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Exploring phytochemical diversity and antioxidant potential in leaves extracts: A comparative study of *Psidium guajava* (Guava) and *Terminalia chebula* (Myrobalan)

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Abstract

The investigation delved into the biochemical analysis of leaf extracts from *Psidium guajava* (Guava) and *Terminalia chebula* (Myrobalan), revealing the presence of vital phytochemicals. Total phenolic contents (TPC) were quantified at 101.791 mg/ml for *Psidium guajava* and 133.795 mg/ml for *Terminalia chebula*, while total flavonoid contents (TFC) exhibited higher values in *Psidium guajava* (42.2869 mg/ml) compared to *Terminalia chebula* (36.4787 mg/ml). Remarkably, *Psidium guajava* leaf extract displayed robust antioxidant activity, displaying a remarkable 93.44% inhibition at 250µg/ml. *Terminalia chebula* extract, on the other hand, demonstrated maximum inhibition (92.55%) at a concentration of 350µl/ml. These findings underscore the distinctive biochemical profiles and potent antioxidant potential of the studied extracts, providing insights into their promising applications in the realm of natural health and wellness. Moreover, the phytochemicals present in *Psidium guajava* and *Terminalia chebula* leaves attributed to antimicrobial properties make them also suitable for treating textile materials, endowing them with antimicrobial activity.

Keywords: Leaves extract, antioxidant, phytochemical, antimicrobial property

Introduction

Phytochemical constituents, complex and bioactive compounds present in diverse plant species, play a crucial role in shaping their biological properties and potential health benefits. Emerging from plant metabolic processes, these compounds encompass a spectrum of functions, from shielding against environmental stressors to regulating interactions with other organisms. Although not essential nutrients, phytochemicals offer potential positive effects on human health through diets rich in fruits, vegetables, and plant-based sources, displaying diverse attributes like antioxidant, anti-inflammatory, and antimicrobial properties. *Psidium guajava* (Guava) and *Terminalia chebula* (Myrobalan) are tropical fruits renowned for their numerous health benefits. While the fruits themselves are widely consumed, the leaves of these plants have garnered significant attention due to their potential medicinal properties. In this article, we will delve into the diverse array of phytochemicals present in *P. guajava* and *T. chebula* leaves. From antioxidant and anti-inflammatory effects to antimicrobial and antidiabetic properties, the phytochemicals in leaves offer a holistic approach to health maintenance and disease prevention. Understanding the composition and potential applications of these phytochemicals can shed light on the remarkable therapeutic potential of *P. guajava* and *T. chebula* leaves, further underscoring the importance of integrating natural remedies into modern healthcare practices.

Psidium guajava, commonly referred to as Guava, belongs to the Myrtaceae family. This distinctive and well-established tropical plant is cultivated for both its nutritional and therapeutic advantages. Guava is a tropical fruit that has been extensively grown and utilized in regions such as South America, Bangladesh, Pakistan, India, and Indonesia. Many cultures have harnessed various parts of the guava tree, including its roots, leaves, bark, stem, and fruits, to address issues like stomachaches, diabetes, and diarrhea. The dark green, elliptical, and oval leaves of *P. guajava* are particularly valued for their applications in managing gastrointestinal and respiratory conditions, as well as for their role in boosting platelet counts in dengue fever patients ^[1].

These leaves also possess antispasmodic, cough sedative, anti-inflammatory, anti-diarrheic, anti-hypertensive, anti-obesity, and antidiabetic properties [2]. Moreover, they exhibit potent antitumor and anticancer characteristics [3, 4]. On the other hand, *Terminalia chebula*, commonly known as Black Myrobalan or Ink Tree, is a moderate-sized tree that holds a significant place in traditional medicines. Belonging to the Combretaceae family, this tree is native to regions such as India, Myanmar, Bangladesh, Iran, Egypt, Turkey, and China. In India, the *T. chebula* tree thrives in deciduous forests, particularly in North India and Southern regions [5]. The leaves of *T. chebula*, measuring 10 – 20 cm long, exhibit sub-opposite arrangement, simplicity, exstipulation, and petiolation. The laminae are broadly elliptic to elliptic-oblong, rarely ovate, with obtuse bases, entire margins, acute tips, and a glabrescent surface. *T. chebula* leaves boast high phenolic content, notably hydrolyzable tannins, anthraquinone, flavonol, carbohydrates, glucose, and sorbitol [6].

Experimental

Materials: Collected locally available *Psidium guajava* and *Terminalia chebula* leaves from Jorhat District of Assam and purchased ethanol and cotton woven fabric from Jorhat local market.

Preparation of leaves extract: To prepare extracts from *Psidium guajava* (Guava) and *Terminalia chebula* (Haritaki) leaves, leaves of each type were cleaned, shade dried and ground into a fine powder using an electric grinder. Subsequently, 20 grams of the powdered leaves were separately placed in glass containers, and 100 ml of ethanol was added to fully submerge the powder. After that the containers were sealed and stored in a controlled environment for 48 hours to facilitate the extraction process. Following this period, the ethanol-leaf mixture was filtered, and the resulting extracts from *Psidium guajava* and *Terminalia chebula* leaves were stored.

Biochemical Analysis of selected plant extracts

Qualitative analysis of Photochemical: Phytochemicals primarily encompass the array of plant-biosynthesized secondary metabolites. The valuable physiological and medicinal effects of plant materials commonly emanate from the intricate amalgamation of these derivative compounds found within the plant matrix [7]. To discern the diverse constituents within the plant extract, including alkaloids, flavonoids, phenols, saponins, tannins, and terpenoids, phytochemical tests were conducted using the methodologies elucidated by [8]. Additionally, the identification of resins and steroids was undertaken through standardized protocols as outlined by [9, 10, 11].

Identification of Alkaloids: The identification of various phytochemical constituents in plant extracts involved a series of tests and methods. Alkaloids, the most prominent class of secondary metabolites, were identified using the Dragendorff and Wagner's tests. In the Dragendorff test, 2 mg of ethanolic extract was mixed with hydrochloric acid and Dragendorff's reagent to yield an orange or orange-red precipitate if alkaloids were present. In the Wagner's test, ethanolic extract was acidified and treated with Wagner's reagent to indicate alkaloid presence through a yellow or brown precipitate.

Identification of flavonoids: Flavonoids, known for their coloration and diverse biological activities, were identified using the ammonia and sodium hydroxide tests. The ammonia test involved applying dilute ammonia to the ethanol extract, followed by sulphuric acid, resulting in a yellow coloration. The sodium hydroxide test required dissolving the extracts in water and adding aqueous sodium hydroxide, leading to a yellow coloration that turned colorless upon addition of dilute hydrochloric acid.

Identification of saponins (Foam test): Saponins were identified through the foam test, where the addition of leaves powder to distilled water followed by vigorous shaking produced foam.

Identification of Phenolic and tannins: Phenolic and tannins tests involved 5% ferric chloride reagent, which induced dark green or intense green coloration for phenolic compounds and purple, blue, or black coloration for tannins. Gelatin reagent and 10% lead acetate reagent also indicated the presence of phenolic or tannin compounds through white precipitates.

Identification of Terpenoids (Salkowski test): The presence of terpenoids was detected through the Salkowski test, where shade-dried leaves powder was mixed with chloroform and concentrated sulfuric acid to yield a reddish-brown coloration.

Identification of Resins: Resins were tested for by mixing ethanol extract with acetic anhydride and concentrated sulfuric acid, resulting in coloration ranging from orange to yellow.

Identification of Steroids: Steroids were identified by dissolving powdered leaves in chloroform and adding concentrated sulfuric acid, leading to a red upper layer and a yellow color with green fluorescence in the sulfuric acid layer.

Quantitative analysis of phytochemicals: Quantitative analysis of phytochemicals included determining total phenolic content using the Folin-Ciocalteu method and total flavonoid content using the aluminum chloride spectrophotometric method.

Total phenolic content: Total phenolic compounds of plant extracts were determined by using the Folin-Ciocalteu method. Various concentrations ranging from 20 to 160 µgml⁻¹ of Standard gallic acid were prepared and mixed with 2.5ml of folin-ciocalteu reagent (1:9 FC: ethanol extract) for 2 min. Then 2ml of 7.5% sodium carbonate (Na₂CO₃) was mixed and incubated at room temperature for 2 hours. At 760nm, the spectrophotometric analysis was done.

Total flavonoid content: For the determination of total flavonoid content, the aluminium chloride spectrophotometric method was used as suggested by +. For the test 0.5ml of plant extract was diluted separately with 2ml of ethanol and mixed with 5% (0.15ml) of sodium nitrite (NaNO₂). Then, 10% of aluminium chloride (AlCl₃) was added to the prepared mixture. After 6 minutes, 1ml (1.0M) of sodium hydroxide (NaOH) and 1.2ml of double distilled water was added and mixed well. After that at 510nm the spectrophotometric analysis was done to measure the absorbance. For the

calibration curve, the standard quercetin solution ranged from 20-160 $\mu\text{g}\text{m}^{-1}$ was used.

3. Evaluation of *in vitro* Antioxidant activity through DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay: The DPPH radical scavenging assay was used to determine the antioxidant activity of leaves extracted from *Psidium guajava* and *Terminalia chebula*. The DPPH assay is based on the assessment of a test compound's free radical scavenging activity [13]. 1 ml of the extract of different concentrations (10-750 $\mu\text{g}\text{ ml}^{-1}$) was mixed with 0.2mM of DPPH ethanol solution. The solution was kept in a shaker and incubated for 30 minutes at 30°C in dark. The absorption of the solution was measured using a UV-Vis spectrophotometer at 517nm and as a positive control, ascorbic acid was used. The percentage of inhibition of free radical (I%) was calculated using the following equation:

$$I\% = (A_{\text{control}} - A_{\text{sample}} / A_{\text{control}}) \times 100$$

Where, A_{control} is the absorbance of DPPH radical (without the test sample) and A_{sample} is the absorbance of DPPH radical with the isolated sample at various concentrations. The extract concentration generation 50% inhibition (IC_{50}) was calculated against the concentration of the plant extract samples from a plotted graph of scavenging activity. Five replications were

taken for the study and mean values were used for the data analysis.

Results and Discussion

Biochemical analysis of the leaves extract: The natural compounds found in various plants, enriched with phytochemicals, play a pivotal role in safeguarding the human body against a spectrum of diseases [14]. Phytochemicals, in general, possess protective attributes and are categorized as non-nutritive plant chemicals. These compounds are broadly classified into two groups based on their roles in plant metabolism: primary metabolites and secondary metabolites [15]. Primary metabolites encompass carbohydrates, amino acids, proteins, and chlorophyll, while secondary metabolites include alkaloids, saponins, flavonoids, and more [16].

Qualitative screening of phytochemicals: Preliminary screening was done to examine the presence of phytochemicals such as alkaloids, flavonoids, saponin, phenolic and tannins in leaves extracts. The results of these tests were exhibited in the Table 2. The phytochemical screening of leaves of *Psidium guajava* and *Terminalia chebula* ethanolic extract revealed the presence of alkaloids, flavonoid, saponin, phenol and tannins compound, terpenoids, resins and steroids.

Table 2: Qualitative analysis of phytochemicals

Phytochemical group	Test	<i>Terminalia chebula</i> leaf	<i>Psidium guajava</i> leaf
Alkaloids	Dragendorff test	+	+
	Wagner's test	+	+
Flavonoid	Ammonia	+	+
	Sodium hydroxide	+	+
Saponin	Foam test	+	+
Phenol and tannins	5% ferric chloride reagent	+	+
	Gelatin reagent	+	+
	10% lead acetate reagent	+	+
Identification of Terpenoids	Salkwski test	+	+
Resins	Acetic anhydride	+	+
Steroids	Chloroform	+	+

[17] also found a wide range of phytochemical constituents in the methanolic extract of *Terminalia chebula* leaves contain alkaloids, flavonoids, saponins, tannins, glycosides, phenols, proteins, triterpenoids, steroids, and fixed oils and fats. [18] concluded *Terminalia* plant contains several constituents like tannins, flavonoids, sterols, amino acids, fructose, resin, fixed oils. It is also found to contain compounds like anthraquinones, 4, 2, 4 chebulyl-dglucopyranose, terpinenes and terpinenols. [19] concluded that guava leaves contains tannins, saponins, steroids, saponin glycosides, flavonoids, balsmas, volatile oil, anthraquinones and alkaloids which help to inhibits bacterial growth.

Phytochemical constituents present within plants play a pivotal role in the intricate process of reducing metal ions and stabilizing resultant metal nanoparticles. Functional groups like phenols, alkaloids, and flavonoids demonstrate an inherent capability to effectuate the reduction and stabilization of metal ions into nanoparticles [20, 21, 22]. This reduction mechanism extends its influence to the precise control of both size and stability of the nanostructures formed. The stability exhibited by nanoparticles can be attributed to the intricate interactions occurring between metallic

nanoparticles and the phytochemicals naturally present in plant extracts [23]. Flavonoids, classified as hydroxylated phenolic substances, steroids, phenols, tannins, anthocyanins, and saponins, are synthesized by plants and are recognized for their expansive biological activities, spanning antimicrobial and anti-inflammatory effects, among others [24, 25, 26, 27].

Quantitative Screening: Quantitative screening of extracts helps in the quantification of the phytochemical *viz.*, alkaloids, flavonoids, saponin, phenolic, tannins, terpenoids, and other secondary metabolites present in the plant parts were expressed in terms of total phenolic content (TPC) and total flavonoid content (TFC).

Total Phenolic and flavonoid content of leaf extract: The Total Phenolic contents (TPC) and total flavonoid contents (TFC) of leaf extract of *Terminalia chebula* and *Psidium guajava* are presented in Table 3. TPC and TFC were determined by Folin-Ciocalteu's method and aluminum chloride spectroscopic method respectively. Gallic acid was used as the standard for TPC and quercetin was used as the standard for TFC. It was observed from Table 3, that the

amount of TPC content by the leaf extract of *Terminalia chebula* and *Psidium guajava* are 133.795 mg/ml and 101.791 mg/ml respectively. The amount of TFC was found highest in *Psidium guajava* (42.2869 mg/ml) than *Terminalia chebula* (36.4787 mg/ml).

Table 3: Total phenolic content (TPC) and total flavonoid content (TFC) of leaves extracts

Extracts	TPC (mg/ml) \pm SD	TFC (mg/ml) \pm SD
<i>Psidium guajava</i>	101.791	42.2869
<i>Terminalia chebula</i>	133.795	36.4787

The constituents present in the leaves extracts exhibit the capacity to enhance antioxidant, cytoprotective, anti-coagulant, anti-inflammatory, and analgesic properties. This multifaceted spectrum of effects contributes to the mitigation of disease risks and extends a range of other health benefits [28,29]. Furthermore, the presence of flavonoids and polyphenolic compounds within certain botanical extracts holds a remarkable potential to actively chelate and reduce metal ions, ultimately leading to the formation of nanoparticles [21].

It's worth noting that the observed variations in the content of phenolic and flavonoid compounds could potentially stem from a multitude of environmental factors. Factors such as maturity time, climate, geographic location, temperature, soil fertility, prevailing illnesses, the portion of the plant analyzed, exposure to pests, and rainfall can all exert significant influences on the bioactive composition [30]. These intricacies in the environment can lead to substantial disparities in the quantified values of phenolic and flavonoid compounds present in leaves extracts. The distinct variations observed in the total phenolic content among different leaves extracts could be attributed to the inherent diversity in the types and concentrations of various phenolic and flavonoid constituents. This variance in the overall content of phenolic compounds is likely reflective of the intricate interplay between the plant's genetics, growth conditions, and environmental interactions.

DPPH (2,2-diphenyl-1-picrylhydrazyl) assay: DPPH is a stable free radical that has gained widespread acceptance as a method for calculating the free radical-scavenging abilities of antioxidants. This is the simplest method wherein the prospective compound or extract is mixed with DPPH solution and absorbance is recorded after a defined period [31, 32]. The free radical scavenging activity of *Psidium guajava* and *Terminalia chebula* leaf extracts was analyzed using DPPH assay. Varying concentrations of the samples were used ranging from 10 to 750 μ g/ml. It was observed from Table 4, that the free radical scavenging activity increases with the increase in the concentration of the leaf extracts. The data showed that the *Psidium guajava* leaf extract has excellent antioxidant activity showing 93.44% at 250 μ g/ml but in *Terminalia chebula* leaf extract, maximum inhibition percentage (93.44%) was shown in 350 μ l/ml concentration whereas lowest antioxidant activity was observed as 70.49% and 69.67% in both *Psidium guajava* and *Terminalia chebula* respectively at 10 μ g/ml concentration. The percentage inhibition with the greatest value indicates the highest antioxidant activity.

The phenolic content in the leaves might be linked with its antioxidative properties [33]. Since *Psidium guajava* and *Terminalia chebula* contains phenolic compound and

flavanoid compound which is responsible for antioxidant activity. As the concentration of leaves extract of *Psidium guajava* and *Terminalia chebula* increases subsequently the amount of phenolic and flavonoid content was increases resultant inhibition percentage value which indicates the highest antioxidant activity. Total phenolic content and total flavonoid content of leaves extracts may also attributed to antioxidant activity.

Table 4: DPPH (2,2-diphenyl-1-picrylhydrazyl) assay

Concentration (μ l)	<i>Psidium guajava</i> % Inhibition	<i>Terminalia chebula</i> % Inhibition
10	70.49	69.67
50	83.61	82.79
150	91.80	85.25
250	93.44	88.52
350	92.62	93.44
500	91.80	92.62
750	90.16	90.98

Conclusion

In conclusion, both *Psidium guajava* (guava) and *Terminalia chebula* (Myrobalan) leaves stand out as remarkable sources of phytochemicals with diverse and potent health benefits. *Psidium guajava* leaves, with their extensive array of phytochemicals such as flavonoids, polyphenols, and alkaloids, have also emerged as a valuable natural remedy. These leaves showcase an impressive range of health-promoting effects, including antioxidant protection, antimicrobial action, and potential antidiabetic properties. The synergy of phytochemicals in *Psidium guajava* leaves has led to their exploration in various health applications, underlining their potential as a complementary approach to modern healthcare. Similarly, *Terminalia chebula*, known for its rich content of tannins, flavonoids, and other bioactive compounds, has been revered in traditional medicine systems for its wide-ranging therapeutic properties. From its potential as an antioxidant and anti-inflammatory agent to its role in digestive health and immune modulation, *Terminalia chebula* has garnered attention for its holistic approach to well-being. Furthermore, *Psidium guajava* and *Terminalia chebula* extracts has antimicrobial activity and it can be applied to textile. This eco-friendly approach not only promotes sustainable textile manufacturing but also addresses the increasing demand for antimicrobial textiles in various applications, including medical, sports, and everyday wear, where hygiene and freshness are paramount. *Psidium guajava* and *Terminalia chebula* leaves offer a promising avenue for developing innovative and environmentally friendly antimicrobial textile solutions.

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