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Evaluation of Synergistic Antioxidant Activity of Psidium Guajava and Raw Honey- An Invitro study

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Abstract

Despite the significant advances in modern medicine over the last few decades, plants continue to play an important role in health care. Antioxidant capabilities have been studied in a large range of medicinal plants. Natural antioxidants, whether in the form of raw extracts or chemical components, are extremely efficient in preventing oxidative stress-related damage. The objective of the study is to evaluate the synergistic antioxidant property of P.guajava and raw honey with Butylated hydroxytoluene as a standard comparison agent. The plant extract (P.guajava) was prepared by the cold infusion method. 1ml (1mg/ ml) of Guava leaf extract (GLE) was added to 1ml of honey and mixed well. 1ml of this sample mixture was taken and diluted with sterile distilled water to attain different concentrations of the sample such as 500 µg/ml,400 µg/ml, 300 µg/ml, 200 µg/ml and 100 µg/ml. The percentage of antioxidant activity (AA %) of each substance was assessed by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. This assay was performed with different concentrations of P.guajava and raw honey combination. The test sample showed [42.8%] antioxidant activity at 500 µg/ml concentration whereas the standard agent showed 99.8% activity at 500 µg/ml. So, the standard extract yielded the greatest percentage, while the combination extract [Guava leaf and raw honey] yielded the lowest. From this study, it is evident that the antioxidant property of the test agent (P.guajava and raw honey) is comparatively less significant than the standard. Overall, the combination of guava leaves and raw honey proved effective in developing antioxidant capabilities. These parameters might be adjusted either directly as a solution to create rapid reactive oxygen species mitigation or indirectly to achieve a delayed, sustained antioxidant effect.

Keywords: *Antioxidant, Free Radical Scavenging Assay, Leaf Extract, P.Guajava, Raw Honey.*

Introduction

Atoms containing unpaired electrons are known as free radicals, and they are less stable. Free radicals are produced as a result of oxidative damage, and antioxidants protect cells from the harmful chemicals. Reactive Oxygen Species (ROS) is both productive and destructive, serving a dual role. At low concentrations, ROS can have a favourable impact on immunological activities and cellular responses; yet, greater levels of ROS can cause oxidative stress, jeopardizing cellular integrity [1].

Free radicals are highly reactive molecules with unpaired electrons, making them unstable and prone to react with other cellular components. These unpaired electrons seek to stabilize themselves by stealing electrons from nearby molecules, which can cause cellular damage. This process is known as oxidative damage. Free radicals and other reactive species collectively referred to as Reactive Oxygen Species (ROS), include molecules

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such as superoxide anions ($O_2\bullet^-$), hydroxyl radicals (•OH), and hydrogen peroxide (H_2O_2).

ROS play a dual role in biological systems. At low concentrations, ROS are involved in signaling pathways that regulate various cellular processes, including immune responses and cellular growth. They can activate transcription factors, modulate enzyme activities, and contribute to cellular homeostasis. For instance, ROS are crucial in the oxidative burst during phagocytosis, aiding in the destruction of pathogens.

However, when ROS levels become excessive, they lead to oxidative stress, which occurs when there is an imbalance between the production of free radicals and the body's ability to neutralize them with antioxidants. High ROS levels can damage cellular components such as lipids, proteins, and DNA, ultimately leading to cell dysfunction, aging, and diseases such as cancer, cardiovascular diseases, and neurodegenerative disorders.

Antioxidants work by preventing the formation of free radicals as intermediate components. Lack of antioxidants or suppression of antioxidant enzymes creates oxidative stress, which damages or kills cells. Oxidative stress occurs when there is an unfavourable balance between free radical production and antioxidant defences [2].

Antioxidants are molecules that neutralize free radicals and mitigate oxidative stress. They work by donating electrons to free radicals without becoming unstable themselves, thereby neutralizing their harmful effects. Antioxidants can be categorized into two main types: endogenous and exogenous.

Endogenous Antioxidants: These are produced by the body and include enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase. They play a crucial role in the detoxification of ROS and maintenance of cellular health.

Exogenous Antioxidants: These are derived from external sources, particularly from the diet. They include vitamins (e.g., vitamin C and E), minerals (e.g., selenium and zinc), and phytochemicals (e.g., flavonoids and polyphenols). These compounds are found in fruits, vegetables, nuts, and other plant-based foods.

A deficiency or impaired function of antioxidants can lead to an accumulation of free radicals, resulting in oxidative stress and associated cellular damage. This underscores the importance of maintaining a balanced intake of antioxidants to support the body's defense mechanisms against oxidative damage.

Medicinal plants have long been used to cure and enhance human health, and plants with strong antioxidant properties can be utilized as natural medications to prevent aging and chronic disease. The traditional medicinal plant guava (*Psidium guajava L.*) thrives in hot climates all over the world, including Africa, Asia, Europe and South America. Two of the most prominent traditional uses are dehydration and diarrhoea treatment. Other conditions where it is used include the treatment of gastroenteritis, diabetes mellitus, stomach pain and wounds. It also has antibacterial, anti-inflammatory and antioxidant properties [3, 19].

Medicinal plants have been used for centuries to treat a variety of ailments, owing to their rich content of bioactive compounds with antioxidant properties. These plants can offer natural remedies for preventing and managing chronic diseases associated with oxidative stress. One such plant is guava (*Psidium guajava L.*), which has a long history of use in traditional medicine across various cultures.

Guava leaves include antioxidant-rich phenolic chemicals and flavonoids. Components such as polyphenols and phenols of Guava leaves are responsible for the strong antioxidant property. Gallic acid, caffeic acid, guaijaverin, tannins, carotenoids, and triterpenoids are the principal active ingredients in guava leaves. Several solvents, including water, ethanol, methanol and hydroethanolic acid were used to extract these compounds [4, 20].

Guava is native to tropical and subtropical regions and is widely cultivated in Africa, Asia, Europe, and South America. Traditionally, guava leaves have been used to treat a range of conditions, including:

Dehydration: Due to its high content of vitamins and electrolytes, guava is used to rehydrate and replenish essential nutrients.

Diarrhoea: The astringent properties of guava leaves help in reducing diarrhoea and gastrointestinal discomfort.

Gastroenteritis and Stomach Pain: Guava leaves have been employed to alleviate symptoms of gastroenteritis and stomach pain.

Wound Healing: The antimicrobial and anti-inflammatory properties of guava leaves contribute to wound healing.

Despite the known antioxidant properties of guava leaves and honey, there remains a scarcity of studies focusing on the most effective solvent for extracting antioxidant compounds from guava leaves. The choice of solvent can significantly impact the yield and efficacy of the extracted antioxidants.

The preparation and evaluation of ethanolic extracts of *Psidium guajava* leaves and raw honey provide valuable insights into their antioxidant properties. While both natural products have demonstrated significant antioxidant activity individually, the study emphasizes the importance of selecting appropriate solvents for optimal extraction and efficacy. By understanding and harnessing the antioxidant potential of these natural sources, researchers can develop effective strategies for preventing and managing oxidative stress-related diseases.

Honey's antioxidant activity is derived from enzymes (catalase, glucose oxidase), phenolic acids, vitamins, flavonoids, Maillard reaction products and organic acids which were all available in nascent form in raw form of honey. As a result, customers would profit greatly from the development of honey drops and lozenges which were the honey-based sweets that keep the good health advantages of their antecedents.[5] The antioxidant activity of raw honey is being investigated in this study. However, there is a scarcity of studies on the most appropriate solvent for guava leaves' antioxidant activity. As a result, ethanolic extracts of guava leaves and raw honey were tested and assessed for antioxidant activities in this synergistic investigation. The optimum extraction solvent for guava leaves was chosen for its excellent antioxidant effectiveness.

In this study, the antioxidant activities of ethanolic extracts of guava leaves and raw honey were evaluated. Ethanol was selected as the extraction solvent due to its ability to dissolve a wide range of phytochemicals, including both polar and semi-polar compounds. The comparative assessment of ethanolic extracts aims to determine their effectiveness in scavenging free radicals and enhancing overall antioxidant activity.

Materials and Methods

Preparation of Plant Extract Using Cold Infusion Method: The cold infusion method is a popular technique for extracting bioactive compounds from plant materials, valued for its ability to preserve the integrity of sensitive constituents. In this process, plant material is immersed in a solvent at a relatively low temperature, which helps to minimize degradation of the bioactive components. Here, we will detail the preparation of an ethanolic extract of *P.guajava* leaves using this method[6].

Selection and Preparation of Plant Material

The first step in the extraction process involves selecting and preparing the plant material. For this study, *P.guajava* leaves were chosen due to their known antioxidant properties. The leaves were collected, cleaned thoroughly to remove any dirt or contaminants, and then subjected to drying. Drying is a crucial step as it helps in reducing the moisture content of the leaves, which can otherwise lead to microbial growth or fermentation during the extraction process [6].

Once dried, the leaves were ground into a fine powder using a suitable grinding apparatus. The grinding process is essential for increasing the surface area of the plant material, which enhances the efficiency of the extraction by allowing the solvent to penetrate more effectively.

Preparation of Extraction Solvent

Ethanol was chosen as the extraction solvent due to its effectiveness in dissolving a wide range of polar and semi-polar compounds. Ethanol is also preferred for its relatively low toxicity and ability to extract a broad spectrum of phytochemicals, including phenolic compounds, flavonoids, and essential oils. The choice of solvent is crucial, as it impacts the type and quantity of bioactive constituents that are extracted from the plant material [6].

Cold Infusion Process

The cold infusion method was employed to prepare the plant extract. In this method, 15 grams of the finely powdered *P.guajava* leaves were placed in a clean, closed container. The container was designed to ensure minimal exposure to external contaminants and to maintain the integrity of the extract [6].

150 ml of ethanol were added to the container with the powdered leaves. The mixture was allowed to sit at room temperature for a period of 24 hours. This prolonged soaking period is important as it allows sufficient time for the ethanol to dissolve the bioactive compounds from the plant material.

The cold infusion process operates at room temperature, which helps in preserving heat-sensitive compounds that might be degraded by higher temperatures. Additionally, the low temperature reduces the risk of unwanted reactions that could occur at elevated temperatures, thus preserving the quality and efficacy of the extract.

Filtration and Collection

After the 24-hour infusion period, the mixture was filtered to remove the solid plant residues. This was achieved using a thin cotton fabric, which acted as a filter to separate the liquid extract from the solid plant material. The choice of filtration method is important to ensure that the extract is free from particulate matter and that only the liquid containing dissolved bioactive compounds is collected.

The filtrate, which contains the dissolved plant extracts, was collected in a clean container. This liquid extract now contains a variety of phytochemicals that were present in the *Psidium guajava* leaves, including those responsible for the antioxidant properties.

Benefits of Cold Infusion Method

The cold infusion method offers several advantages:

Preservation of Bioactive Constituents - By avoiding the use of heat, this method helps in preserving sensitive compounds that might otherwise be degraded by higher temperatures.

Simplicity - The process is straightforward and requires minimal specialized equipment, making it accessible for both small-scale and large-scale extraction.

Efficiency - Extended soaking in ethanol ensures thorough extraction of the plant's bioactive components, leading to a more potent extract.

In summary, the cold infusion method employed for preparing the ethanolic extract of P.guajava leaves is effective in extracting and preserving the bioactive constituents of the plant. By using ethanol as the solvent and allowing a 24-hour soaking period at room temperature, this method ensures that the extract retains its antioxidant properties. The resulting extract can be used for further analysis and applications, including evaluation of its antioxidant activity and potential therapeutic uses.

The plant extract was prepared by the cold infusion method [6].In this method, the *P.guajava* leaves were dried and ground into a fine powder and placed in a clean and closed container. The extraction solvent used here is ethanol. 15g of powdered plant material was soaked in 150ml of ethanol at room temperature over 24 hours and filtered using a thin cotton fabric. This method helps to keep the bioactive constituents of the plant intact.

Sample Preparation: 1ml (1mg/ ml) of Guava leaf extract (GLE) was added to 1ml of honey and mixed well. This mixture was used as the sample. 1ml of this sample mixture was taken and diluted with sterile distilled water to attain different concentrations of the sample such as *500* µg/ml, 400 µg/ml, 300 µg/ml, 200 µg/ml and 100 µg/ml.

DPPH Radical Scavenging Assay (Tailor and Goyal 2014) [7].

By using the DPPH free radical scavenging assay, the percentage of antioxidant activity (AA %) of each substance was assessed. Except blank the sample was added to all the tubes in different concentrations. Then 0.3 ml of 0.5 mM DPPH radical solution in ethanol and 3 ml of ethanol were added. The preparation of control solution was done by mixing DPPH radical solution (0.3 ml) and ethanol (3.5 ml). After 30 min of reaction at 517 nm absorbance was read. By using the below formula, the scavenging activity percentage (AA %) was calculated.

% Antioxidant activity = {(absorbance at blank) – (absorbance at test) / (absorbance at blank)} X 100.

Results

In an in vitro experiment designed to assess the radical scavenging capabilities of various extracts, ethanolic extracts from guava leaves and raw honey were evaluated for their effectiveness. Radical scavenging is a critical measure of an extract's potential antioxidant activity, which is valuable in various applications including medicinal and nutritional contexts.

Percentage Yields of Ethanol Crude Extracts

Table 1 presents the percentage yields of the ethanol crude extracts obtained from both guava leaves and raw honey. The yields varied significantly, ranging from 12.5% to 42.8%. This variation indicates differences in the efficiency of the extraction process or the inherent concentration of bioactive compounds in each source. Lower yields might suggest less effective extraction or lower concentrations of the desired compounds, while higher yields indicate a more successful extraction process.

Table 1. Percentage Yield of Combination Extract [Guava Leaf and Raw Honey]

Blank -0.56

Sample/Concentration (µg)	100 µg/ml	200 µg/ml	300 µg/ml	400 µg/ml	500 µg/ml
Honey with Guava Leaf extract	0.49	0.45	0.41	0.37	0.32
% Inhibition	12.5	19.6	26.7	33.9	42.8

Standard Yields

Table 2 provides the yields of standard extracts, which ranged from 38.9% to 99.8%. These standard extracts are likely reference materials or well-characterized substances used for comparison. The higher yield percentages in this category suggest that these standard extracts either had a higher concentration of active components or were extracted more efficiently compared to the experimental extracts.

Table 2. Percentage Yield of Standard – *Butylated Hydroxytoluene* [BHT-1mg/ml]

Blank -0.59

BHT Concentrations	100 µg/ml	200 µg/ml	300 µg/ml	400 µg/ml	500 µg/ml
O. D	0.36	0.27	0.17	0.15	0.11
% inhibition	38.9	54.2	71.1	74.5	99.8

Comparative Results

The results of the experiment reveal that the standard extracts achieved the highest percentage yields, underscoring their higher radical scavenging potential or more efficient extraction. In contrast, the combination extract of guava leaves and raw honey yielded the lowest percentage. This lower yield could be attributed to a variety of factors, such as the compatibility of the two materials in the extraction process, the presence of interfering compounds, or less effective extraction methods for the combined materials.

Implications

These findings suggest that while individual standard extracts may offer higher radical scavenging capabilities and more efficient yields, the combination of guava leaves and raw honey in the extraction process yielded fewer effective results. This could have implications for their use in antioxidant applications, indicating that further optimization or different extraction techniques may be necessary to enhance the efficacy of the combination extract.

The ethanolic leaf extracts of both guava leaves and raw honey kinds were used to conduct the in vitro experiment for radical scavenging capabilities. The percentage yields of these ethanol crude extracts are presented in Table 1. They ranged from 12.5% to 42.8%. Table 2 shows the standard yields as a percentage. The percentages ranged from 38.9% to 99.8%. As a result, the standard extract yielded the greatest percentage, while the combination extract [Guava leaf and raw honey] yielded the lowest. [Figure 1].

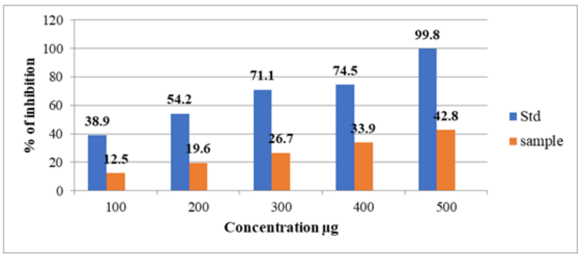

Figure 1. DPPH Assay – Percentage Inhibition of Standard and Sample

This detailed analysis highlights the variability in extract yields and effectiveness, providing insights into the relative performance of different extracts in radical scavenging activities.

This assay was performed with different concentrations of P. guajava and raw honey combination. The test sample showed [42.8%] antioxidant activity at 500 µg/ml concentration whereas the standard agent showed 99.8%

activity at 500 µg/ml. So, the standard extract yielded the greatest percentage, while the combination extract [Guava leaf and raw honey] yielded the lowest. From this study, it is evident that the antioxidant property of the test agent (P. guajava and raw honey) is comparatively less significant than the standard.

Discussion

Plants and plant products have been utilized as natural cures for oxidative stress-related disorders such as cardiovascular and cancer for ages. The researchers were inspired to hunt for natural compounds of plant and marine origin because of the rising resistance, side effects and high cost of presently available chemotherapeutic medications. It has been extensively documented that the synergistic action of a combination of phytoconstituents, rather than a single isolated component, is responsible for many of the therapeutic benefits of plant products [8, 15].

Antioxidants, either externally provided through foods or naturally produced in situ (endogenous antioxidants) are one of the body's methods for combating oxidative stress (exogenous antioxidants). Antioxidants help to prevent disease by neutralizing free radicals, protecting cells from their damaging effects, and neutralizing excess free radicals. The DPPH and FRAP assays are two standard methods for determining whether antioxidants destroy free radicals. Guava extracts in water and organic solvents include a substantial number of antioxidants that can prevent oxidation. As the concentration of these compounds rises, the concentration of these compounds rises as well [9, 16].

Guava and raw honey are abundant in antioxidants, which aid in the prevention of degenerative disorders such as cognitive dysfunction, inflammation, heart disease, cancer, arteriosclerosis and arthritis. Polyphenols and ascorbic acid are the most common oxidants in fruits. Flavonoids make up the majority of polyphenols, which come in glycoside and ester forms. Guava contains free ellagic acid as well as the glycosides of apigenin and myricetin. [10] Honey also includes different amounts of polyphenols which are potent antioxidants that are thought to lower the risk of heart disease and cancer according to researchers [11, 17].

The percentages in this study varied from 12.5% to 42.8%. As a consequence, the standard extract produced the highest percentage, while the combination extract [Guava leaf and raw honey] produced the lowest percentage.

Yadav et al., 2020 revealed that by using the DPPH radical assay technique with ascorbic acid as a control, the leaf extract of *P.guajava* was examined for free radical scavenging activities. The extract’s scavenging activity ranges from 76 to 88 percent [12, 18].

Ahmed et al., revealed that total antioxidant activity of DPPH was found to vary between 223.19 and 958.42 M Fe (II)/kg, showing that raw honey has high antioxidant characteristics. [13, 14].

The exploration of natural compounds for therapeutic applications has a long-standing tradition, particularly in the context of managing oxidative stress-related disorders. As resistance to traditional chemotherapeutic agents rises, and their side effects and costs become prohibitive, the focus has shifted to plant and marine-origin compounds. Notably, antioxidants play a crucial role in combating oxidative stress, which is implicated in a range of degenerative diseases including cardiovascular conditions and cancer. The synergistic effects of various phytoconstituents present in plants like guava and substances like honey can offer substantial health benefits. This discussion will explore the antioxidant properties of guava and honey, individually and in combination, and analyze their implications for health based on current research.

Antioxidants are critical for neutralizing free radicals—unstable molecules that can cause oxidative damage to cells, proteins, and DNA. The body employs both endogenous (internally produced) and exogenous (externally provided) antioxidants to mitigate this damage. Endogenous antioxidants include enzymes like superoxide dismutase (SOD) and catalase, while exogenous antioxidants come from dietary sources, including fruits, vegetables, and other plant products [21, 22].

The DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (Ferric Reducing Antioxidant Power) assays are widely used to assess antioxidant activity. The DPPH assay measures the ability of antioxidants to neutralize DPPH free radicals, while the FRAP assay evaluates the antioxidant's reducing power.[23][24] These assays help quantify the effectiveness of different substances in scavenging free radicals and reducing oxidative stress.

Guava (Psidium guajava) is renowned for its high antioxidant content. Research has demonstrated that guava extracts, whether in water or organic solvents, contain significant amounts of antioxidants, including polyphenols and vitamin C. Guava's antioxidant properties are attributed to its rich array of phytoconstituents, such as flavonoids, ellagic acid, and glycosides of apigenin and myricetin [25, 26].

Yadav et al. (2020) investigated the antioxidant capacity of guava leaf extracts using the DPPH radical scavenging assay. Their results indicated that guava leaf extracts possess a high scavenging activity, ranging from 76% to 88% [27]. This underscores guava's potential in mitigating oxidative stress through its robust antioxidant profile.

Guava's polyphenolic compounds, including flavonoids, are known to play a significant role in neutralizing free radicals. Flavonoids, in particular, contribute to guava's ability to protect cells from oxidative damage and inflammation [28, 29]. The presence of ellagic acid and its glycosides further enhances guava's antioxidant potential, offering a multifaceted approach to oxidative stress management.

Raw honey, another natural product with notable antioxidant properties, has been the subject of various studies. Ahmed et al. have demonstrated that raw honey exhibits substantial antioxidant activity, with total antioxidant capacity ranging from 223.19 to 958.42 M Fe (II)/kg [30]. This high antioxidant capacity is indicative of honey's ability to scavenge free radicals and mitigate oxidative damage.

Honey contains a variety of polyphenols, which are potent antioxidants that contribute to its health benefits. Polyphenols in honey are thought to play a role in reducing the risk of chronic diseases, including heart disease and cancer [31, 32]. The presence of diverse polyphenolic compounds in honey enhances its antioxidant profile and supports its therapeutic potential.

The combination of guava and raw honey has been explored for its synergistic effects on antioxidant activity. However, existing studies have not extensively investigated the combined antioxidant effects of guava leaf extracts and honey. The current study found that the combination extract of guava leaf and raw honey exhibited a lower antioxidant percentage compared to the standard guava extract alone. This observation suggests that the interaction between guava and honey may affect their combined antioxidant activity, warranting further investigation [33, 34].

The lack of significant findings in combining guava and honey could be attributed to various factors, including the specific preparation methods and concentrations used. The interaction between different phytoconstituents might influence their efficacy in scavenging free radicals, highlighting the need for optimized formulation and experimental conditions to fully understand their combined effects [35, 36].

The individual antioxidant properties of guava and raw honey suggest their potential as therapeutic agents in managing oxidative stress-related disorders. Guava's high levels of flavonoids and polyphenols contribute to its ability to combat oxidative damage, while honey's diverse polyphenolic content enhances its antioxidant activity. Both substances have been shown to offer protective benefits against diseases such as cardiovascular disorders, cancer, and cognitive dysfunction [37, 38].

Despite the promising results from individual studies, the combined antioxidant effects of guava and honey require further exploration. Future research should focus on optimizing the combination of these substances, exploring different ratios, and evaluating their efficacy using various antioxidant assays.

The investigation into the antioxidant properties of guava and honey reveals their significant potential in managing oxidative stress and related disorders. While individual studies have highlighted their efficacy, the combined antioxidant effects of guava and honey remain less understood. Continued research is necessary to fully elucidate the synergistic interactions between these natural products and to optimize their use in therapeutic applications.

None of the studies detected antioxidants in guava leaves and raw honey in combination form on the DPPH radical scavenging test. However, to support the current study there is no evidence. Still more studies have to be done to substantiate the above result.

Conclusion

The results of this study contribute to the broader discourse on the effectiveness of natural product combinations. They reinforce the notion that while natural ingredients can offer substantial benefits, their combined use does not always guarantee superior outcomes. This underscores the importance of empirical testing and validation in determining the most effective formulations.

In conclusion, this study illuminates the complex nature of natural product interactions and provides a critical perspective on the efficacy of combining guava leaves and raw honey. While individual substances like guava and honey exhibit strong antioxidant effects, their combination does not necessarily enhance these effects. This finding serves as a reminder of the intricate dynamics involved in natural product research and the need for continued exploration to fully understand and optimize the therapeutic potential of natural remedies. The insights gained from this study may stimulate further research and refinement in the development of natural product-based interventions, ultimately contributing to the advancement of health and wellness strategies.

Though many chemical compositions in pharmaceutical research have been proven and manufactured for its beneficial effect, it is not mandatory that all combinations whether it's a synthetic or natural combination should show pragmatic results. It is the need of the hour to enlighten every human globally what should be and what should not be combined. Recent outbreak of global pandemic has fuelled the mind to search for natural ingredients based on easy availability and accessibility. Inspite of more weightage to natural ingredients, especially honey and combo, this study enhances us that the antioxidant effects of ethanolic extracts of *P.guajava* [leaves], Raw Honey, and their combination consistently outperformed the separate extracts effects. This study helps us to conclude though individual substances have the highest anti-oxidant effect, it is important to avoid combinations, not that it hinders or nullifies each other's effects but "How unlonely being alone can be". This study may quench or fuel many of its kind in research.

Conflict of Interest

There is no conflict of interest.

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