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Assessment of free radical scavenging potential of selected fruit extracts by DPPH method

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Abstract
This study explores the antioxidant potential of various fruits using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay to better understand how fruit-based antioxidants contribute to human health. Antioxidants are vital in neutralizing free radicals that can lead to chronic conditions such as heart disease, cancer, neurodegenerative disorders, and inflammation. The DPPH method is commonly used because it is both simple and effective in measuring antioxidant capacity. It works by observing changes in absorbance at 517 nm as antioxidants interact with DPPH radicals. Results revealed that *Mangifera indica* (mango) showed the highest antioxidant activity at around 95%. Other fruits, including *Actinidia deliciosa* (kiwi), *Carica papaya* (papaya), *Prunus domestica* (plum), *Syzygium cumini* (jamun), *Malus pumila* (apple), *Musa acuminata* (banana), and *Citrus limetta* (sweet lime), also demonstrated high antioxidant levels above 90%. These findings reinforce the importance of fruit-rich diets and support further research into natural antioxidants and their role in disease prevention.

Keywords: Scavenger, DPPH(2,2-diphenyl-1-picrylhydrazyl), Antioxidants, Reactive oxygen species (ROS)

Introduction
As an aerobic organism, we do need Oxygen, but excess of it can become dangerous and threat. Although molecular oxygen in its stable form is not highly reactive, its partial reduction leads to the formation of reactive oxygen species (ROS) such as singlet oxygen, superoxide anion, and hydrogen peroxide. These compounds contribute to oxidative stress, which refers to the harmful impact of oxidants on normal physiological processes. Oxidative stress plays a key role in causing various human diseases, such as cellular necrosis, cardiovascular disease, cancer, neurological disorder, Parkinson's dementia, Alzheimer's disease, inflammatory disease, muscular dystrophy, liver disorder, and even aging [1].
Moreover, oxidative damage to DNA is believed to be a major factor in the aging process. The accumulation of oxygen inside cells can also trigger unwanted chain reactions that harm essential biomolecules. Due to their high reactivity, these radicals pose harm and threat that leads to oxidative stress in we Humans, to manage this, organisms have evolved multiple defence strategies [2]. Several natural processes within the human body lead to the formation of free radicals as secondary products. These include energy production, the breakdown of fats, and the release of catecholamines during stress, and responses linked to inflammation [4] Antioxidants are compounds that protect cells by inhibiting the oxidative damage caused by free radicals [3]. Free radicals, which are atoms or molecules with unpaired electrons, are highly unstable and tend to react quickly with other substances, often leading to the formation of more radicals. In plants, antioxidants play a key role in neutralizing these reactive molecules [2]. It Function as agents that inhibit oxidation processes, even when present in small amounts, and therefore contribute to a range of physiological activities in the body. The antioxidant compounds found in plants act as radical scavengers, neutralizing reactive molecules by converting them into less harmful forms. To combat oxidative stress, plants have evolved complex defence mechanisms collectively known as the antioxidant system. This system is composed of both enzymatic and non-enzymatic components. The non-enzymatic antioxidants include substances such as vitamin C (ascorbic acid), alpha-tocopherol, and carotenoids. On the other hand, the enzymatic defenses comprise enzymes like superoxide dismutase (SOD),

catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX), glutathione reductase (GR), and polyphenol oxidase (PPO). These antioxidant systems play a crucial role in detoxifying reactive oxygen species, thereby enabling plants to withstand and adapt to oxidative stress conditions.

Since certain antioxidants, like vitamin E, β-carotene, and vitamin C, are micronutrients that the human body cannot produce on its own. Therefore, they need to be obtained through a regular diet [6].

Table 1: Comparative analysis of antioxidant compounds and health benefits in common fruits.

Fruit	Antioxidant found	Health benefits
Mango	Vitamin E, Beta-Carotene,	Protects cells, supports immunity and eye health, maintains skin integrity, and helps prevent aging and heart disease.
Kiwi	Vitamin C, Vitamin E	Boosts immunity, aids healing, protects cells, and reduces aging and heart disease risk.
Papaya	Vitamin C, Beta-Carotene	Boosts immunity, supports skin and eye health, aids collagen formation and wound healing, protects against oxidative stress, and improves iron absorption
Plum	Anthocyanins	Reduces inflammation, enhances cognitive function, promotes heart health, and may lower the risk of some cancers
Jamun	Anthocyanins	Reduces inflammation, enhances cognitive function, promotes heart health, and may lower the risk of some cancers
Apple	Quercetin	Helps relieve allergies, supports respiratory health, regulates blood pressure, and promotes cardiovascular well-being
Banana	Carotenoid, dopamine, phenolic acids and vitamin c	Support eye and skin health, enhance mood and brain function, reduce inflammation, protect against oxidative stress, and boost immune defence.
Mousami	Flavonoids, Quercetin and vitamin c	provide antioxidant and anti-inflammatory effects, boost immunity, and support heart and skin health
Pomegranate	Flavonoids	Combats oxidative stress and inflammation, promotes cardiovascular health, enhances vascular function, and helps prevent chronic illnesses
Lemon	Vitamin C	Enhances immunity, supports collagen production, defends against oxidative damage, aids iron uptake, and accelerates wound recovery
Pine apple	Flavanoid, vitamin c and phenoloc acids	Offer antioxidant protection, reduce inflammation, support immune function, and promote overall cardiovascular and skin health
Pear	Anthocyanins, flavanols	Support heart health, provide antioxidant protection, reduce inflammation, and improve blood vessel function
Cucumber	Flavanoids, tannin, Beta-Carotene	Provide antioxidant and anti-inflammatory effects, support immune health, protect against cell damage, and promote eye and skin health.

Materials and Methods

This study employed the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay to evaluate the antioxidant potential of various fruit juice samples. Emphasis was placed on careful sample preparation, accurate formulation of DPPH solutions, and standardized measurement procedures to ensure reproducibility and reliability of the results. Standardization protocols were strictly followed to enhance the assay's credibility and comparability across different trials [6].

Materials

The fruit samples used in this study included the fresh juices of the following plant species:

1. *Mangifera indica (mango)*
2. *Carica papaya (papaya)*
3. *Cucumis sativus (cucumber)*
4. *Musa acuminata (banana)*
5. *Ananas comosus (pineapple)*
6. *Citrus limon (lemon)*
7. *Malus pumila (apple)*
8. *Actinidia deliciosa (kiwi)*
9. *Citrus limetta (sweet lime)*
10. *Punica granatum (pomegranate)*
11. *Prunu sdomestica (plum)*
12. *Syzygium cumini (black plum/jamun)*
13. *Pyrus communis (pear)*

All fruits were purchased fresh from local markets and washed thoroughly with distilled water before juice extraction. The juice was extracted manually or with the aid of a cold press juicer and then filtered to remove pulp and other solid particles. Extracts were stored in airtight containers at 4 °C and used within 24 hours to maintain freshness and bioactive compound integrity [7].

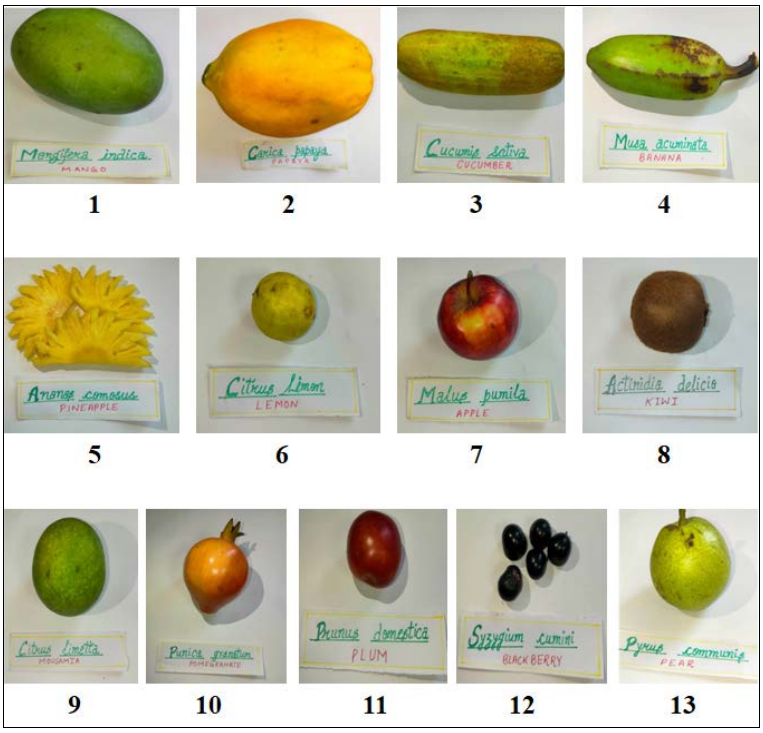

Fig 1: Fruits used in this research

Other chemicals used in the experiment included

Methanol (analytical grade), Ethanol (analytical grade), Distilled water, DPPH (2,2-diphenyl-1-picrylhydrazyl), obtained from a reputable supplier.

Sample Preparation and DPPH Assay Procedure

A 0.1 mM DPPH solution was prepared in methanol and kept in the dark at room temperature until use to prevent degradation. For each fruit juice sample, 100 µL of juice was mixed with 2.9 mL of DPPH solution in a test tube. The mixture was vortexed gently and incubated in the dark at room temperature for 30 minutes to allow for reaction between antioxidants and DPPH radicals [8].

The absorbance of each sample was measured at 517 nm using a UV-Vis spectrophotometer. A blank (methanol only) and a control (DPPH without fruit juice) were also prepared. The percentage of DPPH radical scavenging activity was calculated [9].

Methods

Preparation of Fruit Extracts

Fresh, undamaged fruits were selected for the study, ensuring the absence of microbial infection, physical damage, or decay. Each fruit was thoroughly rinsed with tap water and then wiped with ethanol to eliminate any residual microbial contamination that could not be removed by washing alone. The cleaned fruits were then air-dried at room temperature or in an incubator at 37 °C [10].

Fig 2: Preparation of fruit extract

The edible portions of the fruits were extracted either manually or using a cold press juicer. The resulting juice was homogenized, and any solid residues were removed through filtration. The clear extracts were transferred into 2 mL Eppendorf tubes and stored at 4°C until further analysis.

The fruits used in this study were:

1. *Mangifera indica* (mango)
2. *Carica papaya* (papaya)
3. *Cucumis sativus* (cucumber)
4. *Musa acuminata* (banana)
5. *Ananas comosus* (pineapple)
6. *Citrus limon* (lemon)
7. *Malus pumila* (apple)
8. *Actinidia deliciosa* (kiwi)

9. *Citrus limetta* (sweet lime)
10. *Punica granatum* (pomegranate)
11. *Prunu sdomestica* (plum)
12. *Syzygium cumini* (black plum/jamun)
13. *Pyrus communis* (pear)

Preparation of DPPH Stock Solution

A DPPH stock solution was prepared by dissolving 20 mg of DPPH [8] (2,2-diphenyl-1-picrylhydrazyl) in 100 mL of methanol, resulting in a concentration of 0.2 mg/mL (approximately 0.5 mM). The solution was stored in a dark bottle at room temperature to prevent light-induced degradation [11].

Preparation of Control and Test Solutions

A control was prepared using 3.0 mL of the DPPH solution without any fruit extract to serve as the baseline absorbance value. For each fruit sample, 100 µL of fruit extract was transferred into a 2 mL Eppendorf tube and mixed with 2.9 mL of DPPH solution, resulting in a total volume of 3.0 mL [12].

Incubation

The mixtures were vortexed to ensure proper mixing and allowed to incubate at room temperature in the dark for 30 minutes. This incubation period allowed for sufficient interaction between the antioxidants present in the fruit extracts and the DPPH radicals. A visible color change from deep purple to yellow indicated the reduction of DPPH by antioxidants [12].

Measurement of Absorbance

After incubation, the absorbance of each test solution and the control was measured at 517 nm using a UV-Visible spectrophotometer. A decrease in absorbance relative to the control indicated the radical scavenging activity of the fruit extract [8].

Determination of DPPH Scavenging Activity

The antioxidant activity of each fruit sample was evaluated by comparing the absorbance of the test solution with that of the control. A decrease in absorbance indicates the neutralization of DPPH radicals by antioxidants present in the sample. The greater the reduction in absorbance, the higher the antioxidant potential of the extract [13].

% Scavenging Activity = [(Abc – Abs)/(Abc)] X 100

where: Abc—Absorbance of control; Abs—absorbance of each sample prepared in DPPH.

Result and Discussion

Table 2. Antioxidant activity and standard deviation of different fruit sample

Sl no	Scientific name	Common name	Family	Antioxidant activity in % (mean value ±standard deviation)
01	*Mangifera indica*	Mango	Anacardiaceous	94.58 ± 0.40
02	*Actinidia deliciosa*	Kiwi	Actinidiaceae	93.64 ± 0.70
03	*Carica papaya*	Papaya	Caricaceae	93.64 ± 0.47
04	*Prunus domestica*	Plum	Rosaceae	92.70 ± 0.476
05	*Syzygium cumini*	Jamun	Myrtaceae	92.70 ± 0.94
06	*Malus pumila*	Apple	Rosaceae	92.47 ± 0.470
07	*Musa acuminata*	Banana	Musaceae	91.76 ± 0.475
08	*Citrus limetta*	Mousami	Rutaceae	91.05 ± 0.71
09	*Punica granatum*	Pomegranate	Lythraceae	90.82 ± 0.47
10	*Citrus limon*	Lemon	Rutaceae	86.58 ± 0.478
11	*Ananas comosus*	Pine apple	Bromeliaceae	65.41 ± 0.472
12	*Pyrus communis*	Pear	Rosaceae	20.94 ± 0.47
13	*Cucumis sativus*	Cucumber	Gourd	19.52 ± 0.94

In this research the antioxidant activity of different fruit extract was assessed using the DPPH method. This technique evaluates the extract's capacity to donate hydrogen atoms to DPPH radicals. A higher donation of hydrogen atoms results in a more noticeable colour shift in the DPPH solution, changing from purple to yellow [14]. The absorbance of the DPPH solution was measured using a UV-Vis spectrophotometer. The absorbance data were then used to calculate the percentage of inhibition.

Among the fruits tested, mango (*Magnifera indica),* exhibited the highest antioxidant activity with 94.58% DPPH radical inhibition at 100 µg/mL concentration followed by *Actinidia deliciosa* (kiwi) 93.64%, *Carica papaya* (papaya) 93.64%, *Prunus domestica* (plum) 92.70%, *Syzgium cumini* (jamun), 92.70%, *Malus pumila* (apple) 92.47%, *Musa acuminate* (banana) 91.46% and *Citrus limetta* (sweet lime) 91.05% suggesting their potential as significant sources of natural antioxidants. The results highlight the role of these fruits in protecting against disease caused by oxidative stress and emphasize the health benefits of including antioxidant- rich foods in the diet.

Conclusion

With the growing emphasis on natural healthcare solutions, plant-based products have emerged as key contenders in therapeutic practices. A considerable portion of the global population now opts for herbal and plant-derived medicines, primarily due to their affordability and fewer side effects when compared to synthetic pharmaceuticals. This increasing preference is driven not only by cost and safety considerations but also by a heightened understanding of the long-term health advantages offered by plant compounds. Traditionally, medicinal plants have been instrumental in treating diseases, especially cancer. The toxic effects of conventional cancer treatments have prompted scientific efforts to identify safer, plant-based alternatives. Bioactive substances and antioxidants found in fruits and vegetables are among the most promising of these, given their role in reducing oxidative stress and enhancing overall health. Although antioxidant levels in produce can vary with factors like variety, climate, and ripeness, their consistent health benefits are well-recognized. For instance, mango—a seasonal fruit—has demonstrated exceptionally high antioxidant activity, suggesting its inclusion in the diet may promote a healthier

lifestyle. The significant antioxidant content in such fruits contributes to the prevention of chronic diseases and aligns with the broader shift toward natural and integrative medical approaches. This underscores the need for continued exploration and innovation in plant-based medicine, reinforcing the importance of diet and nature-derived compounds in advancing human health and longevity.

Joint First Authorship

Ayesha Khatoon, Rishita Mathuria contributed equally to this work.

Conflict of Interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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