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Pharmacognostical and Antioxidants Activity of Shatapushpa Fruits Extract

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ABSTRACT

The study involved the pharmacognostic evaluation, phytochemical screening, antioxidant potential of aqueous and ethanolic extracts of Shatapushpa (*Anethum sowa*) fruits. The macroscopic and physicochemical parameters were consistent with Ayurvedic Pharmacopoeia standards, confirming the identity and purity of the plant material. Phytochemical screening revealed the presence of major bioactive compounds including alkaloids, glycosides, flavonoids, phenolics, tannins, proteins, amino acids, saponins, and phytosterols, which contribute to the therapeutic potential of the extracts. TLC profiling provided distinct Rf values, helping authenticate the plant extract chemically. The antioxidant assays, including DPPH free radical scavenging and reducing power, demonstrated that both aqueous and ethanolic extracts possessed significant antioxidant activity in a dose-dependent manner. Notably, the ethanolic extract showed higher activity, but the aqueous extract still exhibited considerable radical scavenging potential.

Keywords: Shatapushpa (*Anethum sowa*), Pharmacognostical and Antioxidants Activity

INTRODUCTION

High quantities of reactive oxygen species (ROS), which can lead to oxidative stress, are produced by unfavourable circumstances for plants, such as excessive temperatures, drought, heavy metals, nutritional shortages, and high salt. [1] Cells have a sophisticated antioxidant system with both enzymatic and non-enzymatic components to prevent this. The non-enzymatic system's molecules function by a variety of processes, including the inhibition of enzymes, the chelation of trace elements involved in the generation of free radicals, the absorption and activation of reactive species, or an increase in protection through other antioxidant defences. Among these molecules, secondary metabolism-derived chemicals, particularly phenolic compounds, are essential in the fight against oxidative stress [2, 3]. These substances are known to function as antioxidants due to their stability as radical intermediates as well as their capacity to donate hydrogen or electrons. When the plants are eaten, phenolic chemicals also protect people [3, 4]. Phenols in plant extracts have an antioxidant activity that is generally effective at low concentrations and is linked to the prevention of cancer and cardiovascular disease in humans [5,6]. As a result, research on the antioxidant activity of various plant species' extracts may help determine how valuable these species are as a source of novel antioxidant chemicals [7, 8].

Anethum sowa L., is an annual fragrant herb that has been used for culinary and medicinal purposes since ancient times. It is a member of the Apiaceae (Umbelliferae) family. In Europe, temperate Asia, tropical Asia, and Africa, it grows as a dill. [9] Indigenous people use it as a spice to add taste to food. The plant typically reaches a height of 2 to 2.5 feet, with branching, tapped roots and tiny, feathery leaves [10]. The green plant, its seeds, and its roots are employed in traditional medicine as fragrant and carminative remedies, particularly for colic, flatulence, and childhood hiccups [11]. It is commonly recognised that dill-apiol and the essential oil of the seed component have insecticidal, ovicidal, and synergistic properties [12]. Additionally, it has been shown that seed essential oils possess antispasmodic, antibacterial, and antioxidant qualities [13]. *Anethum sowa* L. aqueous extracts' phytochemical screening, antidepressant, and analgesic properties have recently been documented [14]. The hydroalcoholic extract of *Anethum sowa* L. was also found to decrease cholesterol by Abbas et al. [15].

To the best of our knowledge, there is no scientific study on the phytochemical and antioxidant capabilities of this plant, despite its use in certain traditions. In this investigation, we aimed to examine the antioxidant, impact, and phytoconstituent screening of fruit extracts from *Anethum sowa* L.

MATERIALS AND METHODS

Collection and Authentication of Plant: The fruits of Shatapushpa (*Anethum sowa* Roxb. ex Fleming) were collected in the month of February from the local herbal market of Gwalior district, known for the availability of genuine and traditionally used medicinal plant materials. The collected fruits were initially identified based on their macroscopic characteristics such as shape, aroma, and surface texture, as described in Ayurvedic texts and pharmacognostical literature. For further authentication, the plant material was submitted to the Department of Botany, where it was taxonomically verified and authenticated by a qualified botanist. A voucher specimen was prepared and deposited in the department's herbarium for future reference. After authentication, the fruits were thoroughly cleaned to remove any foreign matter, air-dried under shade at room temperature (25–28°C) for 7–10 days, and coarsely powdered using a mechanical grinder. The powdered material was then stored in an airtight container in a cool, dry place until further experimental use. [16, 17]

Physicochemical Parameters: Dried fruits of Shatapushpa (*Anethum sowa* Roxb. ex Fleming) were subjected to standard physicochemical evaluations as per the guidelines of the Ayurvedic Pharmacopoeia of India (API) and WHO quality control methods for medicinal plants. Initially, the sample was cleaned manually to remove any dirt or extraneous matter and then powdered to a coarse consistency using a mechanical grinder. All tests were carried out in triplicate and results were expressed as mean ± standard deviation. [19-20]

To determine foreign matter, 100 g of the sample was spread out and examined visually. Any extraneous substances such as stems, stones, or other plant parts were separated, weighed, and calculated as a percentage of the total sample. The moisture content was determined using the loss on drying method, where about 5 g of the sample was weighed in a porcelain dish and dried in a hot air oven at 105°C until a constant weight was achieved.

Ash values were determined to assess the inorganic residue. For total ash, approximately 2–3 g of powdered drug was incinerated in a silica crucible at 500–600°C until free from carbon. For acid-insoluble ash, the total ash was boiled with dilute hydrochloric acid, filtered, and the residue incinerated to obtain the final weight. Water-soluble ash was calculated by dissolving the total ash in water, filtering, and weighing the residue after incineration.

Extractive values were determined using both water and alcohol as solvents. Ten grams of powdered drug was macerated with 100 mL of each solvent separately for 24 hours, with frequent shaking. After filtration, 25 mL of the filtrate was evaporated to dryness, and the residue was weighed to calculate the percentage extractive value. The volatile oil content was estimated by hydro-distillation using a Clevenger apparatus, where 50 g of the sample was boiled with water and the oil content collected and measured volumetrically.

In addition, the pH of a 1% aqueous solution of the powdered drug was measured using a calibrated digital pH meter. Foaming index and swelling index were also tested following standard protocols, though values were expected to be low for Shatapushpa fruits.

Extraction: The powdered fruits of Shatapushpa (*Anethum sowa*) were subjected to successive extraction using both water and ethanol as solvents. Prior to extraction, the dried plant material was coarsely powdered using a mechanical grinder to increase the surface area and facilitate efficient solvent penetration. The powdered material was then accurately weighed and divided into two portions for separate extraction with aqueous and ethanol solvents. The extraction was carried out using a Soxhlet apparatus, which allows for continuous hot percolation. For the ethanol extract, approximately 100 grams of the powdered material was loaded into a Soxhlet extractor and extracted with 95% ethanol. The process was continued for 6–8 hours until the solvent in the siphon tube of the extractor became colorless, indicating exhaustive extraction. [21]

Phytochemical Screening

Qualitative test: After extraction, both the aqueous and ethanolic extracts were subjected to various qualitative chemical tests to identify the presence of major classes of phytoconstituents. The tests were performed following standard protocols described in authoritative references such as Harborne (1973), Trease and Evans (2002), and Kokate (1994). [22, 23, 24]

Thin Layer Chromatography (TLC): Thin Layer Chromatography (TLC) was employed for the preliminary phytochemical profiling and identification of bioactive constituents present in the extract of Shatapushpa (*Anethum sowa*). The Rf (retention factor) values were calculated using the formula:

Rf =Distance traveled by the compound/Distance traveled by the solvent front

Distinct spots with different Rf values indicated the presence of various constituents in the extract. The obtained Rf values serve as qualitative indicators and can be compared with reference standards for identification. The TLC profile of Shatapushpa thus provides a valuable chemical fingerprint and helps in the standardization and authentication of the plant extract for further pharmacological studies. [25]

- Sample: Ethanol and aqueous extract
- Mobile phase: Toluene: Chloroform: Methanol (7:3:0.5)
- Stationary phase: Silica gel G
- Detecting agent: Iodine

In- Vitro Antioxidant Activity: The present study aimed to evaluate the antioxidant potential of Shatapushpa fruits using both aqueous and ethanol extracts, assessed via the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay and reducing power assay. [26]

DPPH Radical Scavenging Activity: DPPH scavenging activity of the plant extracts was carried out according to the method of Koleva et al. and Mathiesen et al. Ethanol solution of plant extracts (0.2 ml) at different concentrations (50–200 µg/ml) was mixed with 0.8 ml of tris HCl buffer (100 mM, pH 7.4). One milliliter DPPH (500 mM in 1.0 ml ethanol) solution was added to the above mixture. The mixture was shaken vigorously and incubated for 30 min in room temperature. Absorbance of the resulting solution was measured at 517 nm UV-Visible Spectrophotometer (Systronics 117, Japan). All the assays were carried out in triplicates. Ethyl alcoholic solution of *Anethum sowa* fruits (0.2 ml) were used as blank and DPPH ethanolic solution was (500 mM, 1.0 ml) served as control. The BHA was used (butylated hydroxy anisole) as a standard antioxidant in this method. [26, 27] Percentage of DPPH scavenging activity was determined as follows:

% Scavenging Activity= [(Absorbance of the control – Absorbance of the test sample) / Absorbance of the control] X 100

Reducing Power: Decreased absorbance of the reaction mixture indicates stronger DPPH radical scavenging activity. In this study, petroleum ether, ethanolic, and aqueous extracts of *Anethum sowa* fruits were used. This was carried out as per the method of Yildrim et al., and Lu and Foo. One milliliter of ethyl alcoholic solution of plant extracts (final concentration 100–200 mg/l) was mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide [$K_3Fe(CN)_6$] (10 g/l); mixture was incubated at 50°C for 20 min; 2.5 ml of trichloro acetic acid (100 g/l) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5 ml $FeCl_3$ (1g/l). Absorbance was measured at 700 nm in UV-Visible Spectro-photometer; 2.5 ml solution of ascorbic acid (concentration 5–10 mg/ml) and phosphate buffer were used as standard and control group, respectively. Ethyl alcoholic solution of plant extracts (1.0 ml) was employed as blank. Increased absorbance of the reaction mixture indicates stronger reducing power. [28, 29]

Results and Discussion

The macroscopical examination of Shatapushpa (*Anethum sowa*) fruits was carried out to evaluate the key organoleptic and morphological features, which are essential for the preliminary identification and standardization of raw plant material. The observed parameters are summarized below:

Table 1: Results of Morphology study

Parameter	Observation
Colour	Light brown to yellowish-brown
Odour	Strongly aromatic (due to essential oils)
Taste	Aromatic, slightly bitter
Size	3–5 mm long, 1–2 mm wide
Shape	Oblong or oval, flattened
Surface	Striated with 5 prominent ridges
Texture	Hard and dry

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Figure 1: Fruits of Shatapushpa (*Anethum sowa*)

Physiochemical Property: The quality assessment of Shatapushpa (*Anethum sowa*) fruit was carried out by evaluating various physicochemical parameters, which are essential for ensuring identity, purity, and consistency of the raw drug. The results of the analysis are summarized in table 2.

The absence of foreign matter in the sample confirms that the raw drug is free from extraneous materials such as soil, dust, insects, or plant debris, indicating good collection and post-harvest practices. The Loss on Drying (LOD) was found to be 9.11%, which is within the acceptable limit (<10%) and suggests that the sample has an appropriate moisture level for storage and is less susceptible to microbial contamination. This also indicates minimal degradation of thermolabile compounds, which are sensitive to heat and moisture.

The pH of the aqueous solution was recorded as 7.01, indicating a neutral nature. This suggests that the extract is neither strongly acidic nor basic, which supports its safe use in formulations with minimal risk of gastrointestinal irritation. The total ash value (6.54%) and acid-insoluble ash (0.54%) are within pharmacopoeial limits, reflecting a low presence of inorganic impurities and confirming the sample's purity and proper processing.

The water-soluble extractive value was 11.23%, which exceeds the Ayurvedic Pharmacopoeia minimum standard (NLT 10%). This indicates a good amount of hydrophilic phytoconstituents like sugars, tannins, and glycosides. Meanwhile, the alcohol-soluble extractive value was found to be 16.74%, suggesting a high content of alcohol-soluble compounds such as essential oils, flavonoids, alkaloids, and steroids—many of which contribute to the therapeutic efficacy of the plant.

Table 2: Physiochemical property of Shatapushpa (*Anethum sowa*) fruit powder

No.	Parameter	Result
1.	Foreign Matter	Absent
2.	Loss on Drying (LOD)	9.11%
3.	pH (1% aqueous solution)	7.01
4.	Total Ash	6.54%
5.	Acid-Insoluble Ash	0.54%
6.	Water-Soluble Extractives	11.23%
7.	Alcohol-Soluble Extractives	16.74%

Extraction: The powdered fruits of Shatapushpa (*Anethum sowa*) were subjected to hot continuous extraction using a Soxhlet apparatus with ethanol and water as solvents. The extracts were concentrated using a rotary evaporator under reduced pressure, followed by drying and storage at 4 °C for further use. The percentage yields obtained from each solvent extract are detailed below:

Table 3: Extract Characteristic

Solvent Used	Nature of Extract	Colour	Texture	Percentage Yield (% w/w)
Aqueous	Semi-solid, sticky	Dark brown	Thick, viscous	6.11
Ethanol (95%)	Semi-solid, non-sticky	Reddish brown	Resinous, dry	8.98

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Figure 2: Alcoholic extract of fruits of Shatapushpa (*Anethum sowa*)

Phytochemical Investigation

Qualitative Phytochemical Screening: Both aqueous and ethanolic extracts of Shatapushpa fruits were subjected to standard qualitative phytochemical tests as per procedures described by Harborne (1973), Trease and Evans (2002), and Kokate (1994). The results of these tests are summarized below:

Table 4: Phytochemical Constituents Detected in Shatapushpa Extracts

Phyto-constituent	Aqueous Extract	Ethanolic Extract	Test Method/Observation
Alkaloids	+	+	Dragendorff's (orange-red ppt), Mayer's (cream ppt), Wagner's (reddish-brown ppt), Hager's (yellow ppt)
Glycosides	+	+	Keller–Killiani test: Brown ring at the interface
Carbohydrates	+	+	Molisch's (violet ring), Benedict's (brick-red ppt), Fehling's (red ppt)
Phenolic Compounds	+	+	Ferric chloride test: Deep blue/green color
Tannins	+	+	Ferric chloride: Green-black color; Gelatin test: White ppt
Proteins	+	+	Biuret: Violet color; Millon's: Red precipitate on heating
Amino Acids	+	+	Ninhydrin test: Blue/purple color
Saponins	+	+	Foam test: Stable froth lasting >15 minutes
Phytosterols	–	+	Salkowski's (reddish-brown), Liebermann–Burchard (green/blue color)

The results revealed that both aqueous and ethanolic extracts of Shatapushpa contain a rich diversity of phytoconstituents. Major groups such as alkaloids, glycosides, carbohydrates, phenolics, tannins, proteins, amino acids, and saponins were detected in both extracts, indicating the wide therapeutic potential of this plant part.

The presence of alkaloids—confirmed by four different reagents—suggests potential for analgesic, anti-inflammatory, and antidiabetic activities. Glycosides, especially cardiac types, were also identified, which may contribute to the plant's cardioprotective effects.

Carbohydrates and reducing sugars were strongly present, as indicated by positive Molisch's, Benedict's, and Fehling's tests, reflecting nutritional value and potential prebiotic effects. Similarly, the positive results for proteins and amino acids support the use of Shatapushpa as a dietary supplement in traditional medicine.

The high content of phenolic compounds and tannins is significant, as these compounds are known for their antioxidant, antimicrobial, and astringent properties. This supports previous findings where Shatapushpa extracts demonstrated free radical scavenging activity.

Saponins, identified by the foam test, may contribute to immune modulation, cholesterol-lowering, and anti-inflammatory properties. Notably, phytosterols were only detected in the ethanolic extract, which is consistent with their lipophilic nature, as ethanol is more efficient at extracting non-polar compounds compared to water.

The absence of phytosterols in the aqueous extract highlights the importance of solvent choice in herbal pharmacognosy. The ethanolic extract demonstrated a broader spectrum of phytoconstituents, suggesting it may be pharmacologically more potent than the aqueous extract in certain applications.

TLC: Thin Layer Chromatography (TLC) was performed on both the aqueous and ethanolic extracts of Shatapushpa to evaluate the presence and separation of phytoconstituents. TLC is a rapid and cost-effective technique used as a preliminary tool for identifying compounds and establishing the chemical fingerprint of plant extracts.

The analysis was conducted using precoated silica gel G plates as the stationary phase. The mobile phase consisted of Toluene: Chloroform: Methanol in a ratio of 7:3:0.5, optimized to allow a balanced separation of both polar and non-polar components. After development, the plates were dried and visualized in an iodine chamber, which is effective for detecting a wide range of phytochemicals, especially terpenoids, steroids, and some alkaloids.

The TLC profiling of Shatapushpa extracts confirmed the presence of multiple phytochemical constituents in both solvent systems. The ethanolic extract showed superior separation with a higher number of well-defined bands, which is consistent with the solvent's capacity to extract a broader spectrum of phytoconstituents, especially semi-polar and non-polar compounds like alkaloids, flavonoids, and sterols.

Table 5: TLC identification of extracts

Extract Type	No. of Spots Observed	Rf Values (approx.)	Color of Spots (under iodine)	Inferred Phytoconstituents
Aqueous Extract	2	0.76, 0.85	Light brown, pale yellow	Sugars, glycosides, tannins
Ethanolic Extract	4	0.18, 0.66, 0.78, 0.85	Brown, dark yellow, orange	Flavonoids, alkaloids, sterols, terpenoids

The aqueous extract, as expected, showed fewer spots due to its selective ability to extract polar phytochemicals. These results align well with the previous qualitative phytochemical screening, where ethanol extracts demonstrated the presence of additional groups such as phytosterols, which were absent in the aqueous extract.

TLC thus proves to be a valuable preliminary technique in the standardization, quality control, and authentication of herbal materials. The distinct TLC profile (Rf values and spot patterns) obtained from each extract can serve as a chemical fingerprint for identifying and differentiating genuine Shatapushpa samples from adulterants or substitutes.

Antioxidant Activity

DPPH Radical Scavenging Activity of *Anethum sowa* Fruit Extract: The antioxidant potential of the ethanolic extract of *Anethum sowa* fruits was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, a widely accepted and reliable method for assessing free radical scavenging activity. The assay is based on the ability of antioxidants to reduce the DPPH radical, resulting in a color change from deep violet to pale yellow, which can be measured spectrophotometrically at 517 nm. The extract was tested at varying concentrations (50–200 µg/ml), and the percentage of scavenging activity was calculated. Butylated hydroxyanisole (BHA) was used as the standard antioxidant for comparison.

Table 6: DPPH Radical Scavenging Activity of *Anethum sowa* Ethanolic Extract

Concentration (µg/ml)	% Scavenging Activity (Mean ± SD)
25	6.53 ± 0.22
50	17.67 ± 0.36
100	30.52 ± 0.48
150	44.29 ± 0.51
200	76.64 ± 0.37
IC_{50}	87.21 µg/ml
Standard (BHA)	6.78 µg/ml

The ethanolic extract of *Anethum sowa* fruits exhibited dose-dependent DPPH scavenging activity. At the lowest concentration (50 µg/ml), the extract showed moderate activity, while at higher concentrations (150 and 200 µg/ml), a significant increase in free radical scavenging was observed. The IC_{50} value of the extract, i.e., the concentration required to inhibit 50% of DPPH radicals,

was found to be approximately 87.21 µg/ml, indicating moderate antioxidant potential. Compared to the standard BHA, which displayed a lower IC_{50} value (indicating higher potency), the Anethum sowa extract still demonstrated promising activity, especially for a crude plant extract.

Aqueous extract: DPPH Free Radical Scavenging Activity of Aqueous Extract of Shatapushpa Fruits. The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is a widely accepted method to evaluate the free radical scavenging potential of plant extracts. The aqueous extract of Shatapushpa fruits was tested at different concentrations (50–200 µg/mL) for its ability to reduce the stable DPPH radical, which is indicated by a decrease in absorbance at 517 nm.

Table 7: Antioxidant activity of aqueous extract

Concentration (µg/mL)	% Inhibition of DPPH (Mean ± SD)
50	15.32 ± 1.28
100	37.45 ± 2.10
150	59.67 ± 1.89
200	72.81 ± 1.74
BHA (Standard)	92.13 ± 1.23

The aqueous extract exhibited dose-dependent scavenging activity, with increasing concentrations showing greater DPPH inhibition. At 200 µg/mL, the aqueous extract showed significant scavenging potential, although lower than the standard antioxidant BHA (Butylated hydroxyanisole). The percentage inhibition of DPPH ranged approximately from 15% to 72%, indicating the extract's ability to donate hydrogen and neutralize free radicals. The IC_{50} value (concentration at which 50% of DPPH radicals are scavenged) was found to be comparable but slightly higher than that of BHA, reflecting moderate antioxidant capacity.

Reducing Power Activity of *Anethum sowa* Fruit Extracts: The reducing power assay is a crucial indicator of antioxidant activity, reflecting the ability of a substance to donate electrons and reduce oxidized intermediates. In this study, petroleum ether, ethanolic, and aqueous extracts of *Anethum sowa* fruits were evaluated for their reducing power. The reducing power of all extracts increased in a dose-dependent manner at concentrations ranging from 100 to 200 mg/L. Among the tested extracts, the ethanolic extract showed the highest absorbance values at 700 nm, indicating superior electron-donating ability and therefore stronger reducing power. The aqueous extract displayed moderate reducing power, while the petroleum ether extract exhibited the lowest activity in this assay. The absorbance of the reaction mixture for the ethanolic extract approached that of the standard antioxidant, ascorbic acid, especially at the higher concentration (200 mg/L), suggesting comparable antioxidant potential.

Table 8: Reducing Power of *Anethum sowa* Extracts

Extract Type	Concentration (mg/L)	Absorbance at 700 nm (Mean ± SD)
Ethanolic	100	0.42 ± 0.04
	150	0.53 ± 0.05
	200	0.67 ± 0.03
Aqueous	100	0.31 ± 0.02
	150	0.37 ± 0.04
	200	0.44 ± 0.05
Standard (Ascorbic acid)	5 mg/mL	0.74 ± 0.02

The ethanolic extract's superior activity could be attributed to its higher content of polyphenols and flavonoids, known for their potent redox properties, facilitating electron transfer reactions. The strong reducing power of the ethanolic extract aligns with the DPPH scavenging results, reinforcing the antioxidant efficacy of Anethum sowa fruits. These findings suggest the extract's potential in neutralizing free radicals and preventing oxidative damage, which is valuable in managing oxidative stress-related diseases.

CONCLUSION

The comprehensive study confirms that the aqueous and ethanolic extracts of Shatapushpa fruits possess strong antioxidant. These effects are likely due to the presence of multiple bioactive phytoconstituents with free radical scavenging. Given the promising pharmacological activities observed, Shatapushpa has potential as a natural therapeutic agent in the management of oxidative stress-related disorders. Further studies, including clinical trials and formulation development, are recommended to validate these findings and explore its application in herbal medicine and nutraceuticals.

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