GAP INDIAN JOURNAL
OF FORENSICS AND BEHAVIOURAL SCIENCES
(ISSN – 2582-8177)
Globally peer-reviewed and open access journal.

FORENSIC AND PHARMACOGENETIC STUDY OF ASPARAGUS OFFICINALIS OF BUNDELKHAND REGION, UTTAR PRADESH, INDIA

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Abstract

Asparagus officinalis is one of the most valuable medicinal plants, regarded as the "Queen of Herbs" in the traditional medical system, and used worldwide to treat a variety of ailments. Keeping this in mind, the purpose of the study is to compare the qualitative and quantitative phytochemical properties of Asparagus officinalis root extract. Methanol, ethanol, water, petroleum ether, and chloroform were utilized as solvents for the Soxhlet extraction process. The qualitative phytochemical examination detected alkaloids, flavonoids, tannins, phenolics, saponins, triterpenoids, and glycosides. Quantitative research demonstrated that ethanol can be used to extract TSC and TPC, whereas ethanol and methanol are suitable solvents for flavonoids. The presence of several phytochemicals in the extracts demonstrates the extracts' potential for application as a medicinal and nutraceutical agent in the industry.

Keywords: *Asparagus officinalis, Shatavari, Phytochemicals, Biochemical Estimation*

INTRODUCTION

Historically, natural products, particularly those of wild origin, have been a significant source of therapeutic agents. It is estimated that 25- 30 % of currently available pharmaceuticals for the treatment of disease are derived from naturally occurring compounds (Krause & Tobin, 2013; Salami et al., 2020). Plant-based medicinal research is frequently based on ethnobotanical information, as of now many of the drugs used are developed from medicinal plants employed in indigenous societies. In recent years major part of ethno-pharmaceutical research has been directed toward a better understanding of the pharmacological effects of individual medicinal plants (Jafarirad & Rasoulpour, 2019). Numerous studies in this field suggest that traditional medicine plants have been evaluated as viable models for pharmacological research(Rivero-Segura & Gomez-Verjan, 2021) Thus, medicinal plants and extracts of natural products have been considered alternative therapies for a variety of ailments (Morilla & Demayo, 2019).

Asparagus officinalis is one of the most valuable medicinal plants, regarded as a “Queen of herbs” in the ancient health system and has been used worldwide to cure various diseases (Singh & Gaikwad, 2020; Srivastava et al., 2018). It is commonly known as Shatavari, Satawar or Satmuli (Ranjan & Prabhakar, 2019; Srivastava et al., 2018) and is widely found in India, Africa, Australia, Nepal, and Sri Lanka (Gupta et al., 2021; Mintah et al., 2019). *A. officinalis* is one of the most important species which belongs to family *Asparagaceae (Boubetra et al., 2017)*. It has abundant therapeutic benefits against a variety of conditions such as, gastric ulcers, dyspepsia, cardiovascular diseases, neurological disorders, cancer, and as a galactagogue (Bopana & Saxena, 2007; Kumari et al., 2014). Owing to its varied applications against several diseases, demand for this plant is continuously increasing. The aforementioned medicinal properties of this plant are the result of their phytochemical content. Phytochemicals are the compounds that are isolated from the plant kingdom. The composition of these chemicals depends on the particular plant's geographical locations and harvesting conditions (Kumari et al., 2014). Hence, quantitative and qualitative investigations are necessary to understand their applications.

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The objective of this study was to perform the phytochemical screening, to determine total phenolic, flavonoid and saponin content of root extract of *A. officinalis* using various extractant.

MATERIALS AND METHODS

Plant Material:
The roots of the *A. officinalis* were procured from the Institute of Pharmacy, Bundelkhand University, Jhansi (Uttar Pradesh). The roots were washed thoroughly with 30%(v/v) hydrogen peroxide and shade dried for six days, powdered and stored in air-tight container till use.

Extraction of Plant Material:
Root extracts using methanol, ethanol, water, petroleum ether and chloroform as extractant, were prepared using sequential soxhlet extraction method. Root pieces were powdered in a grinder and extracted with different solvents. *A. officinalis* root powder (50 g) was packed in a thimble, subjected to soxhlet apparatus, and extracted with solvents (250 ml)(Singh & Sawhney, 1988) i.e., methanol, ethanol, water, petroleum ether and chloroform. Refluxing was carried out for 24 h for each solvent. Distilling off the solvents in a round bottom flask gave crude extracts. The extracts were stored at 4 ˚C for further use. The dried extracts were weighed to determine the percentage of yield of the soluble constituents using the formula;

$$Percentage\ Yield = \frac{Weight\ of\ dry\ extract}{Weight\ \ of\ dry\ root\ \ taken}\ 100$$

Analysis of Asparagus Root Extracts:
The samples were analysed for their phytochemical screening, total phenolic content (TPC), total saponin content (TSC) and total flavonoid content (TFC) using solutions prepared fresh on the day of analysis, kept on ice and protected from light. Each analysis was performed three times to ensure accuracy.

Phytochemical Screening:
Methanol, ethanol, water, petroleum ether, and chloroform root extracts of *A. officinalis* were used for phytochemical screening. The presence of alkaloid, flavonoid, tannin, phenolics, saponin, triterpenoids, and glycosides was indicated by colour changes using the usual approach for identifying compounds.

Tests for Alkaloids:
1 ml of extract was taken and placed into a test tube. To this 1 ml of potassium mercuric iodide solution (Mayer's reagent) was added and shaken. Emergence of whitish or cream precipitate implies the presence of alkaloids (Ansari, 2006).

Test for Flavonoids:
5 ml of dilute ammonia solution was added to the extracts, followed by the addition of concentrated H_2SO_4. Yellow colouration indicated the presence of flavonoids (Czeczot et al., 1990)

Tests for Tannins and Phenolics:
About 0.7 g of the extract was dissolved in 2 ml of methanol and distilled water, further three drops of 0.1% ferric chloride solution was added. The formation of blue-black or brownish green precipitates in the solution was observed, which indicated the presence of tannins and phenols (Mukherjee, 2002).

Test for Saponins:
For the detection of saponins, 1 ml of extract was diluted with 20 ml of distilled water and then agitated for 15 minutes in a graduated cylinder. The presence of saponins is indicated by the formation of an emulsion (Ansari, 2006).

Tests for Glycosides:
About 0.7 g of each extract was dissolved in 2 ml of different solvents and then it was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlaid with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the deoxy sugar characteristic of glycosides (Ebana et al., 1991)

Test for Tri-terpenoids:
In order to test for triterpenoids, 2-3 granules of tin metal were dissolved in 2 ml of thionyl chloride solution. Then, 1 ml of the extract was added to the test tube resulting in pink colouration which represents that triterpenoids are present (Durai Prabakaran, 2015; Rao & Anisha, 2018)

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Total Phenolic Content:
Total phenolic content was determined according to Folin-Ciocalteu method (Lachman et al., 2000). In 100 µl of each concentration of the standard solution, 100 µl of Folin-Ciocalteu reagent and 2 ml of 2% sodium carbonate (Na_2CO_3) were mixed. The resulting mixture was allowed to stand at room temperature for 30 min and the absorbance was measured at 760 nm using a spectrophotometer against a blank prepared similarly but containing distilled water instead of standard solution of gallic acid. A standard curve was obtained by plotting the absorbance against amount of gallic acid. A standard curve was obtained by plotting the absorbance at 760 nm against various concentrations of gallic acid (Fan et al., 2015)

Total Flavonoid Content:
Estimation of total flavonoids in extracts of *A. officinalis* roots was done by the method prescribed by (Eom et al., 2007). To 0.5 ml of extracts, 0.1 ml of 10% aluminium chloride and 0.1 ml of potassium acetate (1M) were added in a test tube. In this mixture, 4.3 ml of 80% methanol was added to make the volume 5ml. The absorbance was measured against a blank containing the respective solvent without extracts at 415 nm using a spectrophotometer. The number of total flavonoids present in the extracts was calculated from the standard curve of quercetin and results were expressed as milligrams of quercetin equivalent per gram (Sun et al., 2007).

Total Saponin Content:
Total saponin content of the extracts was determined based on the colorimetric method of vanillin–glacial acetic acid reagent (Fan et al., 2015). The extract solution was evaporated with nitrogen gas followed by the addition of 10 µl of 5% vanillin–glacial acetic acid. The mixture was left undisturbed for 5 min at room temperature, then 40 µl perchloric acid was added followed by incubation for 20 min at 70 °C. The plate was cooled and 200 µl perchloric acid was added. Ethanol was used as the corresponding blanks. The samples were shaken gently for 5 min, and the absorbance was measured at 435 nm. A standard curve (0-500 µg/ml) was constructed using saponin as the reference standard.

Statistical Analysis
All the experiments were carried out in triplicate. Experimental results are expressed as mean ± standard deviation (SD) of three parallel measurements.

RESULTS

Phytochemical Screening
The different extracts of *A. officinalis* were subjected to qualitative phytochemical screening for the detection of phytoconstituents like alkaloid, flavonoid, tannin, phenolics, saponin, triterpenoids and glycosides. The results for different extracts are summarised in the Table. 1.

Table. 1: Phytochemical screening of root extract of *A. officinalis* prepared in different solvents.

Phytochemicals	Methanol	Ethanol	Water	Petroleum ether	Chloroform
Alkaloids	+	+	+	-	-
Flavonoids	+	+	+	+	-
Tannins	+	+	+	-	-
Phenolics	+	+	+	-	-
Saponins	+	+	+	+	-
Triterpenoids	+	+	-	-	-
Glycosides	+	+	+	-	-

Total Phenolic, Flavonoid and Saponin Content
The total phenolic, flavonoid and saponin content in different extracts of *A. officinalis* is summarised in the Table. 2.

Table. 2: TPC, TSC and TFC of root extract of *A. officinalis* prepared using different solvents.

Solvents	TPC (mg GAE/g)	TFC (mg QE/g)	TSC (mg TSC/g)
Methanol	52.2 ± 1.91	16.4 ± 1.11	9.2 ± 0.25
Ethanol	69.2 ± 1.30	17.9 ± 0.35	10.3 ± 0.5
Water	50.8 ± 1.26	15.5 ± 0.85	4.1± 0.25
Petroleum ether	20.9 ± 1.17	12.3 ± 0.32	4.0 ± 0.25
Chloroform	24.8 ± 0.75	8.7 ± 0.47	2.8 ± 0.10

Comparison of The Total Phenol, Flavonoid and Saponins Content in Root Extract of *A. officinalis* Using Various Extractants
The yield of various extraction methods depends on the kind of solvent, the polarity of the solvent, the chemical composition, the physical features of the sample, and the extraction technique. Under similar extraction conditions (temperature, time, and techniques), the extraction solvent has the greatest impact on the extracted

compounds' composition and quantity. Total phenol, flavonoid, and saponin content in different organic solvents of *A. officinalis* root extract is shown in Figures 1, 2, and 3, respectively.

DISCUSSION

Preliminary phytochemical analysis of root extracts of *A. officinalis* indicated the presence of alkaloids, flavonoids, glycosides, tannins, phenols, saponins and triterpenoids in different solvents. These studies demonstrated that the plant has a variety of chemical components which may be accountable for the numerous pharmacological effects. Alkaloids are crucial in medicine and make up most valuable medications (Edeoga & Eriata, 2001). Various kinds of chemicals have several effects which may be useful or harmful as phenolic compounds are thought to be potentially harmful to the growth and development of pathogens, whereas tannins are relatively powerful bioactive chemicals employed in therapeutics (Khan et al., 2011; Singh & Sawhney, 1988). Glycosides participate in the biosynthesis and remodulation of glycans, mobilization of energy and defense whereas saponins and flavonoids help in regulating plant development, pigmentation and play role in defense and signalling between plants and microorganisms. All these chemical components contribute to the disease resistance of plants.

Polar solvents are widely used in the extraction of TPC from roots of *A. officinalis* because of their phenolic chemical characteristics and polarities. The findings of this investigation indicate that ethanol is a suitable solvent for TPC extraction. Flavonoids act as one of the most diverse and widespread groups of natural compounds and are probably the most important natural phenolics. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties. Such properties are especially distinct for flavonols (Quettier-Deleu et al., 2000). From our study, we can say that both ethanol and methanol are suitable solvents for the extraction of flavonoids. (Fan et al., 2015) found that when *A. officinalis* is extracted with ethanol, it resulted in TFC higher than methanol. There was a significant difference in TSC between several polar solvents, which might be due to the different solubility of saponins in the solvent, but no significant difference in TSC between ethanol and methanol was found. Among five extraction solvents, the lowest efficiency in TSC was observed in $CHCl_3$ extraction. There have been very few studies conducted on TSC in *A. officinalis* root. Our study suggests that ethanol can be chosen in the extraction of TSC, TPC and TFC.

CONCLUSION

In the present investigation, it was discovered that *A. officinalis* root extracts contain several phytochemicals of great importance. The ethanol extract had the greatest number of phytochemicals and the highest concentrations of phenolic, flavonoid and saponin among all the extracts examined. The following is the overall ranking of the secondary metabolites including phenol, saponin and flavonoids in *A. officinalis roots*: Extract of ethanol > methanol > water > chloroform > petroleum ether. Further research comparing the fresh and dried roots of *A. officinalis* will expand its applications and pharmacological investigations, which will play a crucial part in the commercialization of this highly valued medicinal plant due to its efficacious results and therapeutic activities.

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