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Phytochemical Screening of Aerial Parts of *Artemisia parviflora* Roxb.: A medicinal plant

Jitin Ahuja*, J. Suresh, A. Deep, Madhuri, Pratyusha, Ravi

Department of Pharmacognosy, JSS College of Pharmacy, JSS University, Mysore, Karnataka, India

ABSTRACT

The aim of present study was to find out phytochemicals present in various extract of aerial parts of *A. parviflora* and to determine the total phenolic and flavonoid content in ethanolic extract of aerial parts of *A. parviflora*. Total phenol and flavonoid content was determined by folin-ciocalteu assay and aluminum chloride colorimetric assay respectively. Ethanolic extract showed the presence of alkaloids, sterols/ triterpenoids, flavonoids, tannins and coumarins. The phenolic and flavonoid content of ethanolic extract using gallic acid ($Y = 0.01143X - 0.02093$ and $r^2 = 0.9994$) and rutin ($Y = 0.0471X - 0.0056$ and $r^2 = 0.9984$) as standards was found out to be 1.09 ± 0.007 mgGAE/g and 1.163 ± 0.0208 mgRE/g respectively. The study showed significant amount of gallic acid and rutin equivalents were present in extract which may be responsible for valuable pharmacological property of extract. As phenolics and flavonoids are responsible for antioxidant activity of plant, present data implies that *A. parviflora* is a perfect candidate for in-vitro antioxidant activity and isolation of phytochemicals.

Keywords: Phytochemical screening, *Artemisia parviflora* Roxb., Secondary metabolites, Total phenolic content, Total flavonoid content.

INTRODUCTION

Since ancient times, natural products obtained from plant sources remains as a major source of preventive and curative items. This result in the large number of population is still dependent on the medicinal plants for their preventive and curative properties. According to World Health Organization, traditional medicines, including herbal medicine, have been, and continue to be, used in every country around the world in some capacity. In much of the developing world, 70-95% of the population relies on these

traditional medicines for primary care [1]. This may be due to better cultural acceptability, better compatibility with human body and lesser side effects.

India is regarded as a gold mine of well documented traditional medicinal plants. But, unlike China, India has not been able to capitalize its herbal wealth by promoting its use to developed countries despite of renewed interest in herbal medicine as this work requires well documented traditional usage, single plant medicine, free from pesticide residue, heavy metals etc, standardization based in chemical and activity profile, safety and stability. One such plant we are going to discuss here is *Artemisia parviflora* which is claimed to possess number of therapeutic uses in ancient tribal medicine and Indian folklore system of medicine. *Artemisia parviflora* (Asteraceae) is an important medicinal plant found in Western Ghats, northern Himalayas, Coimbatore hills, Nilgiris and hills of Travancore above 3000 feet. *Artemisia parviflora* is an important medicinal plant belonging to family Asteraceae, commonly used for skin diseases, cuts and wounds [2]. It is also considered for the treatment of high blood pressure, diabetes, and anthelmintic [3]. These activities are because of complex mix of phytochemicals present in *A. parviflora*. It also possesses anti-viral properties [4, 5]. The plant is getting renewed interest from natural product scientists all over the world because of phytochemicals present in it. Literature suggests the herb is of high therapeutic and economic value but no scientific work is available that discusses the phytochemicals present in plant *A. parviflora* and the species remains uninvestigated [6]. Hence, the objective of present paper was to investigate the phytoconstituents present in aerial parts of *A. parviflora* and to determine total phenol and flavonoids content in ethanolic extract of aerial parts of *A. parviflora*.

Collection and identification of plant material

The aerial parts of *Artemisia parviflora* (Fig. 1) were collected from Ootacamund, the Nilgiri Hills, Tamil Nadu in the month of June. The plant species was identified by Dr. P. Jayaraman, Plant Anatomy Research center (PARC), medicinal plant research unit, Tamil Nadu, India. The voucher specimen was deposited at the department of pharmacognosy, JSS College of Pharmacy, Mysore for future references. After authentication, the plant material was dried under shade and after optimum drying, coarsely powdered and passed from sieve 40, and stored in air tight, well closed container till further use.

Method of extraction

The coarsely powdered [7] aerial parts of *A. parviflora* (AP) were extracted with different solvents like chloroform, ethyl acetate, methanol, ethanol, distilled water and petroleum ether. The herb to solvent ratio was kept 1:10 to ensure complete extraction. The plant material was extracted by cold maceration for 72 hours with various solvents with intermittent agitation. After incubation, the extracts were filtered through Whatman filter paper and the extracts were collected and stored at 4°C in refrigerator till further use.

Preliminary phytochemical screening

A systematic and complete study of crude drugs includes a complete investigation of both primary and secondary metabolites derived from plant metabolism. Different qualitative test were performed for establishing profiles of various extracts for their nature of chemical composition. The extracts obtained were subjected to following chemical tests for identification of various phytoconstituents as per the methods given by Harborne [8]. There were no previously isolated compounds.

1. Test for Sterols [9]

Different extracts/ fractions were dissolved in chloroform, filtered and the filtrate was tested for sterols and triterpenes.

a. **Salkowski test:** Few drops of concentrated sulphuric acid was added to the chloroform solution, shaken and allowed to stand, appearance of red color in lower layer indicates the presence of sterols.

b. **Liebermann-Burchard test:** To the chloroform solution, few drops of acetic anhydride was added and mixed well. 1 mL of concentrated sulphuric acid was added from the sides of the test tube, appearance of reddish brown ring indicates the presence of sterols.

2. Test for Tri-terpenes [10]

a. **Salkowski test:** Few drops of concentrated sulphuric acid was added to the chloroform solution, shaken and allowed to stand, appearance of golden yellow color indicates the presence of triterpenes.

b. **Liebermann-Burchard test:** To the chloroform solution, few drops of acetic anhydride was added and mixed well. 1 mL of concentrated sulphuric acid was added from the sides of the test tube, appearance of deep red color indicates the presence of triterpenes.

3. Test for Saponins [10]

a. **Foam test:** Small amount of extract/ fraction was shaken with little quantity of water, if foam produced persists for 10 minutes; it indicates the presence of saponins.

b. **Haemolysis test:** To 2 mL of 1.8% sodium chloride solution in two test tubes, 2 mL distilled water was added to one of the test tube and to other 2 mL of 1% sample extract/ fraction was added. 5 drops of blood was added to each test tube and gently mixed the contents. Haemolysis was observed under the microscope on glass slide, indicates the presence of saponins in the extract.

4. Test for Glycosides [11]

a. **Kellar Kiliani test:** The test extract was dissolved in glacial acetic acid and after cooling, 2 drops of ferric chloride solution was added. These contents were transferred to test tube containing 2 mL of sulphuric acid. A reddish brown color ring observed at the junction of two layers.

b. **Conc. H₂SO₄ test:** 1 mL of concentrated H₂SO₄ was added to 1 mL of test solution and was allowed to stand for 2 minutes. The formation of reddish color indicates the presence of glycosides.

5. **Test for Alkaloids:** The various extract/ fractions were basified with ammonia and extracted with chloroform. The chloroform solution was acidified with dilute hydrochloric acid. The acid layer was used for testing the alkaloids.

a. **Wagner's test** (Iodine in Potassium iodide): The acid layer was treated with few drops of Wagner's reagent. Formation of reddish brown precipitate indicates the presence of alkaloids.

b. **Mayer's test** (Potassium Mercuric Iodine solution): The acid layer was treated with few drops of Mayer's reagent. Formation of creamy white precipitate indicates the presence of alkaloids.

c. **Dragendorff's reagent** (Potassium Bismuth Iodide): The acid layer was treated with few drops of Dragendorff's reagent. Formation of reddish brown precipitate indicates the presence of alkaloids.

d. **Hager's test:** The acid layer was treated with few drops of Hager's reagent. Formation of yellow precipitate indicates the presence of alkaloids [11].

6. **Test for Carbohydrates:** Small amount of extracts/ fractions were dissolved in little quantity of distilled water and filtered separately. The filtrates were used to test presence of carbohydrates.

a. **Molisch's test:** The extract was treated with Molisch reagent and concentrated sulphuric acid was added from the sides of the test tube to form a layer. A reddish violet ring shows the presence of carbohydrates.

b. **Fehling's test:** Filtrates were hydrolyzed with dilute hydrochloric acid, neutralized with alkali and heated with equal amount of Fehling's A and B solutions. Formation of green to yellow to red precipitate indicated the presence of reducing sugars.

7. **Test for Tannins** [12]

a. **Ferric chloride test:** To extracts a few drops of 1% neutral ferric chloride solution was added, formation of blackish blue color indicates the presence of tannins.

b. **Gelatin test:** To the extracts added 1% solution of gelatin containing 10% sodium chloride. Formation of white precipitate indicates the presence of tannins.

c. **Vanillin Hydrochloric acid test:** To the solution of extracts 1-2 mL of Vanillin hydrochloride reagent was added. Pink to red color indicates presence of tannins.

d. **Lead acetate test:** To the extract, a few drops of aqueous basic lead acetate solution were added. Reddish brown bulky precipitate indicates presence of tannins.

8. **Test for Flavonoids** [13]

a. **Shinoda test:** To the alcoholic solution of extract a few fragments of magnesium ribbon and concentrated hydrochloric acid was added. Appearance of red to pink color after few minutes indicates the presence of Flavonoids.

b. **Ferric chloride test:** Few drops of neutral ferric chloride solution were added to little quantity of alcoholic extract. Formation of blackish green color indicates the presence of phenolic nucleus.

c. **Lead acetate test:** To the extract, a few drops of aqueous basic lead acetate solution were added. Formation of yellow precipitate indicates presence of flavonoids.

d. **Zinc-hydrochloric acid reduction test:** The alcoholic solution was treated with a pinch of zinc dust and few drops of concentrated hydrochloric acid. Formation of magenta color after few minutes indicates the presence of flavonoids.

e. **NaOH test:** To alcoholic solution added few drops of sodium hydroxide solution. Intense yellow color which disappeared after adding dilute HCl indicates the presence of flavonoids.

9. Test for Lactones

a. **Legal test:** The extract was dissolved in pyridine and a mixture of sodium nitroprusside and sodium hydroxide was added. Deep red color indicates the presence of lactones.

b. **Baljet test:** To the extract, sodium picrate solution was added. Formation of yellow color indicates the presence of lactones.

10. Test for Amino acid/ Protein

a. **Ninhydrin test:** Heated the 3 mL of extract and 3 drops of ninhydrin solution in boiling water bath for 10 minutes. Appearance of purple color shows the presence of amino acids.

b. **Biuret test:** To 3 mL of extract added 4% NaOH and few drops of 1% cooper sulphate solution. Formation of violet color confirms the presence of protein.

c. **Millon's reagent test:** Mixed the extract with millon's reagent. Formation of brick red precipitate indicates the presence of protein.

11. **Test for Coumarins:** 1g of powdered drug kept with water in a test tube, covered with paper soaked in NaOH is diluted and boiled. Yellow fluorescence indicates the presence of coumarins after examination under ultra-violet lamp [14].

12. Test for Lignin [9]

a. **Labat test:** To extract added gallic acid, it develops olive green color indicating the positive reaction for lignins.

b. **Lignin test:** Formation of red color, when treated with 2% furfuraldehyde solution indicates the presence of lignin.

Estimation of total phenol content (Folin-Ciocalteu assay)

Total soluble phenolics in the ethanolic extract of aerial parts of *A. parviflora* were determined by Folin-Ciocalteu reagent, according to the method of Ozsoy et al., 2008 with some minor modifications. Aliquots (0.1mL) of the extract were transferred into the test tubes and their volume was made 4.6 mL with distilled water. After addition of 0.1 mL, 2N Folin-Ciocalteu reagent and 0.3 mL, 2% Na₂CO₃ solution tubes were vortexed and the absorbance of the mixture was recorded after 2 hr at 750 nm, using a Shimadzu 1800 UV-Vis spectrophotometer (Shimadzu Corporation, Japan) against the blank containing 0.1 mL of extraction solvent [15]. The amount of total phenolic compounds was calculated as mg of gallic acid equivalents (GAE) from the calibration curve of gallic acid standard solution (concentration range 10-80 µg/mL) and expressed as mg gallic acid/g dry weight (DW) of the plant material. Total phenolic content in the plant extract was calculated using formula: Total phenolic content = GAE × V/m, where GAE is the gallic acid equivalence (mg/ mL) or concentration of gallic acid established from the calibration curve ($Y = 0.01143X - 0.02093$; $r^2 = 0.9994$); V is the volume of

extract in mL and m is the weight (g) of the pure plant extract. The data were presented as the average of triplicate analyses [16].

Estimation of total flavonoids content

Total flavonoids content of ethanolic extract of *A. parviflora* was determined by using aluminum chloride colorimetric assay. Briefly, extract (0.5 mL each) was mixed with 1.5 mL methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 510 nm with a Shimadzu 1800 UV - Vis spectrophotometer (Shimadzu Corporation, Japan). Using rutin, a standard curve was prepared and linearity was obtained in the concentration range of 4-12 µg/mL. Using the standard curve the total flavonol content of extract was obtained. Results were expressed as rutin equivalent per gram of dry weight mg RE [17, 18]. Total flavonoid content in the plant extract was calculated using formula: Total flavonoids content = $RE \times V/m$, where RE is the rutin equivalence (mg/ mL) or concentration of rutin established from the calibration curve; V is the volume of extract in mL and m is the weight (g) of the pure plant extract. The data were presented as the average of triplicate analyses [16].

RESULTS AND DISCUSSION

Preliminary phytochemical screening

As reported in table 1, phytochemical studies reports the presence of phytoconstituents like sterols/ triterpenoids, flavonoids, phenols, saponin, alkaloids, tannins, carbohydrates, coumarins and lignin. Ethanolic and methanolic extract showed rich in sterols/ triterpenoids, tannins, flavonoids, alkaloids, coumarins and carbohydrates. The same results were obtained for *A. sieversiana* [19]. Ethyl acetate extract and chloroform extract showed the presence of sterols/ triterpenes, tannins, flavonoids and sterols/ triterpenes, alkaloids, carbohydrates, flavonoids and coumarins. When compared to the ethanolic extract of aerial parts of *A. parviflora* showed similar phytochemicals compounds like flavonoids, sterols/ triterpenoids, tannins, alkaloids, carbohydrates but ethanolic extract shows additional compounds like lignin and saponin. The result suggests that ethanol is more efficient solvent for extraction of phytoconstituents from aerial parts of *A. parviflora* as compared to other selected solvents. Petroleum ether extract showed very less extractable phytochemicals.

Total phenolic and flavonoids content

The total phenol content is reported as gallic acid equivalents by reference to standard curve ($Y = 0.01143X - 0.02093$ and $r^2 = 0.9994$). The phenolic content in ethanolic extract of aerial parts of *A. parviflora* was found out to be 1.09 ± 0.007 mgGAE/g. The total flavonoid content is reported as rutin equivalents by reference to standard curve ($Y = 0.0471X - 0.0056$ and $r^2 = 0.9984$). The flavonoids content in ethanolic extract of aerial parts of *A. parviflora* was found out to be 1.163 ± 0.0208 mgRE/g. The percentage phenolic content and flavonoids content was found out to be 0.37 and 0.394 respectively. The results suggest high phenolic and flavonoids content in ethanolic extract. Flavonoids and phenols are considered as anti- capillary fragility and anticancer compound

[20, 21]. Polyphenols have inhibitory effect on mutagenesis and carcinogenesis in humans when ingested in daily diet [22, 23]. As these compounds are responsible for the antioxidant activity of plant material further it is required to perform *in-vitro* antioxidant activity using various models that can establish a good correlation between the data obtained in present experiment. Future prospects include isolation of flavonoids and phenols responsible for the activity and determining structure.

Table 1: Preliminary phytochemical screening of aerial parts of *Artemisia parviflora*

Preliminary phytochemical screening		Aerial part of <i>Artemisia parviflora</i> Roxb.					
		Pet. ether	CHCl ₃	Et.Ac.	MeOH	EtOH	Dis. Water
Sterols/ Triterpenes	Salkowski test	-	+	+	+++	+++	++
	Liebermann-Burchard test	-	++	+	++	+++	+
Saponins	Foam test	+	-	-	+	+	+
	Haemolysis test	-	-	-	-	-	-
Glycosides	Kellar Kiliani test	-	-	-	-	-	-
	Conc. H ₂ SO ₄ test	-	-	-	-	-	-
Alkaloids	Wagner's test	-	++	-	++	++	++
	Mayer's test	-	++	-	+++	++	+
	Dragendorff's reagent	-	+++	-	+++	+++	+++
	Hager's test	-	+	-	++	++	+
Tannins	FeCl ₃ test	-	-	+	++	+++	+++
	Gelatin test	-	-	+	+	++	+
	Vanillin HCl test	-	-	+	++	++	++
	Lead acetate test	-	-	+	+	++	+
Carbohydrates	Molisch's test	-	++	-	+	+++	++
	Fehling's test	-	++	-	-	-	-
Flavonoids	Shinoda test	-	++	+	++	+++	+++
	Ferric chloride test	-	++	+	+++	+++	+++
	Lead acetate test	-	+	+	+	++	+
	Zinc-HCl reduction test	-	++	+	+	++	+
	NaOH test	-	++	+	++	+++	++
Lactones	Legal test	-	-	-	-	-	-
	Baljet test	-	-	-	-	-	-
Amino acid/ Protein	Ninhydrin test	-	-	-	-	-	-
	Biuret test	-	-	-	-	-	-
	Millon's reagent test	-	-	-	-	-	-
Coumarins		-	++	-	++	++	+
Lignin	Labat test	-	-	-	+	++	-
	Lignin test	-	-	-	+	+	-

Note: '+' low; '++' average; '+++' high; '-' absence.

Table 2. Total extractable compounds (EC), total phenolic compounds (PC) (as gallic acid equivalents), and total flavonoids content (FC) (as rutin equivalents) of ethanolic extract of aerial parts of *A. parviflora*.

Extract	EC (mg/g DW)	PC (mg/g DW)	PC/EC (%)	Flavonoids (mg/g DW)	FC/EC (%)
Ethanolic	295	1.09 ± 0.007	0.37	1.163 ± 0.0208	0.394

Values are mean of three replicates ± standard deviation (SD).



Fig. 1 Two shoots of *Artemisia parviflora* showing leaf morphology

CONCLUSION

Artemisia parviflora contains a rich amount of phenolic and flavonoids compound which may be responsible for valuable pharmacological activities. Phenolics identified by Folin-Ciocalteu assay and during preliminary phytochemical screening indicates that this species may have an lower IC_{50} value than the other species of *Artemisia* genus. Flavonoids are responsible for anticancer activity and considered as anti-capillary fragility; the presence of flavonoids in significant amount in *A. parviflora* shows medicinal the importance of the plant. Further the plant could be considered for antioxidant, anticancer, immunomodulatory activity.

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