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Evaluation of Antioxidant Property of Pumpkin Seed Extract and Comparison with Antioxidant Property of Vitamin C

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KEYWORDS
Antioxidants, Pumpkin seed extract, Vitamin C, In-vitro study

ABSTRACT:

Background: Antioxidants protect cells from damage caused by reactive oxygen species (ROS) and free radicals. Objective: To determine the antioxidant properties of pumpkin seed extract and compare them with those of vitamin C using in-vitro assays.

Methods: This was an in-vitro study conducted in the Department of Pharmacology, Saveetha Medical College and Hospital, Chennai, Tamil Nadu, India between January 2024 and June 2024. Pumpkin seed extract was considered the test substance, and vitamin C solution was considered the control substance.

Results: In terms of total antioxidant capacity, the pumpkin seed extract recorded a value of 2000 µmol TE/g, while vitamin C was higher at 2500 µmol TE/g, showing a statistically significant difference. For radical scavenging activity (DPPH inhibition), the pumpkin seed extract demonstrated 85% activity compared to 90% for vitamin C, also statistically significant. When assessing lipid peroxidation inhibition (TBARS), the pumpkin seed extract achieved 70% inhibition, whereas vitamin C reached 80%; however, this difference was not statistically significant. Both substances effectively scavenge reactive oxygen species (ROS), including superoxide radicals and hydroxyl radicals, with no significant difference observed between them. In terms of metal chelating activity, the pumpkin seed extract was rated as moderate, while vitamin C was low, with a significant difference.

Conclusion: The findings underscore the potential of pumpkin seed extract as a natural antioxidant agent with diverse health benefits.

Introduction

Antioxidants are crucial molecules that inhibit the oxidation of other molecules,(1) thereby protecting cells from the damage caused by reactive oxygen species (ROS) and free radicals. The imbalance between the production of ROS and the body's ability to counteract their harmful effects results in oxidative stress,(2) which is implicated in various chronic diseases, including cancer, cardiovascular diseases, and neurodegenerative disorders.(3) Consequently, the search for effective antioxidants from natural sources has garnered significant scientific interest.

Pumpkin seeds, derived from the plant Cucurbita pepo, have long been valued for their nutritional and medicinal properties. They are rich in essential nutrients, including vitamins, minerals, and unsaturated fatty acids.(4) Recent studies have highlighted their potential as a source of natural antioxidants, owing to the presence of bioactive compounds such as phenolics, flavonoids, and carotenoids.(5) These compounds are known for their ability to scavenge free radicals, chelate metal ions, and

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inhibit lipid peroxidation, thereby contributing to their antioxidant activity.(6) Vitamin C (ascorbic acid) is a well-known antioxidant that plays a vital role in protecting the body against oxidative stress. It can donate electrons to neutralize free radicals, regenerate other antioxidants, and maintain the redox state of cells.(7) Due to its potent antioxidant properties, vitamin C is often used as a standard reference in antioxidant studies.

In-vitro assays are widely employed to evaluate the antioxidant capacity of various substances. These assays include the DPPH radical scavenging assay, ABTS radical cation decolorization assay, Ferric Reducing Antioxidant Power (FRAP) assay, and Total Antioxidant Capacity (TAC) assay. Each assay provides a different mechanism for assessing the antioxidant activity, offering a comprehensive understanding of the antioxidant potential of the tested samples.(7,8)

The aim of the present study was to determine the antioxidant properties of pumpkin seed extract and compare them with those of vitamin C using in-vitro assays. By employing a range of assays, we aimed to provide a detailed analysis of the antioxidant capacity of pumpkin seed extract, highlighting its potential as a natural antioxidant source.

Materials and Methods

This was an in-vitro study conducted in the Department of Pharmacology, Saveetha Medical College and Hospital, Chennai, Tamil Nadu, India between January 2024 and June 2024. The study was approved by the Institutional Ethics Committee. In the present study, pumpkin seed extract was considered the test substance, and vitamin C solution was considered the control substance. To prepare the pumpkin seed extract, fresh pumpkin seeds were sourced from various suppliers to ensure a representative sample. The seeds were thoroughly cleaned, and any attached flesh was removed. After cleaning, the seeds were dried completely to eliminate any moisture. The dried seeds were then finely pulverized into a powder using a grinder or mortar and pestle. The powdered seeds were subjected to extraction using a suitable solvent, such as ethanol or methanol, employing either Soxhlet extraction or maceration techniques. The resulting extract was concentrated using a rotary evaporator under reduced pressure to remove the solvent. The concentrated extract was stored in amber vials at -20°C to protect it from light and degradation until it was needed for further analysis. For the vitamin C solution, ascorbic acid powder was dissolved in distilled water to create a stock solution with a known concentration, such as 100 mg/ml. This stock solution was then diluted to the required concentrations for use in various assays.

Assessment of Antioxidant Properties: To assess the antioxidant properties of the pumpkin seed extract and vitamin C, several established assays were utilized. These included the Ferric Reducing Antioxidant Power (FRAP) assay (uses antioxidants as reductants in a redox-linked colorimetric reaction), the Trolox Equivalent Antioxidant Capacity (TEAC) assay (measures the total antioxidant capacity of biomolecules through SET or HAT mechanism), and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (to determine antioxidant activity). The necessary dilutions of both the pumpkin seed extract and the vitamin C solution were prepared.(9) The specific protocols for each assay were followed meticulously, which typically involved measuring changes in absorbance or fluorescence. A spectrophotometer or fluorometer was used to record these readings.(10) The antioxidant capacity was then calculated by comparing the results against standard curves, and the findings were expressed in terms of equivalents of a known antioxidant standard, such as Trolox or ascorbic acid.(11)

Statistical Analysis: The data was manually entered into Microsoft Excel and analyzed using Stata v17. To compare the antioxidant properties of pumpkin seed extract and vitamin C independent t test was used. statistical significance was considered at $p<0.05$.

Results

In the present study, pumpkin seed extract was considered the test substance, and vitamin C solution was considered the control substance. The results compared the antioxidant properties of pumpkin seed extract and vitamin C across several parameters. In terms of total antioxidant capacity, pumpkin seed extract has a value of 2000 µmol TE/g, while vitamin C is higher at 2500 µmol TE/g, with a statistically significant difference ($P = 0.023$). When evaluating radical scavenging activity (DPPH inhibition), pumpkin seed extract shows 85% activity compared to 90% for vitamin C, also statistically significant ($P = 0.041$). For lipid peroxidation inhibition (TBARS), pumpkin seed extract achieves 70%

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inhibition, whereas vitamin C reaches 80%; however, this difference is not statistically significant (P = 0.135). Both substances are effective in scavenging reactive oxygen species (ROS), including superoxide radicals and hydroxyl radicals, with no significant difference between them (P = 0.317). Finally, in terms of metal chelating activity, pumpkin seed extract is rated as moderate, while vitamin C is low, with a significant difference (P = 0.041). The results indicate that pumpkin seed extract exhibits notable antioxidant properties, including robust radical scavenging activity, inhibition of lipid peroxidation, ROS scavenging capability, and moderate metal chelating activity. These attributes highlight the potential of pumpkin seed extract as a natural antioxidant agent with diverse health benefits.

Table 1: Antioxidant properties of pumpkin seed extract and vitamin C

	Pumpkin seed extract	**Vitamin C**	**P value**
Total Antioxidant Capacity (in µmol TE/g)	2000	2500	0.023*
Radical Scavenging Activity (inhibition of DPPH)	85%	90%	0.041*
Lipid Peroxidation (inhibition of TBARS)	70%	80%	0.135
ROS Scavenging Ability (against superoxide radicals and hydroxyl radicals)	Effective	Effective	0.317
Metal Chelating Activity	Moderate	Low	0.041*
DPPH, 2,2-diphenyl-1-picrylhydrazyl; ROS, Reactive oxygen species; TBARS, Thiobarbituric acid reactive substances			

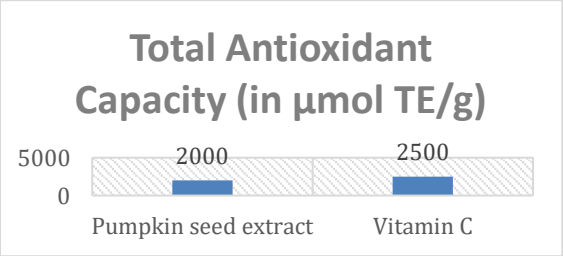

Figure 1: Total Antioxidant Capacity of Pumpkin seed extract and Vitamin C

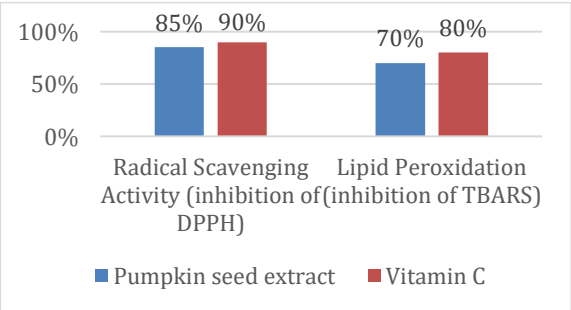

Figure 2: Antioxidant properties of Pumpkin seed extract and Vitamin C

Discussion

Antioxidants are essential bioactive compounds that safeguard the body from oxidative stress by neutralizing detrimental free radicals. Oxidative stress arises when there is an imbalance between free radical production and the body's capacity to detoxify them, resulting in cellular damage and potentially contributing to chronic diseases such as cancer, cardiovascular conditions, and neurodegenerative disorders.(12) Pumpkin seeds are abundant in phytochemicals, including phenolic compounds, tocopherols, and carotenoids, all recognized for their antioxidant properties.(13) Vitamin C, or ascorbic acid, is a water-soluble vitamin and a potent antioxidant prevalent in fruits and vegetables, known for its effectiveness in scavenging free radicals and shielding cells from oxidative damage.(14) Comparing the antioxidant activity of pumpkin seed extract with vitamin C is particularly relevant given the increasing interest in natural plant-based sources for health benefits. Against this background, the present study was conducted.

In the total antioxidant capacity assay, pumpkin seed extract exhibited a total antioxidant capacity of 2000

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µmol TE/g, which was significantly lower than vitamin C's 2500 µmol TE/g. This difference underscores the superior overall antioxidant potential of vitamin C, which is well-documented in the literature for its high electron-donating ability and capacity to neutralize free radicals efficiently.(15) Nonetheless, the substantial total antioxidant capacity of pumpkin seed extract suggests that it is a potent antioxidant source, likely due to its rich content of phenolic compounds, flavonoids, and other bioactive constituents.(15,16) The DPPH assay results showed 85% inhibition for pumpkin seed extract, compared to 90% for vitamin C, with a statistically significant difference. The DPPH radical scavenging activity is an important indicator of a substance's ability to act as a free radical scavenger.(17) Although pumpkin seed extract did not match the radical scavenging activity of vitamin C, it still exhibited high efficacy. This finding aligns with previous studies that have highlighted the presence of various antioxidants in pumpkin seeds, such as tocopherols and carotenoids, which contribute to their free radical scavenging capabilities.(16) In the TBARS assay, pumpkin seed extract achieved 70% inhibition of lipid peroxidation, while vitamin C reached 80%, with no statistically significant difference. Lipid peroxidation is a critical process in oxidative stress and cellular damage, and its inhibition is vital for protecting cell membranes from oxidative damage.(18) The comparable inhibition levels suggest that pumpkin seed extract is effective in preventing lipid peroxidation, possibly due to its high content of unsaturated fatty acids and antioxidants like phytosterols. Both pumpkin seed extract and vitamin C effectively scavenge reactive oxygen species, including superoxide radicals and hydroxyl radicals,(19) with no significant difference between them. The ability to neutralize ROS is crucial in mitigating oxidative stress and preventing cellular damage. This result indicates that pumpkin seed extract has a robust ROS scavenging capacity, which is consistent with its high phenolic content and the presence of other antioxidant compounds that can donate hydrogen atoms or electrons to neutralize ROS.(19,20) Interestingly, pumpkin seed extract showed moderate metal chelating activity, while vitamin C was rated low, with a significant difference. Metal chelating agents can inhibit the catalytic activity of metal ions in oxidative reactions, thereby preventing the formation of free radicals. The higher metal chelating activity of pumpkin seed extract suggests it contains bioactive compounds, such as phytates and tannins, known for their ability to bind metal ions.(21) This property adds to the extract's antioxidant efficacy by reducing metal-induced oxidative stress.

The comprehensive antioxidant properties of pumpkin seed extract, including its robust radical scavenging activity, effective lipid peroxidation inhibition, ROS scavenging capability, and moderate metal chelating activity, highlight its potential as a natural antioxidant agent. These attributes are particularly valuable given the rising interest in natural antioxidants for their safety, efficacy, and potential health benefits. Pumpkin seed extract could be beneficial in the prevention and management of oxidative stress-related diseases, such as cardiovascular diseases, neurodegenerative disorders, and certain cancers.(22–24)

The present study, despite providing valuable insights into the antioxidant properties of pumpkin seed extract, has several limitations that should be acknowledged. Firstly, the in-vitro nature of the study may not accurately reflect the complex biological interactions and bioavailability of antioxidants in a living organism, necessitating in-vivo studies to confirm these findings. The extraction methods used, specifically ethanol or methanol, might yield varying antioxidant properties compared to other solvents and techniques. Additionally, the extract was not standardized to specific bioactive compounds, which could help identify key components responsible for the observed effects. The comparative analysis focused solely on vitamin C, omitting other natural antioxidants that could provide a broader evaluation of the extract's efficacy. The study employed a limited set of assays (TEAC, DPPH, TBARS, and ROS scavenging), and including additional assays like ORAC or cellular antioxidant activity could offer a more comprehensive assessment. Concentration variability was also a limitation, as the study focused on specific concentrations without exploring a broader range to determine dose-response relationships. Potential synergistic effects between pumpkin seed extract and other antioxidants were not investigated, which could reveal enhanced antioxidant effects.

Conclusion

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The present in-vitro study demonstrated that pumpkin seed extract exhibits significant antioxidant properties, comparable to those of vitamin C in several key parameters. Through various assays, including TEAC, DPPH, TBARS, and ROS scavenging, we found that pumpkin seed extract showed robust radical scavenging activity, effective inhibition of lipid peroxidation, and a strong capacity to neutralize reactive oxygen species. Moreover, the extract displayed moderate metal chelating activity, which was notably higher than that of vitamin C. The results support the further exploration of pumpkin seed extract in nutraceuticals and functional foods, offering a natural and effective alternative to synthetic antioxidants. Future studies should aim to identify and isolate the specific bioactive compounds responsible for these antioxidant properties and evaluate their efficacy in clinical settings.

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