Current Trends in Biotechnology and Pharmacy
Vol. 17(3) 1052 - 1059, JUly 2023, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2023.3.43

Phytochemical Analysis in *Pithecellobium dulce* Fruit Peel Extract

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Abstract

The study aimed to reveal the phytochemical profile of *Pithecellobium dulce* fruit peel extract. The *P. dulce* fruit peel extract (PDFE) was obtained by cold maceration technique using ethanol. The qualitative phytochemical study showed that PDFE contains carbohydrates, amino acids and proteins, alkaloids, phenols, flavonoids, glycosides, and anthraquinones. The quantitative phytochemical revealed that one gram of PDFE contains 82.33mg of carbohydrates, 38 mg of proteins, 26 mg of flavonoids (quercetin equivalent), and 19 mg GAE/g of phenolic (gallic acid equivalent) concentration and the quantity is increasing simultaneously with 2 and 3 gms. The study concluded that PDFE is rich in beneficial plant metabolites and which could be highly useful in the medical field to treat oxidative-stress-mediated diseases.

Key words: Ethnopharmacology, *Pithecellobium dulce*, phenols, flavonoids, functional foods.

Introduction

Ethnopharmacology is broadly defined as “the interdisciplinary scientific exploration of the biologically active agents that are traditionally used.” As a result, pharmacology, chemistry, and botany have all contributed to the corpus of research on which the ethno- pharmacological approach is based. This includes field observations, explanations of the application and biological effects of conventional medicines, botanical identification of plant material, and phytochemical and pharmacological investigations. Traditional treatments and their potential consequences have long piqued the interest of many scholars. The evolution of contemporary therapeutic systems has profited significantly from the discovery of new medications derived from natural sources, according to ethnopharmacological research (1-3).

Since ancient times, people have been researching the natural environment, particularly medicinal plants, in an effort to discover new treatments for diseases. Around the world, 80% of people use medicinal herbs to meet their fundamental health needs. India gave rise to the recently rediscovered traditional medical systems like Siddha, Ayurveda, and Unani. Traditional medical systems are constructed with a single plant or combinations of multiple plants. Key elements in establishing this efficacy include our current understanding of the taxonomic traits of plant species, plant parts, and the biological qualities of medicinal plants, which in turn depend on the existence of primary and secondary

metabolites (4,5).

Recent years have seen a surge in interest in phytochemical and pharmacological studies of traditional medicinal plants. Furthermore, a great deal of preclinical and clinical research has examined the biological activity potential of natural medicines, demonstrating a diversity of biological impacts of compounds derived from plants across a wide range of chemical classes. The bulk of natural sources whose active components are now used for an ethnomedical function. Many pharmaceutical companies have recently changed their strategies in the field of natural product research to uncover new sources and potential molecules for medicinal development. Ethnopharmacological knowledge may be useful for the discovery and development of novel, secure, and affordable medicines due to its methodology, which may be supported by experimental evidence (1).

Vegetables and fruits are heavily marketed for their health benefits. The 2010 Dietary Guidelines for Americans recommend that half of your plate consist of fruits and vegetables. Fruits and vegetables should make up half of the plate, according to Myplate.gov. Variable levels of nutrients and energy can be found in a wide variety of plant foods, including fruits and vegetables. Dietary fiber, which is linked to a lower risk of obesity and cardiovascular disease, is also found in fruits and vegetables. Medicinal plants, fruits, and vegetables are good sources of phytochemicals, which include anti-inflammatory, phytoestrogenic, and antioxidant effects in addition to vitamins and minerals (6-10).

In the present study, an attempt was made to reveal the phytochemical profile of *Pithecellobium dulce* fruit peel extract. *P. dulce*, or Manila tamarind, is a member of the Fabaceae plant family. *P. dulce* is a plant that thrives in tropical and subtropical climates, frequently in wetlands with little rainfall. It can be found in Asia, Africa, and South America. It can be considered an invasive species in Australia and New Zealand, where it is also present. There is limited information available specifically regarding the medicinal importance of the outer covering of *P. dulce*. However, various parts of the plant, including the fruit and bark, have been traditionally used in folk medicine for their potential therapeutic properties (11, 12).

In this study, *P. dulce* fruit peel extract was extracted using ethanol by cold maceration technique. The qualitative phytochemical profile of *P. dulce* fruit peel extract was unveiled by determining the various primary and secondary metabolites, which include carbohydrates, amino acids and proteins, alkaloids, phenols, tannins, saponins, flavonoids, glycosides, and anthraquinones. Also, the quantitative phytochemical profile of *P. dulce* fruit peel extract was unveiled by determining the various primary and secondary metabolites, which include carbohydrates, proteins, phenols, and flavonoids.

Materials and Methods

Chemicals and reagents

Ethanol (99.99%), copper sulfate, chloroform, distilled water, Benedict's solution, Wagner's reagent, ferric chloride, lead acetate, Folin's-Ciocalteau reagent, ninhydrin reagent, sodium bicarbonate, sodium hydroxide, gallic acid, sodium nitrite, hydrochloric acid, aluminum chloride, quercetin, and other chemicals of fine grade were obtained from Merck Millipore, Bengaluru, India.

Pithecellobium dulce fruits collection and preparation of peel extract by cold maceration technique

In close proximity to Guntur, Andhra Pradesh, India, the *P. dulce* fruits were collected. The fruits have been identified and preserved. The fruit peel was removed with a sterile knife after the fruits had been cleansed with double-distilled water and left to dry for 15 days in the shade before being electrically mixed into a fine powder. In a clean, amber airtight container, the powder was kept for the preparation of the ethanolic extract.

Then, 100 g of finely powdered *P. dulce* fruit peel underwent a cold maceration process utilizing ethanol as the solvent (13). The resultant mixture was run through Whatman No. 1 filter paper after five days. After being heated to 45 °C using a rotary evaporator, the concentrated filtrate was then placed on glass Petri dishes and left to dry at room temperature. The remaining *P. dulce* fruit peel extract (PDFE) was placed in an amber vial that was tightly sealed for further analysis once the moisture had been eliminated.

Qualitative phytochemical profile

Carbohydrate analysis - Benedict's test

A volume of 0.5 mL of PDFE was combined with 5 mL of Benedict's solution, which was then heated in a water bath. A precipitate that is red, yellow, or green means there are reducing sugars present (14).

Alkaloids analysis - Wagner's test

A reddish-brown precipitate that forms after adding a few drops of Wagner's reagent to about 10 mg of PDFE suggests the presence of alkaloids (14).

Phenols analysis - Ferric chloride test

Briefly, 5 mg of the PDFE was diluted in 0.5 mL of a 20% sulphuric acid solution for the sodium hydroxide test. It becomes blue after being added a few drops of an aqueous sodium hydroxide solution, indicating the presence of phenols (14).

Tannins analysis - Lead acetate test

Briefly, 0.5 mL of 1% lead acetate solution was added to ten mg of PDFE, and a precipitate formed, indicating the presence of tannins and phenolic chemicals (14).

Saponins analysis - Honeycomb test

Briefly, the test tube containing 0.5 mg of PDFE had a few drops of 5% sodium bicarbonate solution added to it. The mixture was violently mixed and left for 3 min. Saponins can be detected by the formation of froth that resembles a honeycomb (14).

Flavonoids test - Shinoda test

Briefly, a pinch of magnesium turnings was combined with 10 mg of PDFE and 1-2 drops of strong hydrochloric acid. The development of a pink hue denotes the presence of flavonoids (14).

Proteins test - Biuret test

Briefly, two drops of 1% copper sulfate solution and an equivalent volume of 40% NaOH solution were added to 0.5 mg of PDFE. The presence of protein is indicated by the emergence of violet color (14).

Glycosides test

Briefly, an aqueous NaOH solution was added after 0.5 mg of PDFE had been dissolved in 1 mL of water. The development of a yellow hue denotes the presence of glycosides (14).

Anthraquinones test - Borntragers test

Briefly, a dry test tube was filled with approximately 0.5 g of the PDFE, and 5 mL of chloroform, and was shaken vigorously for 5 min. After filtering the extract, an equal volume of 10% ammonia solution was mixed into the filtrate. Anthraquinone is present when the bottom layer is colored pink, violet, or red (14).

Amino acids test - Ninhydrin test

Briefly, two freshly made drops of 0.2% ninhydrin reagent were heated and applied to around 0.5 mg of PDFE. Proteins, peptides, or amino acids are present when pink or purple colouring appears (14).

Quantitative phytochemical profile

Carbohydrate determination

A concentration of 100 mg of PDFE was hydrolyzed in a boiling tube using 5 mL of 2.5 N HCl over the course of three hrs. Glucose was used as a standard carbohydrate. The mixture was cooled to room temperature, and when the

effervescence ceased, solid sodium carbonate was added. A volume of 100 ml of the supernatant was produced using distilled water following centrifugation of the contents. The volume was created by pipetting 0.2 mL of the sample out of this and mixing it with 1 ml of distilled water. After that, 5.0 mL of sulfuric acid and 1 mL of phenol reagent were added. Tubes were kept at 25 – 30 °C for 20 min. An absorbance measurement was made at 490 nm using a plate reader (Synergy H1, BioTek, USA) (14).

Protein determination

The PDFE (up to 10 mg) was centrifuged at 7,000 rpm for 10 minutes after being stirred with 50 cc of 50% methanol (1:5 w/v) at 25 °C for 24 hrs. Pipette out 0.2 mL of the PDFE, then add 1.0 mL of distilled water to the mixture. All of the tubes received 5.0 mL of alkaline copper reagent, which was then left to stand for 10 min. Folin's-Ciocalteau reagent was then added, and the mixture was let to sit for 30 min in the dark. At 660 nm, the color's intensity was measured using a plate reader (Synergy H1, BioTek, USA) (14). The protein concentration was expressed as equivalent to bovine serum albumin (mg/g).

Total flavonoid determination

The flavonoid concentration was determined by a slightly modified colorimetry method published by Gunti et al. (15). A volume of 0.5 mL of a 2 mg/2 mL adequately diluted sample solution PDFE (up to 10 mg) was mixed with 2 mL of distilled water before being added to 0.15 mL of a 5% $NaNO_2$ solution. A volume of 0.15 mL of a 10% $AlCl_3$ solution was added to the mixture and allowed to stand for 6 min before 2 mL of a 4% NaOH solution was added. To make the final volume 5 mL, water was added right away. The mixture was then thoroughly mixed and allowed to stand for an additional 15 min. The mixture's absorbance was calculated at 510 nm using a plate reader (Synergy H1, BioTek, USA) and compared to a water blank. Quercetin equivalents per gram of PDFE (mg QUE/g) were used to express the results.

Total phenolics determination

The total phenolic content of PDFE was measured using the Folin-Ciocalteau procedure with some modifications of Nagaraj et al. (16). Briefly, 1 - 10 mg of PDFE and volume was adjusted to 2 mL with distilled water and 1 mL of Folin-Ciocalteau reagent was added in a tube, and then 1 mL of sodium carbonate was supplied. A multimode plate reader was used to measure the absorbance at 765 nm (Synergy H1, BioTek, USA) after the reaction mixture had been incubated for two hours at room temperature. The amount of gallic acid equivalents per gram of PDFE was used to measure the total phenolic content of the test samples (mg GAE/g).

Statistical analysis

Three independent measurements (n = 3) were obtained for each measurement. The data was expressed using the mean and standard deviation. To compare the significant differences between the test samples, appropriate multiple-range Tukey's test and analysis of variance (ANOVA) in a fully randomized design were used. The significance level utilized was the 95% confidence interval ($p \leq 0.05$). The above-mentioned statistical analysis was carried out using the trial version of GraphPad Prism software 8.0

Results and discussion

Qualitative phytochemical profile of P. dulce fruit peel extract

In the present investigation, *P. dulce* fruit peel extract was extracted using ethanol by cold maceration technique (13). The qualitative phytochemical profile of *P. dulce* fruit peel extract was unveiled by determining the various primary and secondary metabolites, which include carbohydrates, amino acids and proteins, alkaloids, phenols, tannins, saponins, flavonoids, glycosides, and anthraquinones. The results were shown in Table 1. The study showed that *P. dulce* fruit peel extract contains carbohydrates, amino acids and proteins, alkaloids,

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phenols, flavonoids, glycosides, and anthraquinones. The tannins and saponins were found to be absent in *P. dulce* fruit peel extract.

Table 1: Qualitative phytochemical profile of *P. dulce* fruit peel extract

S. No	Phytochemical Compounds	End Result
1	Carbohydrates	Present
2	Alkaloids	Present
3	Phenols	Present
4	Tannins	Absent
5	Saponins	Absent
6	Flavonoids	Present
7	Proteins	Present
8	Glycosides	Present
9	Anthraquinones	Present
10	Amino acids	Present

Phytochemical content in P. dulce fruit peel extract by quantitative analysis

Carbohydrate content in PDFE

The amount of carbohydrate in *P. dulce* fruit peel extract was estimated in a dose-dependent manner and the results were depicted in Figure 1. The results showed that *P. dulce* fruit peel extract contains a good amount of carbohydrates. The mean values of carbohydrate content were 82.33, 172 and 249 mg was found in 1, 2 and 3 grams of P. dulce fruit peel extract respectively. From the figure it is evident that ANOVA and Tukey"s tests disclose that there is a significant difference between the amount of test sample at 95% confidence ($p \leq 0.05$) shown in a bar graph with different alphabets.

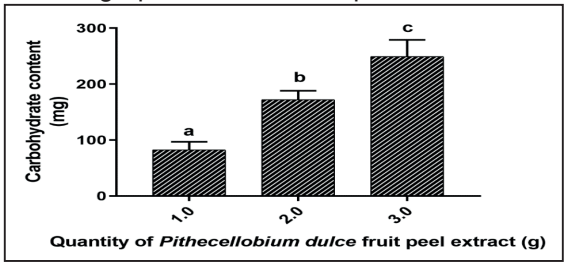

Figure 1: Carbohydrate content in *P. dulce* fruit peel extract

Protein content in PDFE

The findings of a dose-dependent estimation of the protein content in *P. dulce* fruit peel extract is shown in Figure 2. The outcome demonstrated that *P. dulce* fruit peel extract has significant protein content. The mean values of protein content in P. dulce fruit peel extract were 38, 78 and 123 mg were found in 1, 2 and 3 grams respectively.

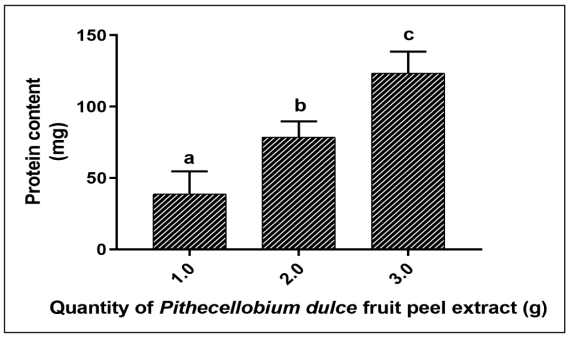

Figure 2: Protein content in *P. dulce* fruit peel extract.

From the figure it shows that ANOVA and Tukey"s test reveal that there is a significant difference between the amounts of test sample at 95% confidence ($p \leq 0.05$) are shown in bar graph with different alphabets.

Flavonoid content in PDFE

Figure 3 displays the results of a dose-dependent assessment of the total flavonoid concentration in *P. dulce* fruit peel extract. The findings showed that there is a substantial amount of flavonoids in the fruit peel extract from *P. dulce* and which could be useful for various biological applications. The mean values of flavonoids content 26, 55 and 73 mg was found in 1, 2 and 3 grams of P. dulce fruit peel extract samples.

ANOVA and Tukey"s test disclose that there is a significant difference between the amounts of test sample at 95% confidence ($p \leq 0.05$) are shown in bar graph with different alphabets.

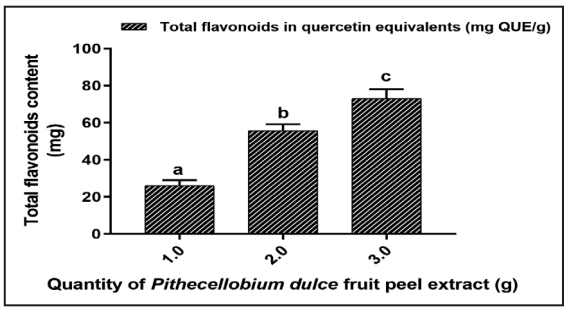

Figure 3: Determination of total flavonoids in *P. dulce* fruit peel extract.

Phenolic content in PDFE

The total phenolics concentration in *P. dulce* fruit peel extract was determined using a dose-dependent analysis, and the findings are shown in Figure 4. The results demonstrated that the fruit peel extract from *P. dulce* contains a significant amount of phenolics, which may be valuable for a variety of biological applications. The mean values of phenolic content were

19, 35 and 64 mg was found in 1, 2 and 3 grams of P. dulce fruit peel extract respectively. ANOVA and Tukey"s test reveal that there is a significant difference between the amount of test sample at 95% confidence ($p \leq 0.05$) are shown in bar graph with different alphabets.

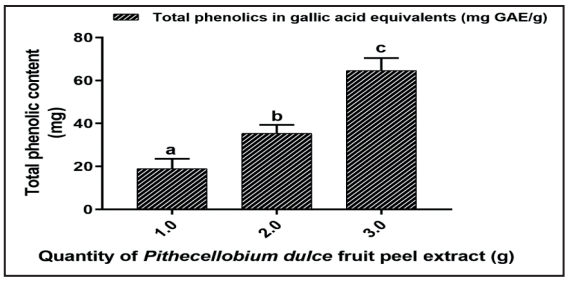

Figure 4: Dose-dependent determination of total phenolics in *P. dulce* fruit peel extract.

As a result, the findings of the present study suggest that *P. dulce* fruit peel extract has the potential to serve as a source of valuable pharmaceuticals due to the presence of numerous phytochemical components including phenols, anthraquinones, flavonoids, alkaloids, and glycosides. Because of their constantly expanding diversity, high level of biological specificity, and potential to operate in additive or synergistic ways, these secondary metabolites have been the source of lead compounds in drug development. The obtained results in the present investigation are quite positive, but they need to be validated by sophisticated techniques before being put into practice.

Current worries about the harmful effects of synthetic chemicals used in medicine have stimulated research efforts globally on natural products and derived structures that may be helpful in the development of alternative treatments for the majority of prevalent ailments linked to the aging process. With the potential, to reduce the consumption of some harmful synthetic antioxidants, phenolic derivatives, flavonoids, and isoflavones have been shown to play preventative roles against degenerative diseases brought on by free radical action. Small modifications to the basic representative structure have been shown to have a significant qualitative and quantitative impact on the biological activity of the majority of secondary metabolite families. Phenolics, flavonoids, terpenoids, and derivatives of nitrogen and sulfur are among those families that are now being studied for their potential to prevent or treat a variety of human disorders (17, 18, 19, and 20).

Conclusion

The study concluded that carbohydrates, amino acids, proteins, flavonoids and phenolic compounds were present in PDFE ethnol extract. Hence, *P. dulce* fruit peel extract could be highly useful for the treatment of various oxidative stress-mediated diseases. However, employing sophisticated analytical techniques, the complete chemical profile of *P. dulce* fruit peel extract must be revealed in order to carry out its functional properties.

Conflict of interest

The authors declare that no conflict of interest

Acknowledgment

The authors were thankful to Acharya Nagarjuna University for providing the facility.

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