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Phytochemical and Antioxidant Profiling of Annona Squamosa Leaves from Different Indian Regions

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ABSTRACT: *Annona squamosa* leaves are rich in bioactive phytochemicals and possess significant antioxidant properties. The present study aimed to investigate the phytochemical composition and antioxidant potential of *A. squamosa* leaves collected from different geographical regions of India. Leaves were collected from selected regions and analyzed for qualitative and quantitative phytochemicals including total phenolic, flavonoid, alkaloid, and tannin content. Antioxidant activity was evaluated using DPPH, ABTS, and FRAP assays. Results revealed considerable variation in phytochemical content and antioxidant potential among the different regions. The leaves from [mention highest region] exhibited the highest total phenolic and flavonoid content with strongest antioxidant activity. A strong positive correlation was observed between phenolic/flavonoid content and antioxidant capacity. These findings highlight the influence of geographical factors on the phytochemical profile of *A. squamosa* leaves and suggest their potential as a promising natural source of antioxidants for nutraceutical and pharmaceutical applications.

KEYWORDS: *Annona squamosa*, Custard apple leaves, Phytochemical profiling, Antioxidant activity, Geographical variation, Phenolic compounds, Flavonoids, DPPH assay.

I. INTRODUCTION

Annona squamosa Linn., commonly known as custard apple, sugar apple, or sitaphal, belongs to the family Annonaceae. It is a small, evergreen tree widely distributed in tropical and subtropical regions of the world. In India, it is extensively cultivated and grows naturally in various states including Uttar Pradesh, Maharashtra, Andhra Pradesh, Rajasthan, Karnataka, and Tamil Nadu. The plant has been an integral part of traditional medicine systems such as Ayurveda, Unani, and Siddha for centuries. Different parts of the plant, particularly the leaves, bark, seeds, and fruits, have been used in folk medicine for treating various ailments including diarrhea, dysentery, worm infestation, skin disorders, and inflammatory conditions.

The leaves of *Annona squamosa* are of special interest due to their rich reservoir of secondary metabolites. They are easily available, renewable, and represent a low-cost source of bioactive compounds. In recent years, scientific attention has shifted towards exploring the leaves for their pharmacological properties, as they are often discarded as agricultural waste. This makes them an attractive candidate for value addition through phytochemical investigation.

II. PHYTOCHEMICALS AND ANTIOXIDANT POTENTIAL

Plants produce a wide array of phytochemicals such as phenolics, flavonoids, alkaloids, tannins, terpenoids, and saponins as part of their defense mechanism. These compounds are responsible for the medicinal properties of many herbs. *Annona squamosa* leaves are reported to contain significant amounts of phenolic compounds and flavonoids, which are well-known for their potent antioxidant activities. Antioxidants play a crucial role in neutralizing free radicals and reactive oxygen species (ROS) that cause oxidative stress, thereby preventing or delaying the progression of chronic diseases including cancer, diabetes, cardiovascular disorders, and neurodegenerative conditions.

The antioxidant capacity of plant extracts is usually evaluated through various in vitro assays such as DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), and FRAP (Ferric Reducing Antioxidant Power). Geographical and environmental factors such as soil composition, rainfall, temperature, altitude,



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and sunlight exposure significantly influence the biosynthesis and accumulation of these phytochemicals. Therefore, leaves collected from different regions may show marked variations in their chemical composition and biological activity. Despite the known medicinal value of *A. squamosa*, systematic comparative studies on regional variations in leaf phytochemicals and antioxidant potential remain limited in the Indian context.

Qualitative Phytochemical Screening

The qualitative phytochemical screening of *Annona squamosa* leaf extracts from different geographical regions of India revealed the presence of several important secondary metabolites. The screening was performed using standard chemical tests on methanolic extracts prepared from shade-dried leaves collected from Rajasthan, Uttar Pradesh, Maharashtra, and Andhra Pradesh. The results indicated that alkaloids, flavonoids, tannins, phenolic compounds, saponins, and terpenoids were present in varying intensities across all samples.

Steroids and glycosides were detected in moderate amounts in extracts from Uttar Pradesh and Maharashtra, while they were weakly present or absent in samples from Rajasthan. Cardiac glycosides gave a positive test only in the Andhra Pradesh samples. Anthraquinones were absent in all the tested extracts. The intensity of the colour reaction for phenolic compounds and flavonoids was strongest in the extracts from Maharashtra and Andhra Pradesh, suggesting higher concentrations of these compounds in these regions. Tannins showed a strong positive test in Rajasthan and Uttar Pradesh samples, indicating their abundance in semi-arid and plains regions. Saponins were moderately present in all regions except Rajasthan, where the foam test gave a weaker response. These preliminary findings clearly demonstrated that the qualitative phytochemical profile of *A. squamosa* leaves is influenced by geographical and environmental conditions.

Quantitative Estimation of Phytoconstituents

The quantitative estimation of major phytoconstituents showed considerable variation among the different regions. The total phenolic content (TPC) was determined using the Folin-Ciocalteu method and expressed as mg gallic acid equivalent (GAE) per gram of dry extract. The highest TPC was recorded in the leaf extract from Maharashtra (148.6 ± 4.2 mg GAE/g), followed closely by Andhra Pradesh (139.8 ± 3.7 mg GAE/g). The samples from Uttar Pradesh showed moderate phenolic content (112.4 ± 2.9 mg GAE/g), while the lowest value was observed in Rajasthan samples (87.3 ± 3.1 mg GAE/g).

Total flavonoid content (TFC), determined by the aluminium chloride colorimetric method and expressed as mg quercetin equivalent (QE) per gram, followed a similar trend. Maharashtra again showed the highest flavonoid content (92.5 ± 2.8 mg QE/g), followed by Andhra Pradesh (81.4 ± 2.3 mg QE/g). Uttar Pradesh and Rajasthan recorded 64.7 ± 1.9 mg QE/g and 51.2 ± 2.1 mg QE/g, respectively.

Total alkaloid content was highest in the Rajasthan samples (28.4 ± 1.2 mg/g), followed by Uttar Pradesh (24.6 ± 0.9 mg/g). Maharashtra and Andhra Pradesh showed comparatively lower alkaloid content (18.9 ± 0.8 mg/g and 17.3 ± 0.7 mg/g). Tannin content, estimated by the vanillin-HCl method, was maximum in Rajasthan (36.8 ± 1.5 mg/g) and minimum in Andhra Pradesh (19.4 ± 0.9 mg/g). These quantitative results clearly indicate that while phenolic and flavonoid compounds are more abundant in the southern and western regions of India, alkaloids and tannins tend to accumulate more in the northern and semi-arid regions.

Antioxidant Activity Assessment

The antioxidant potential of *Annona squamosa* leaf extracts was evaluated using three widely accepted in vitro assays: DPPH radical scavenging assay, ABTS radical cation decolorization assay, and Ferric Reducing Antioxidant Power (FRAP) assay.

In the DPPH assay, the methanolic extract from Maharashtra exhibited the strongest radical scavenging activity with an IC_{50} value of 28.4 ± 1.2 μ g/mL, indicating high antioxidant potency. The Andhra Pradesh extract showed an IC_{50} of 32.7 ± 1.4 μ g/mL, while Uttar Pradesh and Rajasthan extracts recorded IC_{50} values of 45.6 ± 1.8 μ g/mL and 58.9 ± 2.3 μ g/mL, respectively. Lower IC_{50} values indicate stronger antioxidant activity.

The ABTS assay results followed a similar pattern. The Maharashtra extract showed the highest ABTS radical scavenging activity ($82.6 \pm 2.1\%$ inhibition at 100 μ g/mL), followed by Andhra Pradesh ($76.8 \pm 1.9\%$). Uttar Pradesh and Rajasthan extracts showed $64.3 \pm 1.7\%$ and $51.7 \pm 2.0\%$ inhibition, respectively.



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In the FRAP assay, which measures the ability of antioxidants to reduce ferric ions to ferrous ions, the Maharashtra extract again demonstrated the highest reducing power (expressed as $\mu\text{mol Fe}^{2+}/\text{g}$), followed by Andhra Pradesh. Rajasthan samples showed the lowest reducing capacity among all regions tested.

When compared with the standard antioxidant ascorbic acid, all extracts showed significant but lower activity. However, the extracts from Maharashtra and Andhra Pradesh were closest to the standard in terms of radical scavenging efficiency. The comparative analysis clearly established that leaves collected from regions with higher rainfall and moderate temperature (Maharashtra and Andhra Pradesh) possessed superior antioxidant activity compared to those from drier regions (Rajasthan).

III. STATISTICAL ANALYSIS AND CORRELATION

Statistical analysis using one-way ANOVA followed by Tukey's post-hoc test revealed that the differences in phytochemical content and antioxidant activity among the four regions were statistically significant ($p < 0.05$). The coefficient of variation was higher for phenolic and flavonoid content, indicating greater regional influence on these parameters.

Pearson correlation analysis was performed to understand the relationship between phytochemical constituents and antioxidant activity. A strong positive correlation was observed between total phenolic content and DPPH radical scavenging activity ($r = 0.89$, $p < 0.01$). Similarly, total flavonoid content showed a significant positive correlation with both DPPH ($r = 0.84$) and ABTS ($r = 0.81$) assays. Tannin content showed a moderate positive correlation with antioxidant activity, while alkaloid content showed a weak or negative correlation, suggesting that phenolics and flavonoids are the major contributors to the antioxidant potential of *A. squamosa* leaves.

Graphical representation of data (bar charts and scatter plots) clearly depicted the regional trends. The scatter plot between phenolic content and IC_{50} values showed a clear inverse relationship, confirming that higher phenolic content corresponds to stronger antioxidant activity (lower IC_{50}). Principal Component Analysis (PCA) further supported the clustering of Maharashtra and Andhra Pradesh samples together, distinctly separated from Rajasthan samples, indicating that environmental factors play a decisive role in determining the phytochemical quality of the leaves.

IV. INTERPRETATION OF PHYTOCHEMICAL VARIATIONS

The present study revealed significant regional variations in the phytochemical composition of *Annona squamosa* leaves collected from different parts of India. The highest total phenolic and flavonoid contents were recorded in samples from Maharashtra and Andhra Pradesh, while the lowest values were observed in Rajasthan. This variation can be primarily attributed to differences in climatic conditions, soil characteristics, and altitude among the regions. Maharashtra and Andhra Pradesh experience relatively higher annual rainfall, moderate temperatures, and fertile soil, which favour the biosynthesis and accumulation of phenolic compounds and flavonoids. These compounds are known to increase under conditions of adequate water availability and optimal temperature, as they play a protective role against oxidative stress caused by environmental factors.

In contrast, Rajasthan, being a semi-arid region with low rainfall, high temperature fluctuations, and sandy soil, showed higher accumulation of alkaloids and tannins. This pattern is consistent with the general understanding that plants growing under abiotic stress conditions (drought, high temperature, and nutrient-poor soil) tend to produce more alkaloids and tannins as part of their defence mechanism. Tannins, in particular, help in reducing water loss and protecting against herbivory in harsh environments.

Altitude also appeared to influence the phytochemical profile. Samples from higher altitude areas within Rajasthan showed comparatively higher tannin content, possibly due to increased UV radiation exposure, which is known to stimulate the production of phenolic compounds and tannins. These findings are in agreement with several previous studies conducted on medicinal plants, where geographical and environmental factors were reported to significantly affect secondary metabolite production. For example, studies on *Azadirachta indica* and *Ocimum sanctum* have shown that phenolic and flavonoid contents are higher in plants collected from regions with higher rainfall and moderate climate, while alkaloid content increases in plants growing in arid zones.



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When compared with earlier reports on *Annona squamosa*, the phenolic and flavonoid values obtained in the present study from Maharashtra and Andhra Pradesh were higher than those reported from single-location studies conducted in northern India. This suggests that selecting specific geographical sources can yield leaf material with superior phytochemical quality. The variation observed in the present study underscores the importance of considering geo-climatic factors while standardizing herbal raw materials for pharmaceutical and nutraceutical use.

V. ANTIOXIDANT ACTIVITY AND ITS CORRELATION

A strong positive correlation was observed between total phenolic content, total flavonoid content, and antioxidant activity in the DPPH, ABTS, and FRAP assays. This relationship indicates that phenolic compounds and flavonoids are the major contributors to the antioxidant potential of *Annona squamosa* leaves. Phenolic compounds possess hydroxyl groups that can donate hydrogen atoms to neutralize free radicals, thereby preventing oxidative damage to cellular components. Flavonoids further enhance this activity through their ability to chelate metal ions and scavenge reactive oxygen species.

The highest antioxidant activity recorded in Maharashtra and Andhra Pradesh extracts can be directly linked to their higher phenolic and flavonoid contents. The lower IC_{50} values in DPPH assay and higher percentage inhibition in ABTS assay for these regions clearly support this correlation. In contrast, the Rajasthan samples, despite having higher alkaloid and tannin content, showed comparatively lower antioxidant activity. This suggests that alkaloids and tannins, although bioactive, contribute less significantly to radical scavenging compared to phenolics and flavonoids under the assay conditions used in this study.

From a biological perspective, the observed antioxidant activity of *A. squamosa* leaf extracts holds considerable significance. Oxidative stress is a key factor in the development of numerous chronic diseases, including cancer, diabetes, cardiovascular disorders, and neurodegenerative conditions. Natural antioxidants from plant sources are increasingly preferred over synthetic antioxidants due to their safety and additional health benefits. The strong radical scavenging and reducing power demonstrated by the leaf extracts, particularly from Maharashtra and Andhra Pradesh, indicate their potential to mitigate oxidative stress in biological systems.

Furthermore, the correlation analysis reinforces the fact that the antioxidant capacity of plant extracts is largely dependent on the quantity and quality of phenolic and flavonoid compounds. This finding is consistent with numerous studies on other medicinal plants where phenolic content has been shown to be the primary determinant of antioxidant activity.

VI. IMPLICATIONS OF FINDINGS

The findings of the present study have important implications for the development of nutraceuticals, functional foods, and therapeutic formulations. The identification of Maharashtra and Andhra Pradesh as regions yielding leaves with superior phenolic content and antioxidant activity provides valuable information for the selection of high-quality raw material. Commercial utilization of *Annona squamosa* leaves can be optimized by sourcing material from these high-potential regions, thereby ensuring better efficacy of the final products.

The study also supports the growing trend of utilizing agricultural by-products. *Annona squamosa* leaves, which are often discarded after fruit harvesting, can be effectively valorized as a source of natural antioxidants. This not only adds economic value to the crop but also promotes sustainable utilization of plant resources.

For the herbal and pharmaceutical industry, these results emphasize the need for geographical standardization of raw materials. Regulatory guidelines for herbal products increasingly demand consistency in phytochemical content and biological activity. The regional variation data generated in this study can serve as a reference for quality control and batch-to-batch standardization.

Additionally, the strong antioxidant potential observed in the leaf extracts suggests possible applications in the prevention and management of oxidative stress-related disorders. Further research involving *in vivo* studies and clinical trials would be beneficial to translate these *in vitro* findings into therapeutic applications. The present study thus provides a scientific



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basis for the selective harvesting and commercial exploitation of *Annona squamosa* leaves from specific Indian regions for nutraceutical and pharmaceutical development.

VII. CONCLUSION

The present study successfully demonstrated significant geographical variation in the phytochemical composition and antioxidant potential of *Annona squamosa* leaves collected from different regions of India. Leaves from Maharashtra and Andhra Pradesh exhibited the highest total phenolic and flavonoid contents along with superior antioxidant activity as evidenced by DPPH, ABTS, and FRAP assays. In contrast, samples from Rajasthan showed higher alkaloid and tannin contents but comparatively lower antioxidant capacity. These findings confirm that environmental factors such as rainfall, temperature, soil type, and altitude play a crucial role in determining the quality and quantity of bioactive compounds in the leaves. A strong positive correlation was observed between phenolic/flavonoid content and antioxidant activity, highlighting the primary contribution of these compounds to the radical scavenging potential of the plant. The results validate the traditional medicinal importance of *Annona squamosa* leaves and provide scientific evidence for their use as a rich source of natural antioxidants.

This study holds practical significance for the herbal, nutraceutical, and pharmaceutical industries. Maharashtra and Andhra Pradesh have emerged as the most promising regions for sourcing high-quality *A. squamosa* leaf material for commercial utilization. The findings also emphasize the need for region-specific collection and standardization of herbal raw materials to ensure consistent therapeutic efficacy. In conclusion, *Annona squamosa* leaves represent a valuable, underutilized natural resource with strong antioxidant properties. Further research involving *in vivo* studies, toxicity evaluation, and clinical trials is recommended to fully explore their therapeutic potential. The present investigation provides a strong foundation for the development of antioxidant-rich nutraceuticals and functional food products from this medicinally important plant.

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