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Alan Sheeja DB

Department of Chemistry,
Government Arts College,
Thiruvananthapuram, Kerala,
India

Comparison of phytochemical as well as *in vitro* antioxidant, antimicrobial and anti-inflammatory activity studies of the leaves and fruits of *Piper chaba*

Alan Sheeja DB

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Abstract

The chemistry of *Piper* species had been extensively studied, which led to the isolation of various biologically active compounds. *Piper chaba* fruits exhibit properties such as being pungent, aromatic, stimulant, anthelmintic, expectorant, and carminative. They are known to enhance appetite and taste while aiding in conditions such as asthma, bronchitis, fever, inflammation, haemorrhoids, and abdominal pain. This study focuses on examining and comparing the phytochemical, biological, and antioxidant properties of the leaves and fruits of *Piper chaba*, with the findings presented in detail.

Keywords: *Piper chaba*, piperaceae, antioxidant, antimicrobial, anti-inflammatory

Introduction

The rich tapestry of species diversity encompassing plants, animals, microorganisms, and marine organisms gives rise to an astonishing array of secondary metabolites, each characterised by unique and varied chemical structures ^[1]. These complex compounds have historically been crucial in the realms of drug discovery and development, and they are poised to maintain their significance in shaping the future of pharmaceutical advancements. The intricate relationships within ecosystems contribute to the continuous exploration and identification of these vital substances, underscoring their critical role in the quest for new therapeutic agents ^[2].

The diverse species within the genus *Piper* (Piperaceae family) have a long-standing history of traditional medicinal use, recognised for their various health benefits to humans ^[3]. Extensive research into the chemistry of these *Piper* species has been conducted worldwide, resulting in the identification and isolation of numerous bioactive compounds ^[3]. Notably, *Piper chaba* has gained significant attention and is increasingly incorporated into various pharmaceutical formulations.

In traditional Indian medicine, the fruits of *Piper chaba* are commonly utilised as a substitute for *Piper longum* ^[4]. The fruits of *P. chaba* are celebrated for their pungent flavour and aromatic properties, serving multiple roles in health management ^[5]. Additionally, they are considered beneficial in the treatment of various ailments, including asthma, bronchitis, fever, inflammation, haemorrhoids, abdominal pain. Given these versatile uses, a comprehensive study was conducted on the phytochemical, biological, as well as antioxidant properties of both the leaf extract and fruit extract of *Piper chaba* to compare their respective attributes and understand their potential contributions to health and wellness.

Materials and Methods

Plant collection and extraction

Fresh plants of *Piper chaba* were collected from Pappanamcode, Thiruvananthapuram, identified by a botanist. The leaves and fruits were chopped, dried and then powdered. The powdered material was subjected to hydrodistillation using Clevenger apparatus to yield the respective essential oils required for the antimicrobial analysis. The powdered material was effectively extracted with methanol resulting in crude extracts. Following the removal of the solvent, these extracts were carefully diluted with methanol to prepare a range of concentrations. This preparation will facilitate comprehensive studies on phytochemical, antioxidant and anti-inflammatory activities.

Corresponding Author:

Alan Sheeja DB

Department of Chemistry,
Government Arts College,
Thiruvananthapuram, Kerala,
India

Results and Discussion

Preliminary phytochemical analysis

The findings from the initial phytochemical analysis, characterised by various colour reactions referenced in

existing literature ^[6-10], are summarised in the table 1 below. This table provides a comprehensive overview of the results obtained during the analysis.

Table 1: Results of the preliminary phytochemical analysis

Sl. No.	Test for various phytochemicals	<i>P. chaba</i> leaf	<i>P. chaba</i> fruit
1	Cardiac glycosides	x	x
2	Carbohydrates	x	x
3	Flavonoids	✓	✓
4	Alkaloids	✓	✓
5	Terpenoids	✓	✓
6	Quinones	x	x
7	Tannins	✓	✓
8	Resins	✓	x
9	Phlobatanins	x	✓
10	Anthraquinones	x	x
11	Phytosterols	✓	✓
12	Steroids	✓	✓
13	Coumarins	✓	✓
14	Aminoacids, proteins	✓	x
15	Betacyanin	✓	✓

The phytochemical screening revealed that a rich diversity of compounds was present in both the extracts. These phytochemicals, identified during the analysis, likely contribute to the notable bioactivity exhibited by the plant. This bioactivity has led to its application in various medicinal preparations for many years, underscoring the significance of *Piper chaba* in both traditional and contemporary herbal medicine.

Antioxidant activity studies

DPPH[•] scavenging assay

Various concentrations of the extracts were prepared in methanol and subsequently mixed with DPPH[•], a stable free radical commonly used in antioxidant assays. The absorbance of the resulting solutions was measured following established

protocols ^[11], allowing for a thorough evaluation of the antioxidant potential of *Piper chaba*.

This approach is commonly used to assess the effectiveness of antioxidants in neutralising free radicals. In this reaction, the antioxidants engage with the DPPH[•] radical, which clearly indicates their ability to counteract free radicals, thereby underscoring their potential health advantages. The findings are illustrated in figure 1 and detailed in table 2. The EC₅₀ of the leaf extract was determined to be 7560.08±5.12 ppm, indicating its potency, while the EC₅₀ for the fruit extract was recorded as 19015.11±11.56 ppm. The data clearly demonstrates the significant differences in activity between the two extracts, with the leaf extract proving to be the superior scavenger. This finding offers crucial insights into their potential applications.

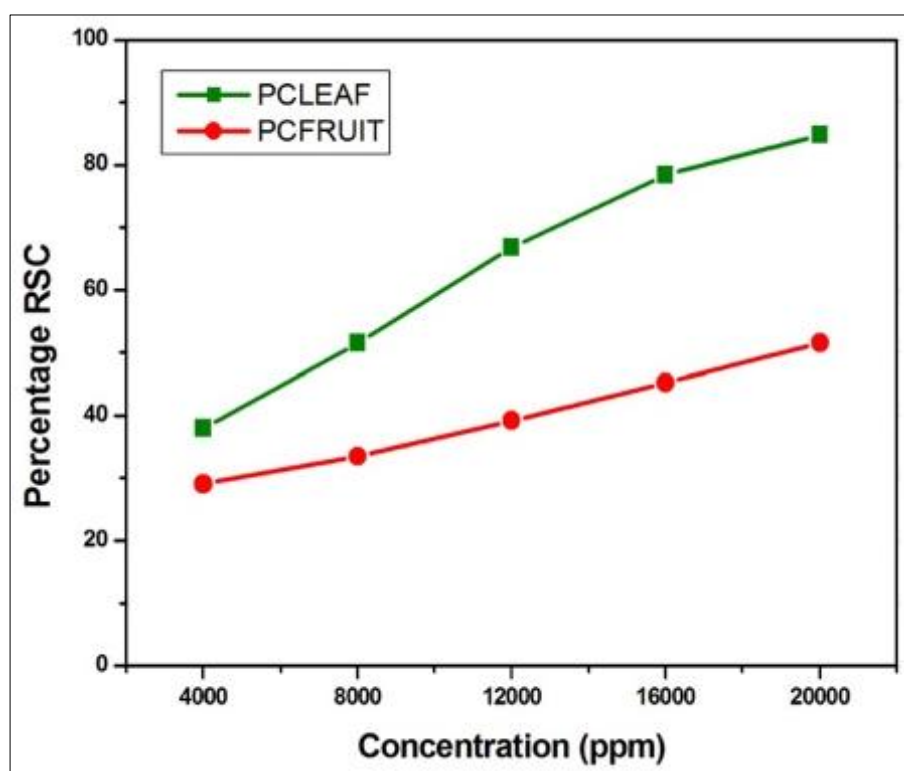


Fig 1: Percentage scavenging of DPPH[•] radical at different concentrations

Total phenolic content: The estimation was conducted by reacting the respective extracts with the Folin-Ciocalteu reagent, following the established protocol documented in the literature [12]. The findings revealed that the total phenolic content in the *Piper chaba* leaf extract was measured to be 1.60 ± 0.02 g of Gallic Acid Equivalents (GAE) per 100 g of

extract. In contrast, the fruit extract demonstrated a higher phenolic content, recorded at 2.34 ± 0.08 g GAE per 100 g of extract. These results which are shown in figure 2 and table 2 highlight the varying levels of phenolic compounds present in various parts of the *Piper chaba*, indicating its potential for further research and applications in health and nutrition.

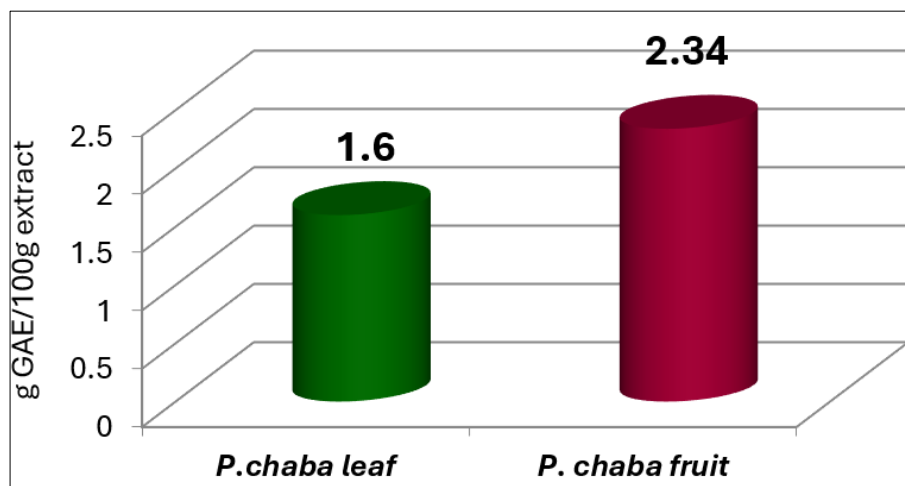


Fig 2: Total phenolic content

Total flavonoid content

The experiment quantified the total flavonoid content of both extracts, following established protocols found in the literature [13]. The results revealed that the *Piper chaba* leaf extract exhibited flavonoid content of 2.51 ± 0.04 g of

Quercetin Equivalents (QE) per 100 g of extract. In contrast, the fruit extract demonstrated a significantly higher flavonoid concentration, measuring 8.90 ± 1.01 g QE per 100 g of extract. The results are depicted in figure 3 and table 2.

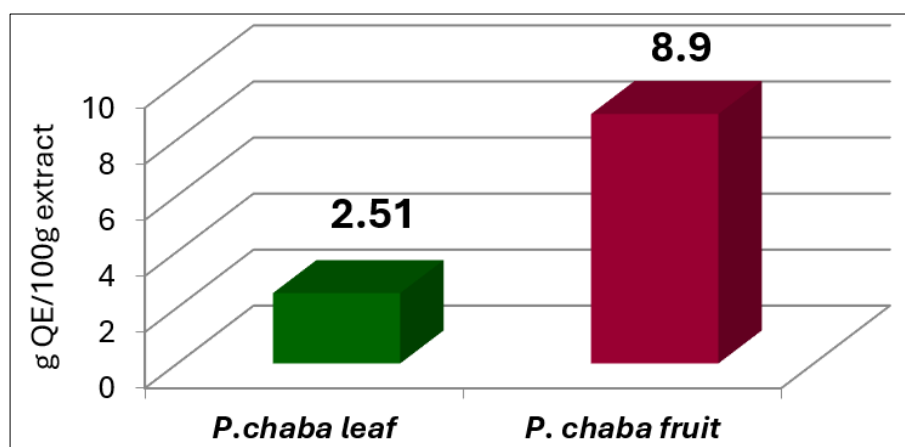


Fig 3: Total flavonoid content

Table 2: Results of antioxidant and anti inflammatory activity studies

<i>Piper chaba</i>	Scavenging of DPPH [•] radical, EC ₅₀ (ppm)	Total phenolic content [g GAE / 100 g extract]	Total flavonoid content [g QE/ 100 g extract]	Nitric oxide scavenging ability, LD ₅₀ (μL)	Superoxide scavenging ability, LD ₅₀ (μL)
Leaf extract	7560.08±5.12	1.60±0.02	2.51±0.04	1024.14±2.58	2943.43±4.52
Fruit extract	19015.11±11.56	2.34±0.08	8.90±1.01	841.65±2.14	2505.90±3.27

Reducing power

To assess the reducing power of both extracts from *Piper chaba*, a range of concentrations were prepared following established protocols for the reaction [14]. The findings from this experiment are depicted in Figure 4, which clearly shows

that the leaf extract demonstrates a significantly greater reducing power in comparison to the fruit extract. This suggests that the leaves may contain more potent antioxidants or active compounds responsible for this enhanced reducing capability.

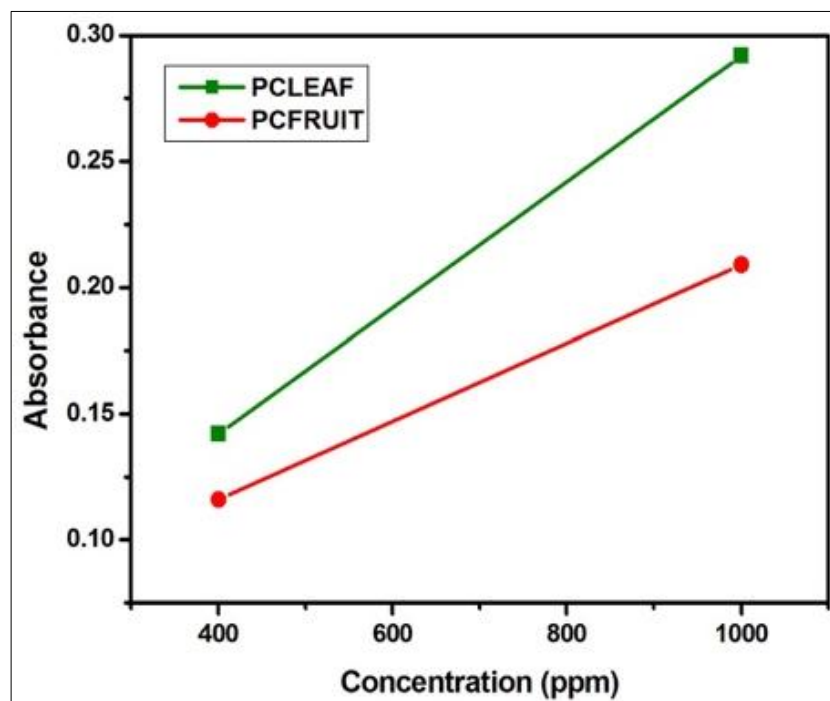


Fig 4: Results of reducing power at concentrations 400 and 1000 ppm

Nitric oxide scavenging activity

The percentage inhibition of nitric oxide scavenging activity was determined through experiments outlined in existing literature ^[15], utilizing various concentrations of both the leaf extract and fruit extract of *Piper chaba* for the analysis. The

results are illustrated in figure 5 and table 2. Notably, the LD₅₀ of the leaf extract was calculated to be 1024.14±2.58 µL, while the LD₅₀ for the fruit extract was found to be 841.65±2.14 µL. These findings highlight the differing degrees of potency between the two extracts.

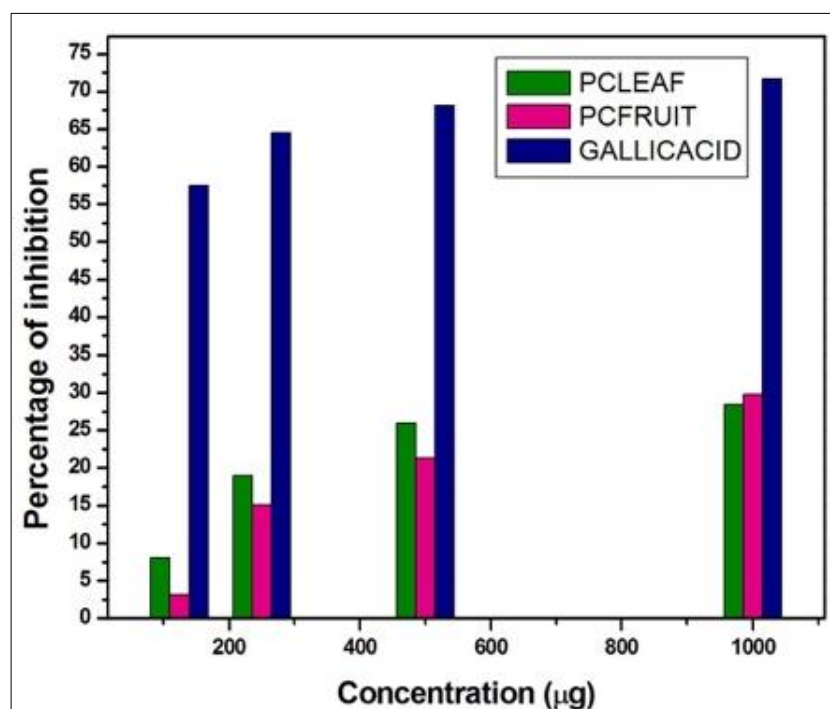


Fig 5: Results of nitric oxide scavenging experiment

Superoxide free radical scavenging activity

The investigation into the superoxide scavenging activity was carried out following established protocols ^[16], yielding significant results. The analysis revealed that the leaf extract demonstrated a Lethal Dose (LD₅₀) of 2943.43±4.52 µL, indicating its potential efficacy as a free radical scavenger. In

contrast, the fruit extract exhibited a slightly lower LD₅₀ of 2505.90±3.27 µL, suggesting it has better free radical scavenging capabilities than the leaf extract. Detailed results are presented visually in Figure 6 and compiled in Table 2, providing a comprehensive overview of the extracts' activities.

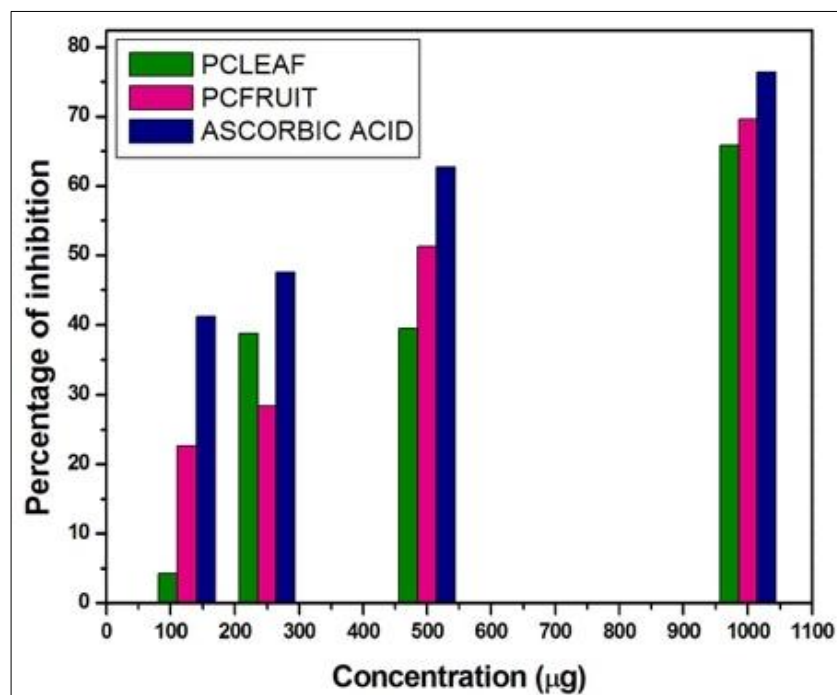


Fig 6: Results of superoxide free radical scavenging experiment

Antimicrobial activity studies

A total of 23 compounds had been reported in literature, comprising 92.5% of the essential oil from *Piper chaba* fruits. The principal constituents of *Piper chaba* fruit oil included germacrene D, beta-caryophyllene, and alpha-humulene [4]. In the present study, the essential oils extracted from *Piper chaba* leaves and fruits were evaluated for their *in vitro* antimicrobial properties against a select group of bacterial and fungal species using the agar well diffusion method [17]. The species tested included:

- Staphylococcus aureus*,
- Streptococcus mutans*,
- Escherichia coli*,
- Pseudomonas aeruginosa*,
- Candida albicans*, and
- Aspergillus niger*.

Among these, the first two species are classified as gram-positive bacteria, while the third and fourth are gram-negative bacteria. The last two species represent fungal organisms. The results of the antimicrobial studies are shown in table 3.

Table 3: Results of antimicrobial assays

Organisms	Essential oil of leaves	Essential oil of fruits
<i>Staphylococcus aureus</i>	+	+
<i>Streptococcus mutans</i>	+	+
<i>Escherichia coli</i>	-	+
<i>Pseudomonas aeruginosa</i>	-	+
<i>Aspergillus niger</i>	-	+
<i>Candida albicans</i>	-	+

The experiment demonstrated that, among the six microorganisms examined, only two were effectively deactivated by *Piper chaba* leaf oil: *Staphylococcus aureus* and *Streptococcus mutans*. In contrast, the essential oil derived from the *Piper chaba* fruits exhibited antimicrobial activity against all six microorganisms tested. Consequently,

it can be concluded that the fruit oil possesses greater efficacy in eliminating microorganisms compared to the leaf oil.

Anti-inflammatory activity studies

In this study, RAW 264.7 cells were stimulated with LPS and treated with distinct concentrations of extracts as well as Diclofenac sodium (standard) and COX activity was definitively measured at 632 nm according to the established method [18]. The results are clearly presented in figure 7.

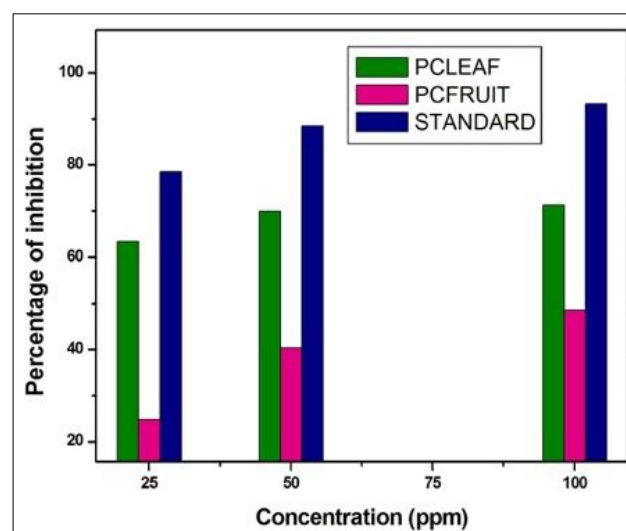


Fig 7: Results of anti-inflammatory activity studies

Conclusion

The current study provides compelling evidence that the fruit extract of *Piper chaba* possesses remarkable antioxidant properties, highlighting its elevated levels of phenolic and flavonoid compounds. These beneficial constituents contribute to its efficacy as a potent scavenger of superoxide and nitric oxide radicals, underscoring its potential role in combating oxidative stress.

In addition to the fruit extract, the leaf extract of *Piper chaba* was found to demonstrate substantial reducing power and

effective DPPH radical scavenging activity, which indicates its strength in neutralizing free radicals. Furthermore, the leaf extract exhibited superior anti-inflammatory effects, suggesting its potential therapeutic applications.

Among the six microorganisms assessed in this study, two were effectively deactivated by the oil derived from the leaves, showcasing its antimicrobial capabilities. On the other hand, the fruit oil demonstrated an impressive antimicrobial activity against all six tested microorganisms, reinforcing its value as a natural antimicrobial agent. Overall, this comprehensive investigation into the phytochemical, biological, and antioxidant properties of both the leaf extract and fruit extract of *Piper chaba* provides a thorough comparison and contributes valuable insights into the potential health benefits of this remarkable plant.

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