobatannins were not detected. The photographic record includes a negative Wagner's test together with positive Shinoda, Keller–Killiani, Liebermann–Burchard and ferric chloride reactions (Figure 4). Vathamkolli showed positive reactions for alkaloids, flavonoids, glycosides, saponins, steroids, tannins, phenols and unsaturated compounds at 80 °C. In contrast, terpenoids, carbohydrates, proteins, coumarins, quinones, anthraquinones and phlobatannins were not detected. The photographs document positive reactions for alkaloids, saponins, phenols and flavonoids and a negative Bornträger's test for anthraquinones (Figure 5). As shown in Figure 3 Representative qualitative phytochemical test photographs of Nirgundi leaf extract. Figure 5 Representative qualitative phytochemical test photographs of vathamkolli leaf extract Figure 4 Representative qualitative phytochemical test photographs of bottlebrush leaf extract.

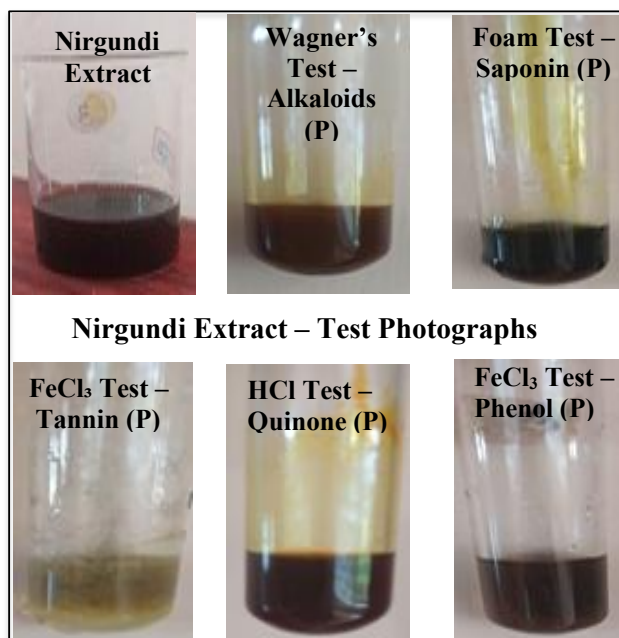


Figure 3 Representative qualitative phytochemical test photographs of Nirgundi leaf extract

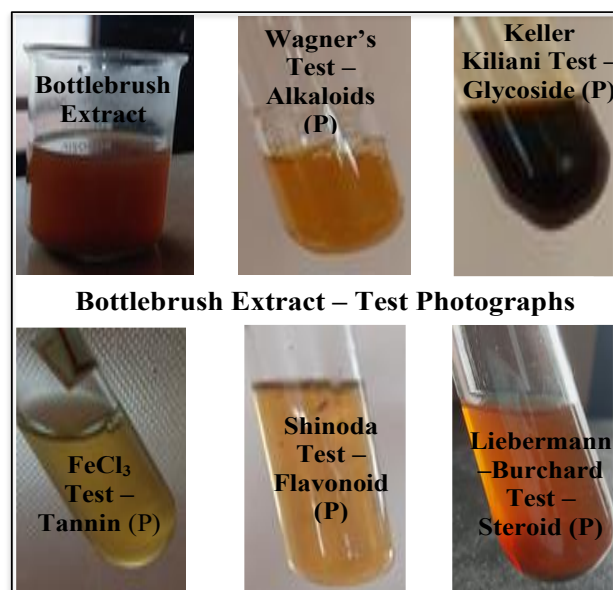


Figure 4 Representative qualitative phytochemical test photographs of bottlebrush leaf extract

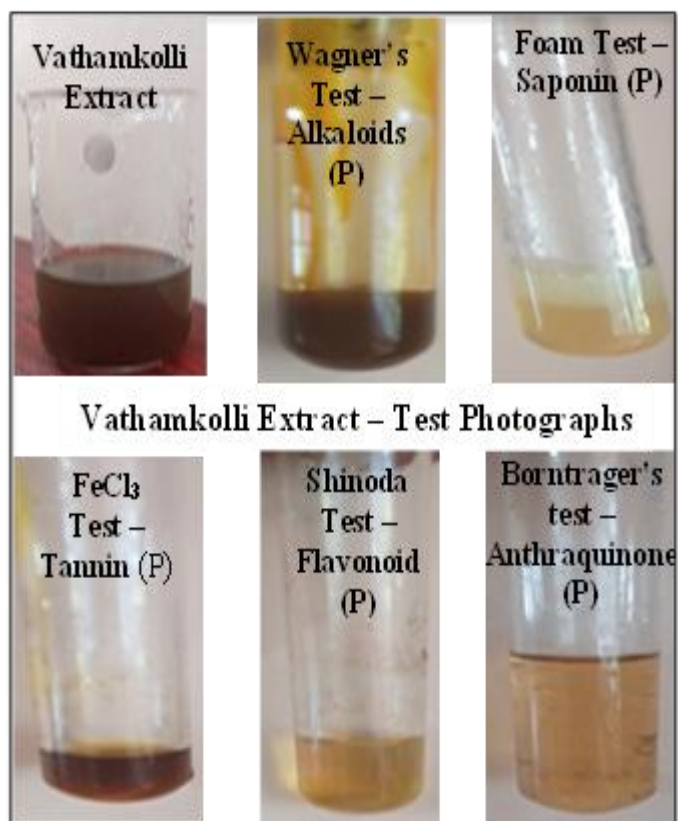


Figure 5 Representative qualitative phytochemical test photographs of vathamkolli leaf extract

Comparison between the two extraction conditions showed that flavonoids, glycosides, saponins, steroids and unsaturated compounds were consistently detected in all four plants. Alkaloids were detected in guava, karinochi and vathakodi at 80 °C but were not detected at 100 °C. Tannins and phenols were detected in all four plants at 80 °C, whereas both were not detected in guava at 100 °C. Terpenoids, carbohydrates, proteins and coumarins showed the same plant-specific pattern under both conditions, with detection in guava, karinochi and bottlebrush and non-detection in vathakodi. Quinones were detected only in karinochi under both conditions. Phlobatannins were detected in karinochi at 80 °C but not at 100 °C. Anthraquinones were not detected under either condition.

3.4. Discussion

3.4.1. Overall phytochemical profile

The qualitative screening demonstrated both common and plant-specific phytochemical patterns among the four aqueous leaf extracts. Flavonoids, glycosides, saponins, steroids and unsaturated compounds were detected in all four plants under both extraction conditions. Such variation in qualitative phytochemical profiles is consistent with the influence of plant species, matrix characteristics, solvent and extraction conditions on the recovery and detectability of secondary metabolites [27,28].

3.4.2. Effect of extraction temperature on alkaloid detection

A clear difference was observed for alkaloids. They were detected in guava, karinochi and vathamkolli after extraction at 80 °C for 30 min, whereas alkaloids were not detected in any of the four plants after extraction at 100 °C for 30 min.

3.4.3. Tannins and phenolic compounds

Tannins and phenols were detected in all four plants at 80 °C. At 100 °C, both groups were not detected in guava, while they remained detectable in karinochi, bottlebrush and vathakodi. Extraction temperature and other extraction parameters can influence the recovery and apparent stability of phenolic constituents, and the effect can vary with the plant matrix and extraction procedure [28,30,31]. Therefore, the present result suggests a temperature-

associated difference in the qualitative recovery of phenolic and tannin-type constituents from guava.

3.4.4. Plant-specific pattern in vathamkolli

Terpenoids, carbohydrates, proteins and coumarins were detected in guava, karinochi and bottlebrush under both extraction conditions but were not detected in vathakodi. The consistency of this pattern suggests that the result is associated with the plant material and/or the ability of the aqueous extraction procedure to recover detectable levels of these phytochemical classes.

3.4.5. Distinctive profile of karinochi

Karinochi showed a distinctive pattern within the present comparison. Quinones were detected only in karinochi under both extraction conditions, while phlobatannins were detected in karinochi at 80 °C but not at 100 °C. The phytochemical diversity reported for *Vitex* species provides a broader context for the occurrence of multiple classes of secondary metabolites in *V. negundo* [29].

3.4.6. Significance of extraction temperature

The observed changes in alkaloid, tannin, phenol and phlobatannin detection indicate that extraction temperature can influence the qualitative phytochemical profile obtained from aqueous leaf extracts. Previous studies have similarly emphasized the importance of extraction temperature and related process parameters in determining the recovery of plant phenolics and other bioactive constituents [28,30,31].

Conclusion

The present study provided a comparative qualitative phytochemical evaluation of aqueous leaf extracts of four medicinal plants commonly available in Kerala, namely *Psidium guajava*, *Vitex negundo*, *Callistemon* sp. and *Justicia gendarussa*, using two extraction temperatures, 80 °C and 100 °C, for 30 min. The screening revealed both common and plant-specific phytochemical patterns among the investigated plants. Flavonoids, glycosides, saponins, steroids and unsaturated compounds were consistently detected in all four plants under both extraction conditions, indicating a common qualitative phytochemical profile within the conditions examined. Distinct differences were observed for several other phytochemical classes.

Alkaloids were detected in guava, karinochi and vathamkolli at 80 °C but were not detected at 100 °C. Tannins and phenols were detected in all four plants at 80 °C, whereas they were not detected in guava following extraction at 100 °C. Terpenoids, carbohydrates, proteins and coumarins were consistently detected in guava, karinochi and bottlebrush but not in vathamkolli. Karinochi showed a distinctive profile, particularly because quinones were detected under both extraction conditions and phlobatannins were detected at 80 °C. Overall, the findings demonstrate clear qualitative differences among the four medicinal plants and indicate that extraction temperature can influence the phytochemical profile obtained from aqueous leaf extracts. The study provides useful preliminary information for comparative phytochemical screening of these commonly available medicinal plants and establishes a basis for further investigations using quantitative assays and chromatographic and spectroscopic techniques to identify and characterize the compounds responsible for the observed reactions.

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