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Screening of phytochemicals and antioxidant activity of *Psidium guajava* fruit pulp

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Abstract
Psidium guajava or typically recognized as guava is useful to deal with gastroenteritis, dysentery, stomach pain and indigestion. In this study, the leaves of this species were screened for its phytochemical and antioxidant activity. The phytochemicals have been extracted by sequential maceration with the aid of the usage of n-hexane, chloroform and methanol, whilst phytochemical screening was once performed the usage of various chemical tests. Meanwhile, its antioxidant exercise was assessed by using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay. Steroids and terpenoids were located to be existing in the n-hexane extract, whilst phenols and terpenoids had been detected in the chloroform extract. The methanol extract contained flavonoids, steroids, saponins, phenols and terpenoids. Among the tested extracts, the methanolic extract confirmed robust DPPH radical scavenging undertaking with an IC50 value of 44.72 µg/mL.

Keywords: *Psidium guajava*, phytochemical screening, antioxidant, scavenging

Introduction
Reactive oxygen species (ROS) such as superoxide (O-2˙), hydroxyl (HO˙) and peroxide radicals (ROO˙) are chemically reactive molecules that are produced from oxygen metabolism [1]. Their excessive manufacturing can purpose severe harm to cells and tissues, loss of cellular function, oxidative stress, and ultimately, apoptosis or necrosis. Collectively, these damages are linked to many fitness issues, such as coronary heart disease and carcinogenesis [2, 3]. In order to counteract the biomolecules towards a ROS attack, antioxidants are wanted to neutralise the immoderate free radicals, guard the cells in opposition to their toxic outcomes and forestall diseases such as arthritis, stroke and persistent bronchitis [4]. In recent years, an intensive interest to search and identify herbal and protected antioxidants, particularly those from a plant source has grown rapidly. These natural antioxidants can also be both phenolic compounds (i.e. flavonoids, phenolic acids and tannins), nitrogen-containing compounds (i.e. alkaloids, amino acids, peptides and amines), carotenoids, tocopherols, or ascorbic acid and its derivatives [5].
Psidium guajava is typically recognized as guave, goyave, or goyavier in France; guave, Guavenbaum, or Guayave in Germany; banjiro in Japan; goiaba or goiabeiro in Portugal; araçà-goiaba, araçà-guaçú, or guaiaba in Brazil; guayaba, or guayabo in Spain and guava in English [6]. This small tree is a native plant of tropical American regions, which is disbursed at some point of Southern Mexico, South America, European, Africa and Asia [6, 7]. Its leaves, barks, fruits, shoots and flower buds are utilized for ethnomedicinal purposes, especially in the therapy of several ailments such as gastroenteritis, dysentery, bacterial infection, diabetes, hypertension, caries and wounds, as well as a pain reliever and fever reducer. Meanwhile, this plant is also utilised commercially as food and in carpentry, house building and toy manufacturing [6]. Biological investigations on P. guajava have revealed that its one-of-a-kind extracts and remoted compounds exhibited antimicrobial, antihyperglycaemic, anti-inflammatory, analgesic, antipyretic, spasmolytic, central frightened device (CNS)–depressant, antidiarrhoeal, antimalarial, hepatoprotective, antigenotoxic, anti-mutagenic, antiallergic, antioxidant, anticancer, cardiovascular, hypotensive, anti-nociceptive and wound healing activities [6-8].

Furthermore, phytochemical studies undertaken via preceding researchers have disclosed that this species can produce flavonoids, triterpenoids, tannins, phenolics, carotenoids and fundamental oils [6, 7, 9- 14]. To date, there is only one study determined detailing the phytochemical screening and antibacterial undertaking of P. guajava fruit pulp extract, which have been accumulated from Rajshahi, Bangladesh [14]. Accordingly, geographical distribution impacts the chemical compositions of plants, whilst their organic activities are impacted conversely. In this study, the fruit pulp of P. guajava had been amassed from Rajshahi, Bangladesh. The investigation was carried out to pick out phytochemicals in the P. guajava fruit pulp extracts and their relationship with the resulting antioxidant activity.

Materials and Method

Plant material and preparation of extracts

The fruit pulp of P. guajava were accumulated in July 2022 from Chapai Nawabganj, Rajshahi, Bangladesh. The fruit pulp have been subjected to air-dried and floor into first-rate powder. The powdered fruit pulp (200 g) had been soaked sequentially with n-hexane (2 L), chloroform (2 L) and methanol (2 L) for three days each. The extracts have been filtered using Whatman paper No. 1 and targeted using a rotatory evaporator to come up with the money for n-hexane, chloroform and methanol extracts.

Phytochemical screening

The extracts were tested for the presence of alkaloids, flavonoids, phenolic compounds, terpenoids, steroids and saponins.

Test for alkaloids (Wagner's test)

Six drops of Wagner's reagent have been delivered to 2 mL of crude extract. The Wagner's reagent was as soon as geared up via mixing iodine in potassium iodide solution. The formation of brown or reddish precipitate suggests the presence of alkaloids [15].

Test for flavonoids (Ferric chloride test)

Crude extract 2 ml was treated with a few drops of ferric chloride solution. The excessive green coloration of the solution suggests the presence of flavonoids [16].

Test for phenolics (Ferric chloride test)

A few drops of ferric chloride solution had been delivered into every crude extract (2 mL). The appearance of a bluish black colour suggests the presence of phenolic compounds [17].

Test for terpenoids (Salkowski test)

Each crude extract (1 mg) was once combined with chloroform (2 mL) and focused sulphuric acid (1 mL). The formation of reddish-brown colour at the interface suggests the presence of terpenoids [2].

Test for steroids (Salkowski test)

About one hundred mg of dried crude extract used to be dissolved in 2 mL of chloroform. About 2-3 drops of sulphuric acid was as soon as carefully brought to the extract to form a lower layer. A reddish-brown coloration at the interface was once indicative of the presence of steroidal ring [2, 18].

Test for saponins (Foam test)

Distilled water (2 mL) was once delivered to two mL of crude extract. The aggregate used to be shaken vigorously for 15 minutes period. The persistence of foam produced after 15 minutes shows the presence of saponins [19].

Antioxidant assay

Antioxidant activity was performed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The method used to be performed as described by means of the preceding learn about with moderate modifications [20]. Each sample (1.0 mg) was once dissolved in methanol (1 mL) to obtain a inventory concentration of one thousand µg/mL. The inventory answer was once in addition diluted to last concentrations of 500, 250, 125, 62.5, 31.3, 15.63 and 7.81 µg/mL in methanol. The DPPH solution was once freshly prepared in MeOH to acquire remaining awareness of 50 µM. Then, 3.8 mL of DPPH answer used to be added to 0.2 mL of pattern answer of exceptional concentrations. The combination was once allowed to react at room temperature in the dark. After 30 minutes, the absorbance of the response combination was once recorded at 517 nm. Ascorbic acid was once used as positive control, whilst the DPPH solution (3.8 mL DPPH and 0.2 mL methanol) was once used as DPPH blank. The percentage inhibition (%) was calculated the usage of the following method [20]:

Percentage Inhibition (%) = [A DPPH blank – (A Sample - A blank sample)] / A DPPH blank x 100 (1)

Where

ADPPH blank is the absorbance of DPPH reagent and methanol, a sample is the absorbance of sample answer with DPPH reagent and a blank sample is the absorbance of pattern answer with methanol. The IC50 value was described as the concentration of every pattern required to scavenge 50% of the DPPH radical and was determined the use of GraphPad Prism 5. The assay was once carried out in triplicate and the outcomes have been reported as capacity ± standard deviation. The Independent t-test was once performed by the usage of SPSS for Windows (version 22) software for evaluation between samples and high quality control. A price of p was viewed significantly different.

Results and Discussion Percentage yield of extracts

The removing of solvents under reduced stress yielded n-hexane, chloroform and methanol extracts as shown in Table 1. The methanol extract gave the easiest extraction yield of 9.65%, determined thru chloroform (2.22%) and nhexane (1.49%) extracts. These consequences indicate that the extraction yield will increase alongside the developing polarity of solvent utilized in the extraction method [21]. Therefore, the larger proportion yield proven via the methanol extract may also be due to the immoderate polarity of the methanolic solvent, which can extract ample kinds of compounds [22].

Table 1: Yield of P. guajava fruit pulp extracts

Extract	Yield (g, %)
n- Hexane	3.04, 753
Chloroform	4.65, 489
Methanol	19.83, 371

Preliminary phytochemical screening

The outcomes of qualitative phytochemical evaluation for the n-hexane, chloroform and methanol extracts are introduced in Table 2 From the findings, it was once determined that n- hexane extract contained steroids and terpenoids, while chloroform extract confirmed the presence of phenols and terpenoids. Flavonoids, steroids, saponins, phenols and terpenoids have been detected in methanol extract. Furthermore, the current find out about printed the existence of terpenoids in all extracts in which a reddish-brown shade was observed. However, these three extracts did now not supply brown or reddish precipitate when dealt with Wagner's reagent, thereby indicating the absence of alkaloids. The qualitative phytochemical evaluation undertaken in this find out about confirmed equal findings for the existence of flavonoids and phenols in contrast to the reviews via earlier researchers [11, 12, 14].

Table 2: Phytochemical screening of P. guajava fruit pulp extracts

Phytochemicals	Extract		
	n- Hexane	Chloroform	Methanol
Alkaloids	-	-	-
Flavonoids	-	-	+
Steroids	+	-	+
Saponins	-	-	+
Phenols	-	+	+
Terpenoids	+	+	+

+ = present; - = absent

The phytochemicals detected in the fruit pulp of P. guajava have been reported to provide limitless benefits of medicinal importance. For examples, phenolics and flavonoids exhibit many organic activities, such as antioxidant [23], antimicrobial [24] anticancer, anti-inflammatory and wound restoration homes [25]. On the different hand, steroids are recognized to have antibacterial, insecticidal and cardiotonic homes [2], whilst saponins can treat diabetes [26]. Similarly, terpenoids have been used to alleviate human illnesses such as cancer, malaria, inflammation and quite a number infectious diseases [27].

DPPH radical scavenging activity

Data on the DPPH radical scavenging undertaking of n-hexane, chloroform and methanol extracts of P. guajava fruit pulp along with the popular antioxidant, ascorbic acid is presented in Table three The samples that confirmed p had been considered to have a statistically large difference as in contrast to ascorbic acid as the effective control. Among the extracts, solely methanol extract established radical scavenging activity with a share inhibition of 86.33% and an IC50 value of 44.72 µg/mL. However, the IC50 value was once higher than that of ascorbic acid, which has an IC50 value of 24.75 µg/mL. The other extracts did now not exhibit radical scavenging exercise in view that their respective proportion inhibitions were less than 50% at the attention of 1000 µg/mL. The potent antioxidant undertaking of the methanol extract may additionally be due to the existence of flavonoids and phenolics, which are acknowledged to be successful of donating their hydrogen atoms [23].

Table 3: DPPH radical scavenging activity of P. guajava fruit pulp extracts

Samples	Inhibition at 1000 µg/mL	IC50(µg/mL)
n- Hexane extract	16.15± 0.73	ND
Chloroform extract	42.32±0.94	ND
Methanol extract	86.33±0.65	44.72±3.36
Ascorbic acid	94.47±1.23	24.75±0.94

Data represent mean ± standard deviation of three replicate experiments; ND = not determined a = $p < 0.01$, b = $p < 0.001$

Conclusion

The phytochemical screening printed that the fruit pulp extracts of P. guajava from Rajshahi, Bangladesh comprise of various really helpful phytochemicals such as flavonoids, steroids, saponins, phenols and terpenoids. This find out about also disclosed that the methanol extract yielded the best extraction yield and top DPPH radical scavenging activity. The existing statistics may additionally be useful as a preliminary step to determine the conceivable natural antioxidants that can be applied in pharmaceutical and therapeutic industries. In order to discover these antioxidants, in addition purification and characterization steps are advised to be carried out in future study.

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