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Mahinder Singh Rathour
 Research Scholar, Department of
 Pharmaceutical Science, Lords University,
 Ahwar, 301028, Rajasthan, India.

Mukesh Kumar Gupta
 Professor, Department of Pharmaceutical
 Science, Lords University, Ahwar, 301028,
 Rajasthan, India.

Correspondence

Mahinder Singh Rathour
 Research Scholar, Department of
 Pharmaceutical Science, Lords University,
 Ahwar, 301028, Rajasthan, India.

Email: rathore.ms2009@gmail.com

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EVALUATION OF PHYTOCHEMICAL AND ANTIOXIDANT ACTIVITY OF LEAF EXTRACT OF *ANNONA SQUAMOSA* AND *OCIMUM SANCTUM*

Mahinder Singh Rathour and Mukesh Kumar Gupta

ABSTRACT

Present study was a comparative analysis of the morphological, microscopic, physiochemical, and phytochemical characteristics of the leaves of *Annona squamosa* and *Ocimum sanctum*, alongside their antioxidant potential. Morphologically, *A. squamosa* and *O. sanctum* leaves were studied. Microscopic examination revealed anisocytic stomata and raphide-type calcium oxalate crystals in *A. squamosa*, whereas *O. sanctum* showed diacytic stomata, rosette-type crystals, and abundant essential oil glands. Physiochemical screening indicated moderate moisture content and ash values, with *O. sanctum* showing higher water-soluble ash and foaming index. Extractive yield was greater in *O. sanctum* (16.27%) compared to *A. squamosa* (13.28%). Quantitative estimation revealed significantly higher total flavonoid (43.23 mg/100 mg quercetin) and phenol content (26.43 mg/100 mg gallic acid) in *A. squamosa* than in *O. sanctum*. Phytochemical screening confirmed the presence of alkaloids, glycosides, carbohydrates, phytosterols, saponins, tannins, and flavonoids in both species, with proteins absent. Antioxidant assays using DPPH and ABTS methods demonstrated dose-dependent radical scavenging activity. *O. sanctum* exhibited superior antioxidant efficacy with lower IC₅₀ values (< 200 µg/ml) compared to *A. squamosa* (< 300 µg/ml), indicating its potential as a natural antioxidant source. These findings support the therapeutic relevance of both plants and provide a foundation for further pharmacological exploration.

Key words: Morphological, Microscopic, Physiochemical, Antioxidant, *Annona squamosa* and *Ocimum sanctum*.

1 INTRODUCTION

The exploration of plant-based compounds has gained momentum in recent years, driven by the growing demand for natural remedies and sustainable healthcare solutions¹. Among the vast array of medicinal plants, *Annona squamosa* (commonly known as custard apple) and *Ocimum sanctum* (holy basil) stand out for their rich phytochemical profiles and traditional therapeutic applications. These plants have been revered in various indigenous systems of medicine for their ability to combat infections, inflammation, and oxidative stress. Oxidative stress, caused by an imbalance between free radicals and antioxidants in the body, is a major contributor to cellular damage and the progression of chronic diseases². Antioxidants derived from plants play a crucial role in neutralizing these reactive species, thereby offering protective benefits³. Phytochemicals such as flavonoids, alkaloids, tannins, and phenolic compounds are known to exhibit potent antioxidant properties, making them valuable targets for scientific investigation⁴.

This study focuses on the qualitative and quantitative evaluation of phytochemicals and the antioxidant activity present in the leaf extracts of *Annona squamosa* and *Ocimum sanctum*. By employing standard phytochemical screening methods and antioxidant assays like DPPH radical scavenging, the research aims to uncover the therapeutic potential of these

botanicals. The comparative analysis not only highlights their individual efficacy but also contributes to the broader understanding of plant-derived antioxidants in disease prevention⁵.

In an era where synthetic drugs often come with adverse effects, the identification of natural antioxidants from readily available plants offers a promising alternative⁶. This investigation seeks to bridge traditional knowledge with modern scientific validation, paving the way for future applications in nutraceuticals and herbal medicine.

2 MATERIALS AND METHODS

2.1 Collection of Plant Materials

The Leaves of plant *Annona squamosa* and *Ocimum sanctum* were collected locally from Jodhpur, Rajasthan, India.

2.2 Authentication of Plant Material

The plant *A. squamosa* and *O. sanctum* was authenticated via order No.: भा. व. स./शु. आ. क्षे. के./A.I2012/Tech.2024-25(PI. Id)/ 520 dated 01/11/2024 of Botanical Survey of India, (Ministry of Environment, Forest and Climate Change) Arid Zone Regional Centre, Jodhpur, Rajasthan, India.

2.3 Drying and Size Reduction of Plant Material

Leaves (1kg) of *A. squamosa* and *O. sanctum* were collected & washed with distilled water to get rid of dirt and soil. The collected leaves were dried under shade at room temperature for 10 to 15 days. The dried plant leaves was powder by using a grinding mill to obtain a coarse powder and then passed through 40 mesh sieve⁷.

2.4 Physiochemical Analysis of Powder

Physiochemical screening of powdered leaves was done by the standard reported methods. Physiochemical screening was also uses as standardization of the plant, the obtained values of these parameters were useful in the characterization of plant⁸.

2.5 Extraction of Leaves of *A. Squamosa* and *O. Sanctum*

The powdered material was subjected to extraction using methanol by solvent extraction method. Initially 250 gm

of crude powder was taken and packed in a packing paper. This pack was placed in a Soxhlet extractor & extract with solvent (ethanol for *A. squamosa* and water for *O. sanctum*) the extraction was carried out until the extract becomes colorless. The extract was then filtered with Whatman filter paper (No.1) and the filtrate was evaporated to dryness in rotary evaporator at 40 °C. The gained crude extract was stored in a refrigerator at 4° C until time of use⁹.

2.6 Extractive Value of Extracts

To calculate the extractive value of extract following formula was employed¹⁰:

$$\text{Extractive value (\% Yield)} = \frac{\text{Weight of plant extract obtained}}{\text{Weight of plant material used}} \times 100$$

2.7 Phytochemical Analysis of Crude Extract

Standard screening test of extract was performed for various plant constituents. The crude extract was analyzed for the absence or presence of secondary metabolites such as alkaloids, glycosides, carbohydrates, proteins, flavonoids, tannins steroidal compounds, phenolic compounds, flavonoids, and saponins by using standard procedures¹¹.

2.8 Estimation of Total Flavonoids Content

Estimation of total flavonoids content was based on aluminum chloride method. Accurately weighed 10 mg of pure quercetin was solubilized in 10 ml methanol and various aliquots of 5- 25µg/ml were prepared in methanol. Accurately weighed 10 mg of dried extract was solubilized in 10 ml methanol then filter it. From this filtrate of 1mg/ml concentration, 3ml was used for estimation of flavonoids. The 3 ml of extract was mixed with 2% AlCl₃ (1 ml) solution and kept aside for 15min at ambient temperature; absorbance was recorded at 420 nm¹².

2.9 Estimation of Total Phenol Content

The total phenolic content in extract was estimated by the modified Folin-Ciocalteu method. Accurately weighed 10 mg Gallic acid was solubilized in 10ml methanol, various aliquots of 10- 50µg/ml was prepared in methanol. Accurately weighed 10 mg of dried extract was solubilized in 10ml methanol then filter it. From this filtrate of 1mg/ml concentration, 2ml was utilized for the estimation of phenol. The 2 ml of extract and standard were added 1ml of Folin-Ciocalteu reagent (prepared in distilled water 1:10 v/v) and 1ml (7.5g/l) of sodium carbonate. The obtained mixture was

vortexes for 15s and kept for 10min for development of color. Absorbance was obtained at 765 nm using spectrophotometer¹³.

2.10 Antioxidant Activity

Antioxidant activity by DPPH assay method: The antioxidant activity of the extracts was determined using the DPPH free radical scavenging assay. Briefly, the universal bottle was contained 50 µL of tea infusions in concentrations from 1 to 5 mg/ml and 5 ml 0.004% (w/v) solution of DPPH was added. The obtained mixture was vortexed, incubated for 30 min in room temperature in a relatively dark place and then was read using spectrophotometer at 517 nm. The blank was 80% (v/v) methanol. Ascorbic acid (Vitamin C) was used for comparison. Measurements were taken in triplicate. DPPH scavenging effect was calculated using following equation:

$$\text{DPPH Scavenging (\%)} = \frac{A_0 - A}{A_0} \times 100$$

Where, A₀ is the absorbance of negative control (0.004% DPPH solution) and A is the absorbance in presence of extract. The results were reported as IC₅₀ values and ascorbic acid equivalents (AAE, mg/g) of tea infusion extracts¹⁴.

2.11 Reducing Power by ABTS Radical Scavenging Method

The ABTS radical (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) scavenging activity was carried out based on the method of Gan and Latiff with some modifications. Briefly, ABTS⁺ was produced directly by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate and allowing the mixture to stand for 16 h at room temperature in the dark. Prior to beginning the assay, the ABTS solution was diluted with methanol. One milliliter of sample solution with different concentrations (0.1, 0.2, 0.3, 0.4, and 0.5 mg/ml) was added to 2 ml of the ABTS solution mixed solution was observed at 734 nm. The sample absorbance was read at 734 nm after 30 min incubation at room temperature. Ascorbic acid was used as a standard¹⁵. The ABTS radical-scavenging activity was calculated according to the following equation:

$$\text{ABTS + scavenging activity (\%)} = 1 - \frac{A_2 - A_1}{A_0} \times 100$$

Where, A₀ was defined as the absorbance of control at 734 nm, and A₁ and A₂ were defined as the absorbance of the sample without the ABTS⁺ solution and with added ABTS⁺ solution, respectively.¹⁵

3 RESULTS AND DISCUSSION

3.1 Morphological Characters of The Leaves of *Annona Squamosa* and *Ocimum Sanctum*

Preliminary plant materials were morphologically studied as the leaves of *A. squamosa* were dull green in color, faint odor on crushing, leaves were oblong to lanceolate with pointed tip, 6-17 cm long and 2-4 cm broad and smooth margin. The leaves of *O. sanctum* were arranged opposite with 2-5 cm long and about 1cm broad, green or purple in color, strong aromatic herbal scent.

Table 1: Morphological study of plant

Plant parts	<i>Annona squamosa</i>	<i>Ocimum sanctum</i>
Leaves arrangement	Alternate	Opposite
Leaves size	6-17 cm long and 2-4 cm broad	2-5 cm long and about 1cm broad
Leaves shape	Oblong to lanceolate with pointed tip	Ovate
Margin	Smooth margin,	Serrated
Color	Dull green	Green or purple
Crushed leaf Odor	Faint	Strong aromatic scent
Flower	Greenish-yellow with three outer fleshy petals	Small, purplish or white, five petals,
Fruit	Round or heart-shaped with a knobby surface with creamy pulp	Small and brown, Nutlets
Seed	Hard, black or dark brown	Small black
Stem and bark	Woody, gray	Hairy, Quadrangular

3.2 Microscopic Analysis of The Leaves of *Annona Squamosa* and *Ocimum Sanctum*

3.2.1 *Annona squamosa*

Upper epidermis was single-layered, covered with a thick cuticle, and lower epidermis was contains numerous multicellular, uniseriate trichomes. Anisocytic type stomata was present mostly on the lower epidermis. Mesophyll was differentiated into palisade and spongy parenchyma. Oil cells were scattered in mesophyll. Collateral type vascular bundles observed that surrounded by a bundle sheath with parenchymatous cells. Calcium oxalate crystals presented in the form of raphides.

3.2.2 *Ocimum sanctum*

Both upper and lower epidermis contains glandular and non-glandular trichomes, important for essential oil secretion. Diacytic type stomata present on both surfaces, more abundant on the lower side. Two layers of tightly packed cells was found in mesophyll, also contains large intercellular spaces and oil glands. Vascular bundle observed as collateral and surrounded by bundle sheath and Xylem and phloem well differentiated. Essential oil glands were abundant and rosette-type calcium oxalate crystals were present.

3.3 Physiochemical Screening of Powders

Physiochemical screening of dried leaves powders was performed for standardization purpose.

Table 2: Physiochemical screening of leaves powder of selected plants

S. No.	Parameters	<i>A. squamosa</i>	<i>O. sanctum</i>
1	Loss on drying (%)	8.84 %	6.65
2	Total ash value (%)	15.12	14.25
3	Acid insoluble ash value (%)	4.26	3.14
4	Water soluble ash value (%)	3.15	4.45
5	Foaming index	14 (ml)	16 (ml)

3.3.1 Extractive value of *Annona squamosa* and *Ocimum sanatum* leaves

The extractive value of water extract of *O. sanctum* powder was 16.27% and methanol extract of *A. squamosa* was 13.28%.

Table 3: Percentage yields of extracts

S. No.	Extracts	Yield	Color
1	Methanol extract of <i>A. squamosa</i>	13.28 %	Dark green
2	Water extract of <i>O. sanctum</i>	16.27 %	Green

3.4 Estimation of Total Flavonoids Content and Total Phenol Content

Total flavonoids content (TFC) and total phenol content (TPC) of Methanol extract of *A. squamosa* were found as 43.23 mg equivalent to per 100 mg of quercetin and 26.43 mg equivalent to

per 100 mg of Gallic acid. Total flavonoids content (TFC) and total phenol content (TPC) of Water extract of *O. sanctum* were found as 3.65 mg equivalent to per 100 mg of quercetin and 2.14 mg equivalent to per 100 mg of Gallic acid.

Table 4: Total flavonoids content (TFC) and Total phenol content (TPC)

S. No.	Extracts	TFC (equivalent to per 100 mg of quercetin)	TPC (equivalent to per 100 mg of Gallic acid)
1	Methanol extract of <i>A. squamosa</i>	43.23 mg	26.43 mg
2	Water extract of <i>O. sanctum</i>	3.65 mf	2.14 mg

3.5 Phytochemical Screening of Extracts of *Annona Squamosa* and *Ocimum Sanctum* Leaves

The crude extract obtained was subjected to various qualitative tests to detect presence of phytochemicals, study revealed the presence of Alkaloids, Glycosides, Carbohydrates, Phytosterols, Saponins, Tannins, Flavonoids but the Proteins were not found.

Table 5: Phytochemical screening of extracts

Chemical Tests	<i>Annona squamosa</i>	<i>Ocimum sanctum</i>
Alkaloids		
Dragendorff's Test	(+)	(+)
Mayer's Test	(+)	(+)
Hager's Test	(+)	(+)
Glycosides		
Legal Test	(+)	(+)
Baljet Test	(+)	(+)
Borntrager's Test	(+)	(+)
Carbohydrates		
Molisch's Test	(+)	(+)
Benedict's test	(+)	(+)
Fehling's Test	(-)	(+)
Steroids and Sterols		
Salkowski Test	(+)	(+)
Libermann-Burchard	(+)	(+)
Proteins and Amino Acids		
Biuret Test	(-)	(-)
	(+)	(-)

Ninhydrin Test	(-)	(-)
Millon's Test		
Tannins		
5% ferric chloride solution	(+)	(+)
10% aqueous K ₂ Cr ₂ O ₇ solution	(+)	(+)
10% lead acetate solution	(+)	(+)
Flavonoids		
Shinoda's Test	(+)	(+)
Alkaline reagent test	(+)	(+)
Lead acetate test	(+)	(+)
Saponins		
Foam taste	(+)	(+)

(+) = Present, (-) = Absent

3.6 Antioxidant Activity

3.6.1 Anti-oxidant activity by DPPH assay method

Antioxidant activity was obtained by using three methods. Free radical scavenging activity of samples was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH). It was observed that the IC₅₀ value for *A. squamosa* (<300 µg/ml) and for *O. sanctum* (< 200 µg/ml) and for extract mix (< 200 µg/ml). It was observed that the antioxidant activity increased with the concentration of extract.

Table 6: Inhibitions (%) of DPPH by leaves extract

Conc. (µg/ml)	% Inhibition of DPPH by extract			
	Ascorbic acid	<i>Annona squamosa</i>	<i>Ocimum sanctum</i>	Extract Mix (1:1)
100	54.13 ± 0.73	36.24 ± 1.37	38.25 ± 0.63	40.24 ± 0.82
200	68.26 ± 1.06	45.43 ± 0.22	52.33 ± 0.86	57.18 ± 1.29
300	74.77 ± 1.32	65.25 ± 1.63	70.06 ± 0.93	74.25 ± 0.89
400	86.82 ± 0.81	73.13 ± 1.21	81.57 ± 0.72	84.26 ± 0.22
500	97.73 ± 1.02	88.36 ± 0.06	93.21 ± 1.22	96.12 ± 1.32

3.6.2 Reducing power by ABTS radical scavenging method

The ABTS radical (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) scavenging activity was carried out based on the method reducing power by ABTS radical scavenging method observed as the IC₅₀ value of *A. squamosa* (<300 µg/ml) and for *O.*

sanctum (< 200 µg/ml). for extract mix (< 200 µg/ml). It was observed that the antioxidant activity increased with the concentration of extract.

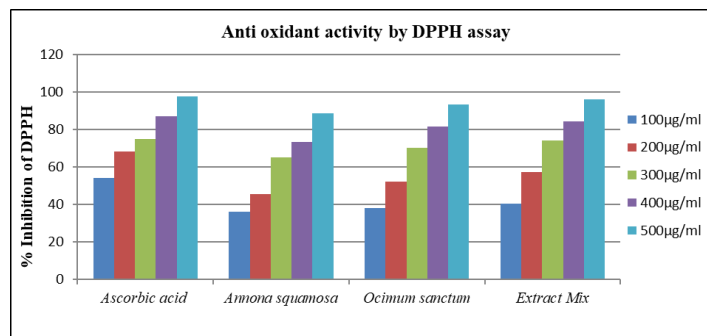


Figure 1: Anti-oxidant activity by DPPH assay method

Table 7: Inhibitions (%) of ABTS by leaves extract

Conc. (µg/ml)	% Inhibition of ABTS by			
	Ascorbic acid	<i>Annona squamosa</i>	<i>Ocimum sanctum</i>	Extract Mix (1:1)
100	55.42 ± 0.34	31.11 ± 0.74	38.32 ± 0.91	39.81 ± 0.44
200	64.68 ± 1.05	42.41 ± 0.57	54.71 ± 1.08	57.19 ± 1.02
300	72.41 ± 0.75	61.45 ± 1.63	73.43 ± 0.92	75.11 ± 0.99
400	85.46 ± 0.52	70.56 ± 0.81	83.62 ± 1.06	85.22 ± 1.13
500	96.64 ± 1.14	79.16 ± 1.67	94.12 ± 0.84	96.26 ± 1.42

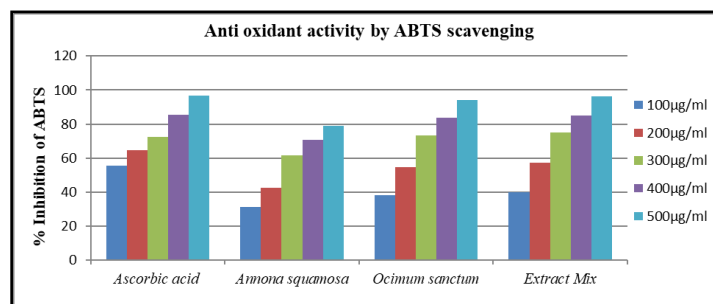


Figure 2: Anti-oxidant activity by ABTS scavenging.

CONCLUSIONS

The comparative evaluation of *Annona squamosa* and *Ocimum sanctum* leaves revealed distinct morphological, anatomical, and phytochemical characteristics that contribute to their medicinal significance. Morphologically, both plants exhibited unique leaf structures and organoleptic properties, aiding in their identification and authentication. Microscopic analysis

confirmed the presence of diagnostic features such as trichomes, stomatal types, and calcium oxalate crystals, which further support their taxonomic differentiation. Physiochemical parameters and extractive values provided essential insights into the quality and purity of the plant materials. Notably, *A. squamosa* demonstrated higher total flavonoid and phenolic content, suggesting a richer phytochemical profile. However, *O. sanctum* exhibited superior antioxidant activity in both DPPH and ABTS assays, indicating its potential as a potent natural antioxidant source.

The presence of diverse bioactive compounds such as alkaloids, glycosides, saponins, tannins, and flavonoids in both species underscores their therapeutic relevance in traditional medicine. Overall, this study establishes a foundational understanding of the pharmacognostic and phytochemical attributes of *A. squamosa* and *O. sanctum*, supporting their continued exploration in herbal drug development and standardization.

CONFLICT OF INTERESTS

There are no any conflicts of interests.

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