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Assessment of Total Flavonoid Content and Antioxidant Activity of Mango Pulp Extracts

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ABSTRACT

Antioxidants can control the progression of many chronic diseases by shielding the body from the oxidation caused by free radicals. Antioxidant properties of flavonoids enable them to function as defences against free radicals. Two extraction procedures, such as maceration and Microwave-Assisted Extraction (MAE), are used to extract flavonoids from mango pulp. Total flavonoid content is estimated by total flavonoid content assay as milligrams of Quercetin Equivalent (QE). Antioxidant activity of extract of mango pulp in four different solvents using two extraction techniques can be estimated by the Ferric Reducing Antioxidant Power (FRAP) assay. Antioxidant activity of pulp extracts has been expressed as mg of Ascorbic Acid Equivalent (AAE). All four solvent extracts of mango pulp have shown high amounts of total flavonoid contents as well as high reducing power. Hence, it may be concluded that eating mango pulp can render its antioxidant activity against free radical-associated diseases due to the presence of flavonoids as bioactive compounds.

Keywords: *Extraction Methods; Ferric Reducing Anti-Oxidant Power (Frap) Assay; Flavonoids; Maceration; Microwave-Assisted Extraction (Mae); Total Flavonoid Content Assay*

Introduction

Plants contain secondary metabolites called flavonoids, which serve a variety of purposes in their organs. Vegetables, wine, fruits, and drinks (tea) have all been claimed to contain them. Flavonoids' chemical structures are made up of C6-C3-C6 rings, which stand for two aromatic rings A and B, connected by three carbon atoms. A third ring (C) develops as a result (Jucá *et al*., 2020). The several subclasses of flavonoid substances are derived from variations in this fundamental structure. These include chalcones, flavonols, anthocyanins, flavonones, isoflavones, and flavones (catechins). According to reports, the kingdom Plantae has about 10,000 distinct types of flavonoids (Ekalu & Habila, 2020).

Numerous biological investigations have demonstrated the health benefits of flavonoids, including the prevention of disease. They have demonstrated anti-inflammatory, antioxidant, antibacterial, antiviral, and antiallergic qualities as well as anticarcinogenic ones (Mir *et al*., 2024). Flavonoids have been shown to have protective effects via

lowering oxidative stress in the body. The hydroxyl group in flavonoids is more important for these biological actions.

Compounds known as antioxidants delay or stop oxidation in living cells. They counteract free radicals' actions. The body is shielded from reactive oxygen species by flavonoids. Flavonoids are chemically capable of scavenging free radicals because of their highly conjugated π-electron system and hydroxyl groups. Flavonoids' antioxidant properties enable them to function as defences against free radicals. Antioxidants slow the progression of many chronic diseases (Basu, 2020; Tungmunnithum *et al*., 2018) by shielding the body from the oxidation caused by free radicals.

Reactive oxygen species (ROS) are created when electron acceptors, including molecular oxygen, react quickly with free radicals. These ROS comprise non-radical hydrogen peroxide (H_2O_2), hydroxyl radicals ($.OH^-$), and free radicals with unpaired electrons, e.g. superoxide anions. Enzymatically produced in aerobic living organisms, ROS are vital cellular components that are crucial to a variety of physiological and pathological processes. Excess ROS can harm cellular macromolecules including DNA, proteins, as well as lipids. This is primarily caused by external factors like radiation, infections, UV light, medications, etc. Nearly 200 illnesses, including cancer, diabetes mellitus, atherosclerosis, hypertension, cardiovascular disease, ischemia, neurological diseases such as Parkinson's disease, Alzheimer's disease (Ji *et al*., 2025), and rheumatoid arthritis, may be linked to oxidative stress.

Antioxidants are compounds that, when present in low quantities, considerably slow down or stop the oxidation of an oxidisable substrate. Numerous health issues, such as infectious, systemic, and neurological disorders, have been demonstrated to respond well to antioxidant treatment. Flavonoids' distinct antioxidant qualities make them the most studied class of phytochemicals.

One of the most widely consumed tropical fruits worldwide is the mango (*Mangifera indica* L.). It is valued for its nutritional content as well as its sensory qualities. In addition to being high in phytochemicals such as phenolic compounds, flavonoids, and carotenoids, mango pulps also supply energy, dietary fibre, carbs, protein, and lipids. The medicinal qualities and high antioxidant activity of these bioactive chemicals make them fascinating (Basu, 2024).

Identifying and characterising flavonoids is the most difficult part of the extraction process, which is dependent on the polarity of the solvents and involves solvent extraction using a variety of solvents and an appropriate isolation methodology. To the best of our knowledge, this is the very first study to extract and characterise flavonoids from the Himsagar variety, despite the fact that other research groups have examined antioxidant activity and the phenolic content's reducing effect on various mango components (Umamahesh, Sivudu & Reddy, 2016; Fonseca *et al*., 2011; Ma *et al*., 2011).

Methodology

Plant Material

Fresh pulp samples of *Mangifera indica* L. are collected and cut into pieces to perform extraction of bioactive compounds.

Extraction Procedure

The process used to extract flavonoids from plant material is important and is determined by the objectives of the study. Typically, methanol, ethanol, acetone, acetonitrile, or their combinations with water are used to extract flavonoids. The type of flavonoid determines the necessary solvent polarity. Acetone, methylene chloride, chloroform, and diethyl ether are suitable solvents for less polar flavonoid structures such as flavanones, isoflavones, and flavones; alcohol or an alcohol–water mixture is typically used for more polar flavonoid fractions (Rodríguez De Luna, Ramírez-Garza & Serna Saldívar, 2020).

Optimization of Extraction Condition

The extraction techniques are classified into two groups: modern techniques (extraction by pressurised liquid, ultrasound, microwaves, enzyme assistance, supercritical fluid, matrix solid-phase dispersion, etc.) and conventional techniques (reflux, maceration, and Soxhlet extraction).

The conventional methods are simple to use, don't require specialised tools, may be used to extract a lot of samples, and produce a high yield of extract. They aren't selective, though. Depending on the polarity property of the target flavonoid fraction, the appropriate solvent can provide a certain level of selectivity (Tzanova *et al*., 2020).

Maceration

The process of maceration involves soaking the plant material in an appropriate solvent in a stoppered container. The sample is shaken regularly and incubated at room temperature for at least three days. The soluble phytochemicals are released as the solvent breaks down and softens the plant's cell wall. The mixture is then filtered after that.

Green Extraction Techniques

Long extraction times, expensive solvent requirements, massive solvent evaporation, poor extraction selectivity, and automation challenges are the main drawbacks of classical extraction methods. The limits of traditional extraction technologies are being overcome by the development of innovative and promising procedures (Chávez-González *et al.*, 2020; Chaves *et al.*, 2020). They fall within the category of unconventional, environmentally friendly extraction methods. They offer numerous benefits: a decrease in the amount of used organic solvents and sample deterioration,

avoidance of contamination, removal of superfluous sample concentration and cleanup procedures, enhancement of extraction efficiency and selectivity, and automation potential.

Enzyme-assisted extraction (EAE), pressurised liquid extraction (PLE), ultrasound-assisted extraction (UAE), supercritical fluid extraction (SFE), microwave-assisted extraction (MAE), and matrix solid-phase dispersion (MSPD) are a few of the most promising techniques.

Microwave-Assisted Extraction (MAE)

The extraction method known as MAE encourages the analytes to pour out of the sample into the solvent by using microwave power. Polar and polarisable materials' dipoles are affected by microwave radiation, which heats the materials close to their surface. Conduction is how the heat is transmitted. When dealing with polar compounds and liquids that have a high dielectric constant, MAE might be regarded as a selective technique. In contrast to traditional extraction techniques, MAE provides a number of benefits: enhanced recovery, decreased extraction time, solvent volume, energy and processing costs, and the potential to utilise fewer hazardous solvents. However, only tiny compounds that are stable under microwave heating conditions can be used using this approach.

Mango peels are removed by using a sharp knife, and the underlying mango pulp is recovered by scraping with the sharp edge of the knife, and the pulp is washed with distilled water and air-dried. 8.5 gm, 7.4 gm, 6.9 gm and 7.1 gm of mango pulp are successively extracted by maceration with water, methanol, acetone and ethanol, respectively, for 72 h stirring. A second extraction method, Microwave-Assisted Extraction (MAE), is carried out with 5.6 gm, 7.0 gm, 5.6 gm and 6.3 gm of mango pulp with the same four solvents, respectively. The obtained extracts are stored under dark conditions until biological assay.

Determination of Total Flavonoids Content

According to Pękal and Pyrzynska's description (Pękal & Pyrzynska, 2014), aluminium chloride is used to colorimetrically assess the total flavonoid concentration. Typically, quercetin is utilised as a standard. Methanol is used to dissolve 10 mg of quercetin, which is further diluted to 10, 20, 50, and 100 µg/ml. After vigorously shaking the diluted standard solution (1.0 ml) and sample solutions with 1.0 ml of water and 0.5 ml of 2% (w/v) aluminium chloride, they are incubated for 30 minutes at room temperature. For 300–600 nm, the absorbance is estimated using a blank. Except for test samples (extracts, standard), all reagents are added in blank. The milligrams of quercetin equivalent per gram of dry mass (mg of QE/g) are calculated to indicate the overall flavonoid content. In another procedure, 1 ml sample solutions are added with 0.3 ml $NaNO_2$ (5% w/v) and kept for 5 minutes. Then 0.5 ml of 2% (w/v) aluminium chloride is mixed with the mixtures, and after 6 minutes, 0.5 ml of 1M NaOH solution is added. The whole mixtures are incubated at room temperature for 10 minutes and finally scanned

for 300-600 nm.

Ferric-Reducing Antioxidant Power (FRAP) Assay

The change of iron (III) to iron (II) in the presence of plant extracts using two distinct extraction methods, water, methanol, acetone, and ethanol, is the basis for the reducing power. The development of Perl's blue at 700 nm can be used to measure iron (II). With a few minor adjustments (Oyaizu, 1986), the process approach outlined by Saeed, Khan and Shabbir (2012) is used to reduce ability. In short, 1 millilitre of the aforementioned extracts (100 µg/ml) is combined with 2.5 millilitres of phosphate buffer (0.2 M, pH 6.6) and 2.5 millilitres of potassium ferricyanide (10 mg/ml) using Vertex. After 20 minutes of incubation at 50°C, 2.5 millilitres of trichloroacetic acid (100 mg/l) are added to the solutions.

The mixtures are centrifuged for 10 minutes at 3000 rpm in order to obtain the supernatant layer. 2.5 ml of each of the aforementioned mixes is combined with 0.5 ml of 0.1% (w/v) fresh ferric chloride solution and 2.5 ml of distilled water. The absorbances are measured at 700 nm following a 10-minute incubation at room temperature. The calibration curve is made with standard ascorbic acid. In triplicate, the iron (III) reducing activity is measured and reported as µMol Ascorbic Acid Equivalent (AAE)/g of extract. A higher reducing power is demonstrated by an increase in absorbance.

Results and Discussion

Total Flavonoids Content

A quercetin standard solution is considered as a guide to correlate between quercetin concentration and its absorbance value on a calibration curve, as shown in Figure 1. In order to prove the existence of active compounds in mango pulp, the content analysis had been done targeting flavonoids using a colorimetric assay. A colorimetric assay using aluminium chloride is a method that can detect flavonoids in the flavone and flavanol groups of flavonoids. Aluminium chloride in the assay will react with flavonoid and create a stable, colour-signature complex. The reaction involved in the keto group at carbon number 4 and the hydroxyl group in either carbon number 3 or 5 forms an acid-stable complex; meanwhile, the reaction within the ortho-dihydroxyl group present in the A- and B-rings of a flavonoid could also be responsible for several acid-labile complexes. These resulting complexes formed by flavone have a maximum absorbance at around 400 nm. By applying an aluminium chloride colorimetric experiment to the extracts of mango pulps, the total flavonoid content can be detected. Using the calibration equation ($y = 0.0005x + 0.2762$) and a correlation coefficient of 0.9012, the quercetin concentration of the samples can be calculated.

The Total Flavonoid Content (TFC) for each sample for various extraction methods can be calculated using Equation 1 as milligrams of Quercetin Equivalent (QE) per gram of mango pulp by multiplying the quercetin concentration of the sample solution in µg/ml (C) by the volume of the sample solution in mL (V) and then dividing it by pulp mass expressed in grams (W).

TFC= CXV/WX1000 (1)

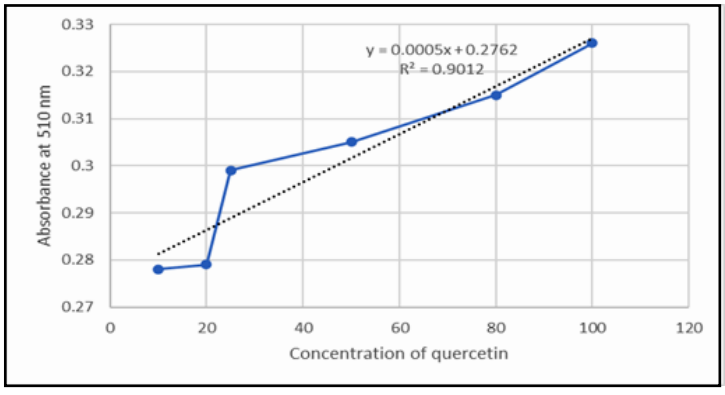

Figure 1: Standard Curve of the Quercetin Solutions

Ferric Reducing Antioxidant Power (FRAP) Assay

The reducing power method estimates the electron donation ability of the antioxidant agent present in the extracts for conversion of ferric ion Fe^{3+} into ferrous form Fe^{2+}. The amount of Fe^{2+} complex (formed as Pearl's Prussian blue colour complex) is calculated at 700 nm (Amarowicz *et al*., 2010), and the increase in absorbance is proportional to the increase in the reducing power activity of the sample solution. The antioxidant activity of different extracts of mango pulp can be calculated with a standard curve of ascorbic acid, as shown in Figure 2. The results of the FRAP assay are often expressed as AAE, which represents the concentration of ascorbic acid (vitamin C) that would produce the same absorbance change as the sample being tested. The reducing power is shown to be in the following order: Ethanolic extract from MAE > Methanolic extract from MAE > Methanolic extract from maceration > Aqueous extract from MAE > Ethanolic extract from MAE > Acetone extract from maceration > Aqueous from maceration, respectively. Considering the reducing power of bioactive compounds using the FRAP assay, the MAE technique has higher extraction power compared to maceration.

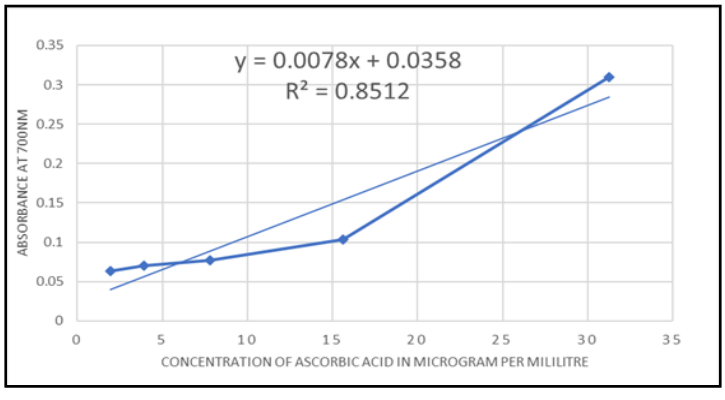

Figure 2: Standard Curve of Ascorbic Acid Solutions

Conclusion

The present study shows that all four solvent extracts of mango pulp for two different extraction methods have shown high amounts of total flavonoid contents, which can impart antioxidant activity. Hence, it may be concluded that eating mango pulp can render its antioxidant activity against free radical-associated diseases due to the presence of flavonoids.

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