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Research Article

Phytoconstituents analysis of ripe *Pithecellobium dulce* seeds and coats: Insights from Gas Chromatography-Mass Spectrometry (GC-MS) and bioactivity assessment

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Abstract
Pithecellobium dulce is a tropical tree known for its medicinal properties, which are traditionally used in folk medicine to treat ailments such as diabetes and inflammation. Recent research has focused on exploring its bioactive compounds to validate its therapeutic potential. This study investigates the antioxidant and amylase inhibitory potential of methanolic extracts from the ripe seeds and coats of *P. dulce,* previously overlooked plant parts. Phytochemical analysis of both seed and coat extracts was conducted using standard qualitative methods, revealing the presence of alkaloids, flavonoids, glycosides, saponins, steroids, tannins, proteins, phenols, and terpenoids. The Total Phenolic Content (TPC) in ripe seeds was 9.11±0 mg GAE/g, while the fruit coat measured 3.056±0.5 mg GAE/g. Quantitative assessment of Total Flavonoids Content (TFC) indicated the presence of 36.4±0.8 mg RE/g in the seed extract and 31.64±0.4 mg RE/g in the fruit coat. The seed extract displayed substantial saponin content (118.4±14.1 mg/g dry extract saponin equivalents). Phytoconstituents identified with GC-MS showed antioxidant, anti-inflammatory, and anticancer properties, including Phytol, n-Hexadecanoic acid, cis-Vaccenic acid, 9,11-Octadecadienoic acid, methyl ester (E,E), Oleoyl chloride, Eugenol, Benzofuran, 2,3-dihydro, and 5-Hydroxymethylfurfural. Antioxidant activities, assessed through DPPH, reducing power, and metal chelating assays, demonstrated IC_{50} values of 1378.3 µg/mL and 36.7 µg/mL (DPPH scavenging) for seed and coat extracts, respectively. Significant inhibition of α-amylase activity was observed, with the coat extract exhibiting the highest inhibition at 55%. This study enhances the understanding of the phytochemical composition and bioactivities of ripe seeds and coats of *P. dulce*, providing a foundation for future research to fully realize the plant's therapeutic benefits.

Keywords: Bioactivities, Medicinal potential, Methanolic extracts, Phytoconstituents, *Pithecellobium dulce*

INTRODUCTION

Phytochemicals, the bioactive compounds found in plants, have emerged as key players in promoting health and combating chronic diseases. Extraction, isolation, and identification of these compounds are crucial for uncovering their therapeutic potential. Recent studies have highlighted their diverse medicinal properties. For instance, flavonoids have anti-inflammatory and antioxidant effects, emphasizing their

potential to prevent cardiovascular diseases and cancer (Patel *et al.*, 2021). Alkaloids have demonstrated antimicrobial properties, proving effective against multidrug-resistant bacteria (Smith *et al.*, 2020). Terpenoids have exhibited significant anticancer activities (Lee *et al*., 2022), and phenolic compounds have been linked to managing type 2 diabetes by modulating glucose metabolism (Wang *et al.*, 2023). These findings reinforce the therapeutic potential of phytochemicals and highlight advancements in their study.

Phytochemicals found in *Pithecellobium dulce (P. dulce*), commonly known as manila tamarind, have garnered global recognition for their significant health benefits (Selvakumar *et al.*, 2019). Recent scientific investigations have highlighted its diverse pharmacological properties, aligning with traditional claims (Kondabolu *et al.*, 2023; Kumari, 2017). Chemical analyses have identified a range of compounds in *P. dulce* responsible for its therapeutic benefits, including antimicrobial, antioxidant, and anti-inflammatory properties (Getie *et al.*, 2002). Moreover, *P. dulce* shows promise in diabetes management, with certain extracts demonstrating inhibitory effects on key enzymes (Nagmoti and Juvekar, 2013). The presence of specific bioactive compounds further bolsters these observed pharmacological activities (Niranjan *et al.*, 1999; Yoshikawa *et al.*, 1997). The present study aimed to characterize the bioactive compounds in ripe seeds and coats of *P. dulce*, focusing on antioxidant activity, amylase inhibition, and compound identification using GC-MS, to explore its pharmacological properties and therapeutic potential.

MATERIALS AND METHODS

Sample collection

Fruit of *P. dulce* was collected from the *P. dulce* tree present in the Pravara Institute of Medical Sciences campus, Loni, Maharashtra, and has a latitude and longitude of 19.5792° N, 74.4539° E.

Sample preparation

The fruit coat and seed were separated from the freshly collected fruit of *P. dulce* and air-dried at room temperature for 1 week. The dried sample was ground into a fine powder with the help of a mechanical grinding machine. The dry powder was homogenized in a gradient to prepare the extract and use for analysis.

Maceration extraction process

The coat and seed extracts were prepared using a maceration process. 27.06 grams of fruit coat and 36.98 grams of seed powder were weighed and immersed in 270 ml and 360 ml of methanol (1:10 w/v). The solution was kept in a shaking Incubator for 24 hours and after incubation, it was filtered using Whatman Paper no 42 (125mm). The filtrate was collected in separate glass beakers and allowed to dry to form the extract.

Qualitative analysis of phytochemicals

The extracts were analyzed for various bioactive compounds, including saponins, phenols, tannins, terpenoids, flavonoids, glycosides, proteins, alkaloids, and steroids (Semwal *et al.*, 2014; Nahrin *et al.*, 2020).

Determination of total phenolic content (TPC)

The TPC was determined according to a previously described procedure (Bao *et al*., 2005). The methanolic extracts of ripe seeds and coats of *P. dulce* (0.1mg/mL) were mixed with 0.5 mL of Folin- Ciocalteu reagent (previously diluted in a ratio of 1:3v/v with distilled water) and it was allowed to incubate at room temperature for 3 min. Then, 2 mL of 7% sodium carbonate solution was added and the mixture was placed in a boiling water bath for 1 minute. The absorbance was measured at 650 nm by a UV-visible spectrophotometer. Using the gallic acid standard curve (20-100 µg/ml), the total phenol content was obtained. The total phenolic content was expressed as gallic acid equivalent (GAE) in mg/g of the plant extract.

Determination of total flavonoid content (TFC)

Aluminum chloride colorimetric method was used for TFC determination (Chang *et al*., 2002). Methanolic extracts of ripe seeds and coats of *P. dulce* (2 mL of 1:10 w/v) was separately mixed with 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 425 nm by using a Spectrophotometer. The flavonoid content was calculated from a calibration curve using Rutin as a standard. The analysis was performed in triplicates and the results were expressed as milligrams of rutin equivalent /gram dry weight extract.

Determination of saponins content

The vanillin-sulfuric acid method was used to determine the total saponin content of methanolic extracts of ripe seeds and coats of *P. dulce* (Chen *et al*., 2011). A methanolic extracts (0.5 mL) was placed in a test tube containing 0.5 mL vanillin (8% w/v) and 4.0 mL sulphuric acid (72% w/v). The mixture was incubated in a water bath at 60 ºC for 15 minutes before being chilled in an ice bath for 10 minutes. The absorbance of the sample was measured at 560 nm using aescin as a reference standard. The results are expressed as milligrams per gram of dry extract Standard Saponin equivalents (Segaran and Chua, 2020).

Determination of antioxidant activity

DPPH radical scavenging assay

Total DPPH (2,2-diphenyl-1- picrylhydrazyl) radical

scavenging activity of methanolic extracts of ripe seeds and coats of *P. dulce* was estimated according to the previously reported method with slight modification (Bhalodia *et al*., 2013). The antiradical activity was measured by a decrease in absorbance of colored DPPH in methanol brought about by the sample. Test solutions of different concentrations were added to 2 mL of Methanolic DPPH. The mixture was shaken vigorously and kept at room temperature for 30 min in the dark. The absorbance of the reaction mixture was measured at 517 nm spectrophotometrically. The absorbance of the DPPH radical without antioxidants, i.e. blank was also measured. A solution of methanol and DPPH in equal amounts served as a control. The percentage inhibiting activity was calculated from below formula:

DPPH scavenging (%) = O.D. Control-O.D. Sample/ O.D. Control X 10 Eq.1

Reducing power assay

This assay was done using method described previously (Güder and Korkmaz, 2012). The reaction mixture was prepared by combining 0.1 mL of the methanolic extracts of ripe seeds and coats of *P. dulce* in phosphate buffer (0.25 mL, 0.2 M, pH 6.6) and separately potassium ferricyanide (0.25 mL, 1%). The reaction mixture was incubated at 50°C for 20 min. After incubation, trichloroacetic acid (0.25 mL, 10%) was added and the reaction mixture were centrifuged for 10 min. at 3000 rpm. The top layer of the reaction mixture (0.25 mL) was recoverd and 0.25 mL of distilled water and 0.05 mL of ferric chloride (0.1%) were mixed. The conversion of Fe^{3+} to Fe^{2+} in the presence of methanolic extracts of ripe seeds and coats of *P. dulce* or standards was observed, and the optical density was recorded at 700 nm.

Reducing Power (%) = (*A*s / *A*c) × 100 Eq. 2

Where,

Ac is the absorbance (optical density) of the control (L-ascorbic acid), and

As is the absorbance (optical density) of samples (extracts) or standard.

Metal chelating activity

Metal chelating activity of methanolic extracts of ripe seeds and coats of *P. dulce* was performed according to the previously reported method with slight modification (Chelliah and Oh, 2022). The metal chelating activity was measured by adding 0.2 ml of 0.1mM ferrous sulfate (FeSO4) solution and 0.4 ml of 0.25mM concentration ferrozine solution subsequently into different volumes of methanolic extracts of ripe seeds and coats of *P. dulce* (40, 80, 120, 160 and 200µl). After incubating for 10 minutes at room temperature, the absorbance was taken at 562 nm. A solution without extract is taken as a control. The chelating activity of the sample was calculated by the formula:

Metal Chelating Activity (%) = O.D. Control - O.D. Sample/O.D. Control (562 nm)X 100 Eq. 3

Amylase inhibitory activity by starch plate method

The starch agar plate method was employed to assess the amylase inhibitory activity of methanolic extracts of ripe seeds and coats of *P. dulce* (Hemlata *et al*., 2019). About 1.5 grams of agar were heated and allowed to cool to approximately 60°C, after which 0.1 grams of starch were incorporated, and the mixture was poured into plates. Wells were then created on the solidified agar using a well-borer. Three different concentrations of both enzyme and extracts (T1-3:1, T2-1:1, and T-3 1:3 v/v) were prepared separately. The volume of the reaction mixture was adjusted with 0.1 M phosphate buffer (pH 6.8). Subsequently, the wells were individually loaded with these mixtures (100µl), with salivary amylase serving as a positive control and buffer as a negative control. The plates were then incubated at 37° C overnight and the following day, flooded with iodine and potassium iodide solution (Lugol's reagent). The hydrolysis of starch was visually monitored around the wells as starch hydrolysis zones. The well displaying the minimum hydrolysis zone compared to the control amylase indicates inhibition and confirms the presence of amylase inhibitors.

Gas Chromatography/Mass Spectroscopy (GC-MS) analysis

Approximately 20 mg of methanolic extracts of ripe seeds and coats of *P. dulce* was mixed with 10–15 mg of graphitized carbon black to remove excess pigments. The mixture was then reconstituted with 1 mL of a suitable solvent (methanol extract in methanol) and vortexed until completely dissolved. Subsequently, the mixture underwent centrifugation at 8000 rpm for 5 minutes, and the supernatant was filtered using a membrane filter (AllPureTM syringe filter, 25 mm diameter, 0.45 µm pore size). The resulting filtrate was stored in amber vials at 4°C for further analysis using GC-MS.

For the GC/MS analysis, a Shimadzu GCMS-TQ8040 instrument equipped with a RESTEK Rxi-5ms column and an auto-injector (AOC-20i+s) was employed. Methanolic extracts of ripe seeds and coats of *P. dulce* (1 µL) were injected in splitless mode at an injection temperature of 280°C and analyzed in scanning mode. The carrier gas flow (He) was set at 1.2 mL/min with a linear velocity of 39.5 cm/s. The column temperature was initially held at 40°C for 1 minute, then increased at a rate of 5°C/min to 270°C, and finally maintained at 270°C for 5 minutes. The transfer line and ion source temperatures were set at 270°C and 220°C, respectively. Ionization was performed using electron ionization (EI) at 0.94 kV+0.2 kV, and EI mass spectra were

recorded at 10,000 u/s over the mass range m/z 45–550. The total sample run time was 52 minutes. System control and data acquisition were managed using Shimadzu's GC-MS solution software. Data processing, including peak selection, peak identification, and peak integration, was carried out within the GCMS software application for post-run analysis, with compound identification performed using the NIST library (Hallur *et al*., 2021).

Statistical analysis

The experimental procedures were carried out and analyzed three times to guarantee the reliability and precision of the outcomes. Mean values and standard deviations were computed for each data set. Statistical comparisons were undertaken using Microsoft Excel 2013 to evaluate the significance of the results.

RESULTS AND DISCUSSION

This study evaluated the phytochemical composition, antioxidant, and antidiabetic activities of methanolic extracts from the ripe seeds and coats of *Pithecellobium dulce*, alongside a detailed GC-MS analysis of their phytoconstituents. The findings reveal a significant presence of bioactive compounds, supporting the pharmacological potential of these underexplored plant parts.

Phytochemical composition

Phytochemical screening revealed that both seed and coat extracts contained phenolic compounds, terpenoids, flavonoids, glycosides, proteins, and alkaloids. Notably, saponins were exclusively present in the seed extract (Table 1), which has not been previously reported in such detail for *P. dulce*. This finding expands the known phytochemical profile of the species and suggests the potential for unique therapeutic applications. These results are consistent with earlier studies, which also reported a diverse array of phytochemicals in root, bark, seeds, fruits and leaves of *P. dulce* (Murugesan *et al*., 2019; Kumari, 2017).

Table 1. Qualitative analysis of phytoconstituents of methanolic extracts of ripe seeds and coats of *P. dulce*

Phytoconstituents	Fruit Coat	Seed
Saponin	-	+
Phenol	+	+
Tannins	-	-
Terpenoids	+	+
Flavonoids	+	+
Glycosides	+	+
Protein	+	+
Alkaloids	+	+
Steroids	+	+

+Indicates Presence - Indicates Absence

Total phenolic content (TPC) and Total flavonoid content (TFC)

The TPC was measured as 9.11 ± 0.5 mg GAE/g in the seed extract and 3.06 ± 0.5 mg GAE/g in the coat extract. Similarly, the TFC was found to be 36.4 ± 0.8 mg RE/g in the seed extract and 31.6 ± 0.4 mg RE/g in the coat extract (Figures 1 and 2). These values suggest a higher concentration of phenolic and flavonoid compounds in the seed, which may contribute to the plant's antioxidant activity. Previous studies have demonstrated the presence of these compounds in *P. dulce* (Semwal *et al*., 2014; Nahrin *et al*., 2020). However, our comparative quantification of these compounds specifically in the ripe seed and coat, provides new insights into the phytochemical composition.

Total saponin content

The total saponin content in the seed extract was quantified at 118.4 ± 14.1 mg/g of dry extract (Figure 3). This high saponin content in seed extract was noteworthy, as previous studies on *P. dulce* have not reported such detailed data on saponin levels (Murugesan *et al*., 2019). The presence of saponins suggests potential health benefits, including cholesterol-lowering and immune-modulating effects, which warrant further investi-

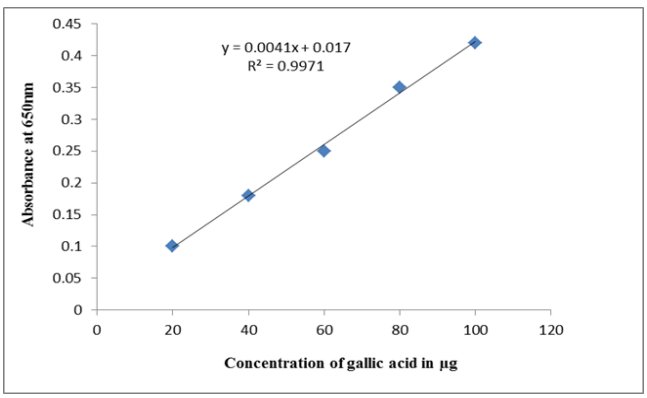

Fig. 1. *Regression line for gallic acid*

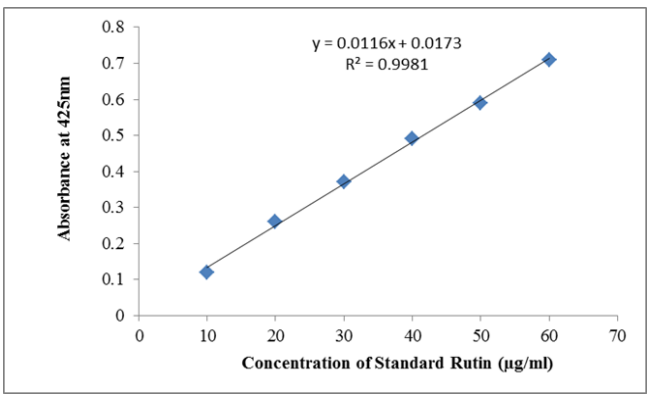

Fig. 2. *Regression line for rutin*

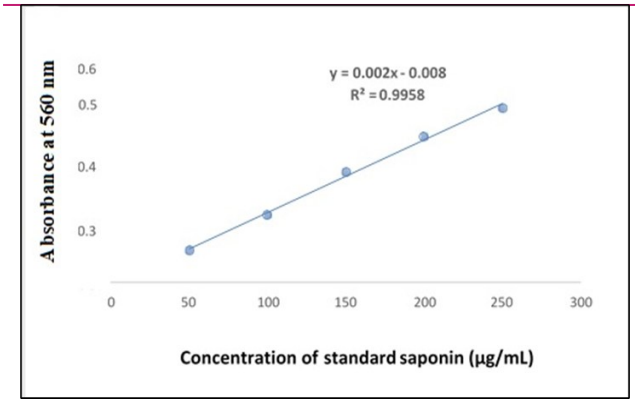

Fig. 3. *Regression line for saponin*

gation. This finding adds a new dimension to the known pharmacological profile of *P. dulce*.

Antioxidant activity

The antioxidant potential was assessed using three in vitro assays: DPPH scavenging activity, reducing power, and metal chelating activity. DPPH Scavenging Activity: The coat extract exhibited stronger DPPH scavenging activity (IC50 = 36.7 µg/mL) compared to the seed extract (IC50 = 1378.3 µg/mL), indicating a higher radical scavenging potential in the coat (Figure 4). This result aligns with previous studies suggesting significant antioxidant potential in leaves, seeds, fruits, and barks of *P. dulce* (Kumar *et al*., 2017; Nagmoti *et al*., 2013). The present study showed that, at a concentration of 100 µg/mL, the coat extract demonstrated higher reducing power (O.D. = 0.44) than the seed extract (O.D. = 0.19), indicating a greater ability to donate electrons (Figure 5). This dose-dependent activity is comparable to that of ascorbic acid, a known antioxidant. Metal Chelating Activity: The seed and coat extracts exhibited metal chelating activity with IC50 values of 254.53 µg/mL and 391 µg/mL, respectively, compared to the standard EDTA (IC50 = 89.68 µg/mL) (Figure 6). These assays collectively demonstrate significant antioxidant properties, consistent with the bioactive potential of other tropical plants reported in recent literature (Pío-León *et al*., 2013; Hallur *et al*., 2021).

α-Amylase inhibitory activity

The extracts also showed promising α-amylase inhibitory activity, with the coat extract displaying higher inhibition (55.55% at T3) compared to the seed extract

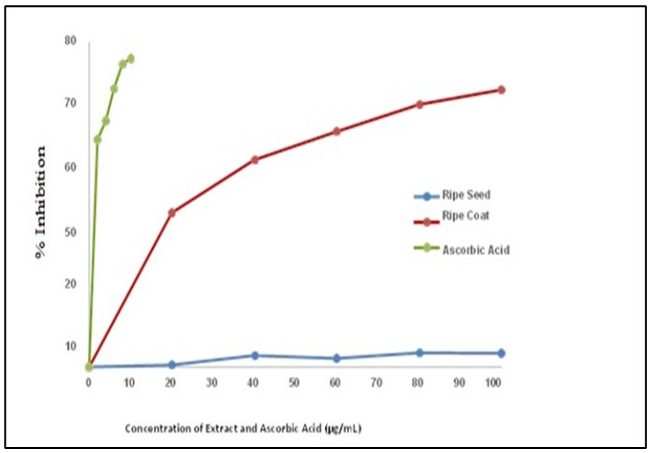

Fig. 4. *Antioxidant activity (DPPH) of methanolic extracts of ripe seeds and coats of Pithecellobium dulce*

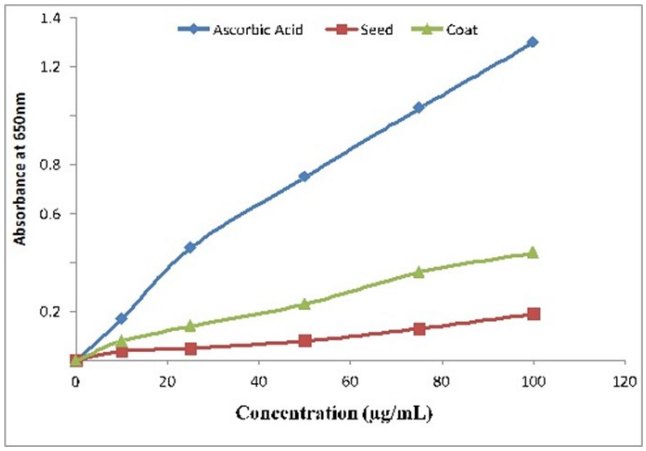

Fig. 5. *Reducing power potential of methanolic extracts of ripe seeds and coats of Pithecellobium dulce*

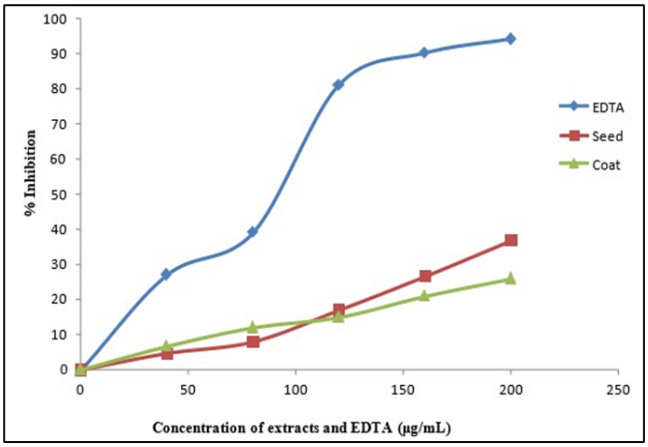

Fig. 6. *Metal chelating activity of methanolic extracts of ripe seeds and coats of Pithecellobium dulce*

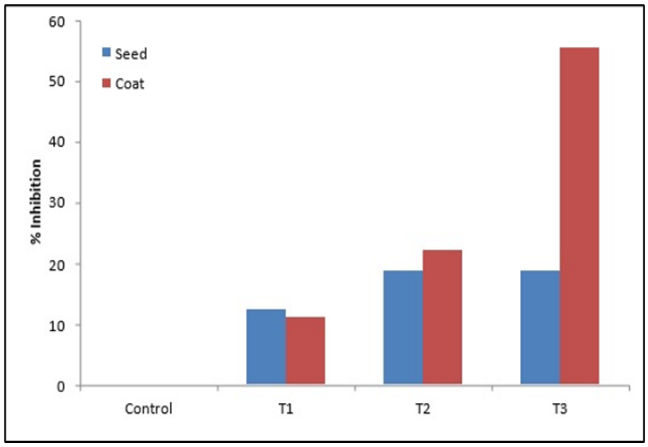

Fig. 7. *α-amylase inhibitory activity of methanolic extracts of ripe seeds and coats of Pithecellobium dulce. T1-3:1, T2-1:1, and T-3 1:3 (amylase: extract v/v)*

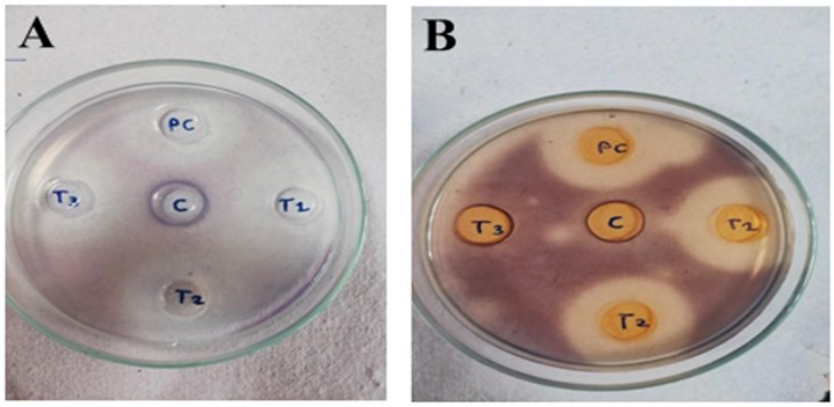

Fig. 8. *α-amylase inhibitory activity of methanolic extracts of ripe seeds (A) and coats (B) of Pithecellobium dulce on starch agar plate. C-Buffer control, PC-Positive control (amylase), T1-3:1, T2-1:1, and T-3 1:3 (amylase: extract v/v)*

Table 2. Major chemical compound identified in methanolic extracts of ripe seeds and coat of *Pithecellobium dulce* by GC/MS in present study

Sr. no.	Extract	Name	Category	MF	Rt	MW	RA %	Biological activity
1	Seed	n-Hexadecanoic acid	Fatty acid	$C_{16}H_{32}O_2$	26.120	256	11.96	Anti-tumor and anti-oxidant activity (Harada, 2002)
2	Seed	cis-Vaccenic acid	Trans fatty acid and omega-7 fatty acid	$C_{18}H_{34}O_2$	29.495	282	12.34	Anti-cancer & Antimi-crobial activity (Liu *et al*., 2011)
3	Seed	Phytol	Diterpene alcohol	$C_{20}H_{40}O$	28.561	296	0.01	Anxiolyt-ic,cytotoxic,antioxidant activity (Islam, 2018)
4	Coat	9,11-Octadecadienoic acid, methyl es-ter, (E,E)	Fattyacid methyl ester	$C_{19}H_{34}O_2$	28.227	294	0.12	Anti-inflammatory ac-tivity
5	Seed	Oleoyl chloride	Oleic acid chloride (Fatty ac-id)	$C_{18}H_{33}ClO$	37.721	300	0.12	Antioxidant activity (Visioli *et al*., 2002)
6	Coat	Eugenol	Aromatic organic compound	$C_{10}H_{12}O_2$	14.579	164	0.06	Antioxidant activity (Marchese *et al*., 2017)
7	Coat	Benzofuran, 2,3-dihydro-	Heterocy-clic aro-matic com-pound	C_8H_8O	9.404	120	1.88	Antioxidant activity (Diaz *et al*., 2009)
8	Seed	5-Hydroxymethyl-furfural	Organic compound	$C_6H_6O_3$	9.469	126	2.88	Anticancer activity (Zhang *et al*.,2020), Antioxidant activity (Liu *et al*., 2021)

Note- MF: Molecular Formula, Rt: Retention Time (minutes), MW: Molecular Weight (g/mol), RA %: Relative Abundance Percentage

(18.7% at T2 and T3) (Figures 7 and 8). This suggests potential use in managing type-II diabetes mellitus by controlling postprandial hyperglycemia. These findings support earlier reports on the antihyperglycemic properties of seed extract of *P. dulce* and other medicinal plants used for diabetes management (Kumar *et al*., 2017; Nagmoti *et al*., 2013).

GC-MS analysis

GC-MS analysis identified 60 phytoconstituents in the seed extract and 41 in the coat extract, including significant compounds such as n-Hexadecanoic acid, cis-Vaccenic acid, Phytol, 9,11-Octadecadienoic acid methyl ester (E,E), Oleoyl chloride, Eugenol, Benzofuran

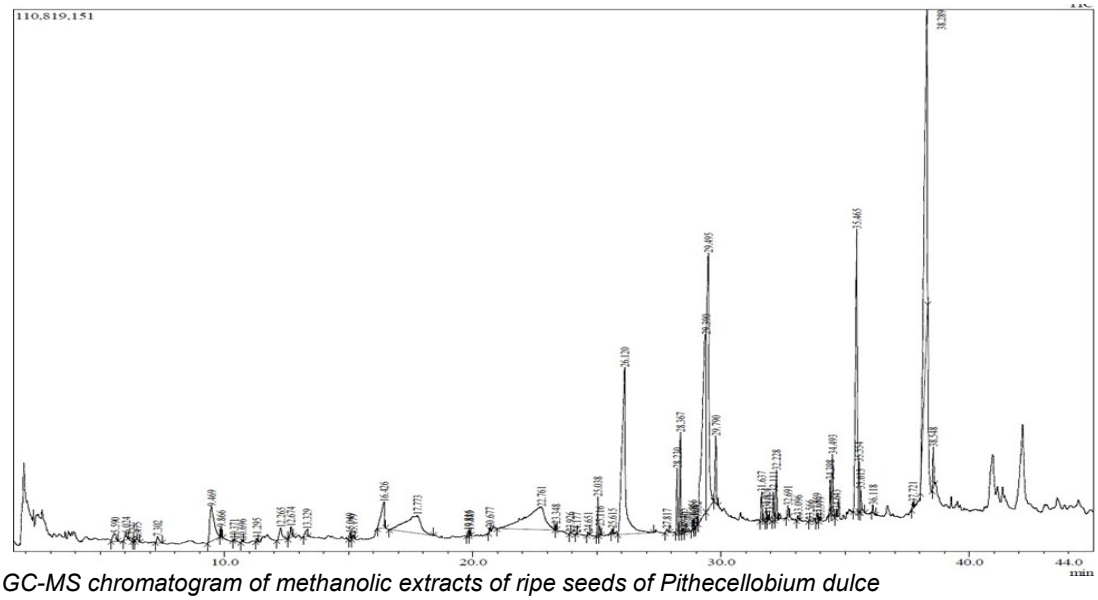

Fig. 9. *GC-MS chromatogram of methanolic extracts of ripe seeds of Pithecellobium dulce*

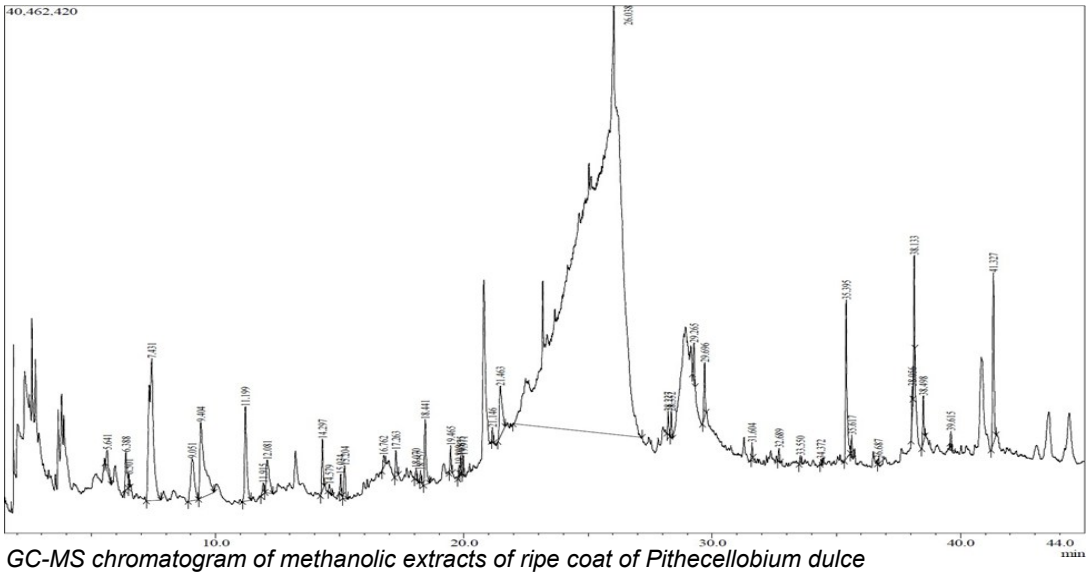

Fig. 10. *GC-MS chromatogram of methanolic extracts of ripe coat of Pithecellobium dulce*

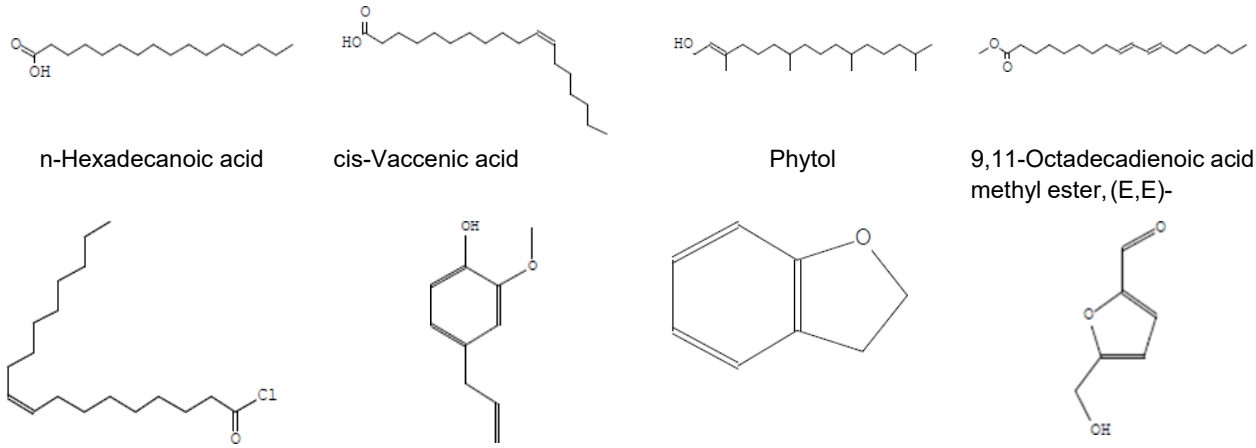

Fig. 11. *Structures of the chemical compounds identified from extracts of ripe seeds and coat of Pithecellobium dulce by GC/MS*

2,3-dihydro, and 5-Hydroxymethylfurfural (Table 2, Figures 9-11). These compounds are known for their diverse biological activities, such as anti-tumor, antimicrobial, anti-inflammatory, and antioxidant properties, which may contribute to the therapeutic potential of ripe seeds and coat of *P. dulce* (Aldarhami *et al*., 2023; Murugesan *et al*., 2019). The presence of these bioactive compounds reinforces the plant's potential use in both traditional and modern medicine.

Though the phytochemicals from various parts of *P. dulce* such as the root, bark, seeds, fruits, and leaves have indeed been documented earlier. However, the present study uniquely focuses on the phytochemical profile of the ripe seeds and coats, which has not been thoroughly investigated previously, especially concerning their amylase inhibitory and antioxidant activities.

Conclusion

This study provides a novel insight by evaluating antioxidant and α-amylase inhibitory for the first time in *P. dulce* ripe seeds and coats, addressing potential applications in managing hyperglycemia and oxidative stress. Additionally, the findings reveal distinct phytochemicals with possible therapeutic implications, expanding the understanding of the species' phytochemical diversity and biological potential.

Conflict of interest

The authors declare that they have no conflict of interest.

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