



QUALITATIVE AND QUANTITATIVE ANALYSIS OF PHYTOCHEMICALS IN AERVA JAVANICA (BURM. F.) SHULT

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ABSTRACT

The aim of the present study was to find out the presence of phytochemicals in the aqueous and alcoholic extracts of *Aerva javanica* by both qualitative and quantitative screening methods. In qualitative analysis, the phytochemical compounds such as steroids, reducing sugar, triterpenoids, alkaloids, phenolic compounds, flavonoids, saponins, tannins, cardiac glycosides and anthroquinones were screened in crude extracts by using standard methods. The methanol extract showed positive results for maximum number of compounds. In quantitative analysis the important secondary metabolites such as phenolic compounds, flavonoids and tannins were tested in all the three extracts. The methanol extract of showed highest amount of phytochemicals when compared with other solvent extracts.

INTRODUCTION

Plant based drugs have been used world-wide in traditional medicines for treatment of various diseases. The world is now looking towards India for new drugs to manage various challenging diseases because of its rich biodiversity of medicinal plants and abundance of traditional know how such as Siddha, Ayurveda etc., to cure different diseases. Phytoconstituents are the natural bioactive compounds found in plants. These phytoconstituents work with nutrients and fibers to form an integrated part of defense system against various diseases and stress conditions. The plant *A. javanica* (vernacular names : Telugu-TellaBuraga, English-Kopak Bush, Hindi-Safedshamli, Gujarati-SafedShimlo, Marathi-Shamil, Bengali-Swetshimul, Kannada-Dudi, Tamil-Panchu) belonging to Amaranthaceae family is an erect perennial herb and widely distributed in the various parts of the world. It is native to Africa and also found in some Asian countries. In traditional medicine, the herb is used as diuretic, diabetic demulcent and decoction of plant is used

to ameliorate swellings and urinary disorders. Powders of the plant are applied externally to treat ulcers in domestic animals. The seeds are used to relieve head ache and also rheumatism. In Ayurveda the leaves, seeds and roots are used for treatment of kidney stones, and as astringent [1]. In view of this *A.javanica* plant was studied exhaustively for its potential phytochemical constituents to prove its medicinal values.

MATERIALS AND METHODS

Collection of the plant samples

The plant materials were collected from in and around Tiruchirappalli District, Tamil Nadu dust free samples. Soon after collection, the leaves were dried in shade for 20 days and then powdered to get a coarse powder. This powder was stored in air tight container and used for further successive extraction. The collected plant was authenticated by Dr. S. Soosai Raj, Assistant Professor, PG & Research Department of Botany, St. Joseph's College (Autonomous), Tiruchirappalli-2, Tamil Nadu and a voucher specimen was deposited at Department herbarium.

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Preparation of Extracts

Aqueous extraction

25 g of air-dried powder was taken in 100 ml of water in a conical flask, plugged with cotton wool and they were shaken at room temperature for 2 days. After 2 days hours the supernatant was collected and the solvent was evaporated to make the final volume one fourth of the original volume (12) and stored at 4⁰ C in airtight bottles.

Solvent extraction

25 g of air-dried powder was taken in 100 ml of methanol in a conical flask, plugged with cotton wool and they were shaken at room temperature for 2 days. After 2 days the supernatant was collected and the solvent was evaporated to make the final volume one fourth of the original volume and stored at 4⁰ C in airtight bottles. In the same conditions was obtained Ethanol extract. The percentage yield of the extracts from each solvent extraction was calculated. The crude extract was crushed into powder and then kept in desiccators.

Phytochemical screening

Test for Alkaloids

Hager's Test: About 1 ml of leaf extract and 1ml of Hager's reagent (Saturated solution of picric acid) are added and mixed. A crystalline yellow precipitates indicate the presence of alkaloids

Wagner's test: About 1 ml of leaf extract and 1ml of wagner's reagent (dilute iodine solution) are added and mixed. Formations of reddish brown precipitates indicate the presence of alkaloids.

Test of Flavonoids

Shinoda test: To 1ml of the extract add 8-10 drops of conc. HCl and a pinch of magnesium powder or filings. Boil for 10-15 min and cool. A red for coloration indicate the presence of flavonoids.

Ammonia test: A small piece of filter paper is dipped to about 1 ml of the extract and is exposed to ammonia vapour. Formation of yellow spot on filter paper indicates the presence of flavonoids.

Test for steroids

Liebermann Burchard test: To 0.5 ml of the extract add 2 ml of acetic anhydride and 2 ml of concentrate H₂SO₄ along the sides of the tube. The formation of green colour indicates the presence of steroids.

Test for Cardiac glycosides

Keller-Killani test: 5 ml of the extract is treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution and one ml of concentrated sulfuric acid. A brown ring at the interface indicates the presence of deoxysugar, a characteristic feature of cardenolides. A violet ring may

appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Baljet test: To 1ml of the extract, add 2 ml of sodium picrate solution formation of yellow to orange color shows the presence of aglycones or glycosides.

3, 5-Dinitro benzoic acid test: To 1 ml of the extract, add few drops of NaOH followed by 2% solution of 3, 5, dinitro benzoic acid. Formation of pink color shows the presence of cardiac glycosides.

Test for terpenoids

Salkowski test: To 5 ml of the extract, add 2ml of chloroform and 3 ml of concentrated H₂SO₄. Formation of yellow color ring at the two liquids that turns reddish brown color after two min, showed presence of terpenoids.

Test for Triterpenoids and Steroids

Antimony trichloride test: To 1 ml of the extract, add saturated solution of SbCl₃ in chloroform containing 20% acetic anhydride. Formation of pink color on heating indicates the presence of steroid and triterpenoids.

Test for Tannins

Modified pressian blue test: To 1ml of the extract, add 1ml of 0.008 M potassium ferricyanide and 1ml of 0.02M ferric chloride in 0.1 M HCl. Appearance of blue color indicates the presence of tannins.

Test for Saponins

Froth test: About 2g of the powder sample is boiled with 20 ml of distilled water in a water bath and filter. 10 ml of the filtrate is mixed with 5 ml of distilled water and shake vigorously for a stable persistent forth the frothing is mixed with 3 drops of olive oil and shake vigorously. The formation of emulsion for the positive result can be observed.

Test for Phlobatannins: 1 ml of the extract was boiled with 1% HCl. The formation of red precipitate indicates the presence of phlobatannins.

Test for reducing sugars

Fehling's test: To 1ml of the extract, 8 drops offehling's (A) and 5 drops of fehling's (B) solution are added. The tubes are heated in a boiling water bath for few minutes. Observe red precipitate for the positive result.

Borutrager's test for anthroquinones : To 1 ml of the extract, add 1 ml of 10% ferric chloride adds 0.5 ml concentrated HCl. Boil in a water bath for few min. Filter it and the filtrate with 1ml of diethyl ether and concentrated ammonia. Appearance of pink or drop red color indicates the presence of anthroquinones.



Determination of total phenolic compounds

100 mg of the extract of the sample was weighed accurately and dissolved in 100 ml of triple distilled water (TDW). 1 ml of this solution was transferred to a test tube, then 0.5 ml 2N of the Folin-Ciocalteu reagent and 1.5 ml 20% of Na₂CO₃ solution was added and ultimately the volume was made up to 8 ml with TDW followed by vigorous shaking and finally allowed to stand for 2 hours after which the absorbance was taken at 765 nm. These data were used to estimate the total phenolic content using a standard calibration curve obtained from various diluted concentrations of gallic acid [2].

Determination of total flavonoids

The method is based on the formation of the flavonoids - aluminium complex which has an absorptive maximum at 415nm. 100µl of the plant extracts in methanol (10 mg/ml) was mixed with 100 µl of 20 % aluminum trichloride in methanol and a drop of acetic acid, and then diluted with methanol to 5ml. The absorption at 415 nm was read after 40 minutes. Blank samples were prepared from 100 ml of plant extracts and a drop of acetic acid, and then diluted to 5ml with methanol. The absorption of standard rutin solution (0.5 mg/ml) in methanol was measured under the same conditions. All determinations were carried out in triplicates [3].

Determination of tannins

500 mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtered was pipetted out into a test tube and mixed with 2 ml of 0.1 M FeCl₃ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min [4].

Results and Discussion

The present study carried out on the *Aerva javanica* revealed the presence of medicinal active constituents. Preliminary phytochemical screening revealed the presence of Alkaloids, flavonoids, cardiac glycosides, Phenol, Tannins, Saponins and minute amount of terpenoids as shown in table 1. The aqueous fraction of leaves revealed the presence of alkaloids, flavonoids, glycosides, phenols, saponins, tannins, cardiac glycosides and carbohydrates but negative results were obtained for steroids, resins, gum and mucilages, anthroquinones and terpenoids. In ethanolic extract, alkaloids, flavonoids, terpenoids, saponins, tannins and cardiac glycosides were present in moderate concentrations. However, it was negative for, steroids, phlobatannins, anthroquinones and gum and mucilages. The methanolic extract showed the presence of flavonoids, alkaloids, cardiac glycosides, trace amounts of steroids, phenols, saponins, resins and whereas was negative for phlobatannins, anthroquinones and gum

and mucilages. Flavonoids are naturally occurring phenols which possess numerous biological activities including anti-inflammatory, antiallegic, antithrombotic and vasoprotective effects [5].

Total Flavonoid Content

The results of total flavonoid are shown in Table 2. A total flavonoid content of the extract were expressed in quercetin equivalents and was found to be 135 mg/g in alcoholic extract, 75 mg/g in methanolic extract and 49mg/g in aqueous extract. Flavonoids are naturally occurring phenols which possess numerous biological activities including anti-inflammatory, antiallegic, antithrombotic and vasoprotective effects. Flavonoid is one of the main groups of Phenolic compounds and widely distributed Flavonoid, Flavones and Flavonols. Many flavonoids and related compounds are reported to possess strong antioxidative characteristics [6]. In this results revealed the same that of Antioxidant Capacity of Macaronesian Traditional Medicinal Plants [7].

Total Phenolic Content

The results of total phenolic content are shown in Table 3. Total phenol contents of the extract were expressed in gallic acid equivalents and were found to be 56 mg/g in alcoholic extract, 62.4 mg/g in methanolic extract and 32mg/g in aqueous extract. Phenolic compounds are known to be a powerful chain breaking antioxidants; they possess scavenging ability due to their hydroxyl groups [8]. Studies have shown that the polyphenols found in dietary and medicinal plants could inhibit oxidative stress by antioxidant mechanism [9]. So also, in the present study, the pronounced antioxidant activity of the extract of *Aerva javanica* as reducing ferrous ions was possibly due to its high Phenolic content.

Total Tannin Content

The results of total tannin content are shown in Table 4. The total tannin content of the extract were expressed in Tannic acid equivalents and was found to be 36mg/g in alcoholic extract, 38mg/g in methanolic extract and 31 mg/g in aqueous extract. Tannins are astringer, bitter plant polyphenols that either bind and precipitate or shrink proteins and various other organic compounds including amino acids and alkaloids. Tannins have shown potential antiviral, antibacterial and antiparasitic effects (Kolodziej et al., 2005). In the past few years tannins have also been studied for their potential effects against cancer through different mechanisms [10].

For the pharmacological study of novel drugs, the essential information's regarding the chemical constituents are generally provided by the qualitative phytochemical screening of plant extracts. In the present study the physicochemical parameters can be used as significant parameters of plant identification. The qualitative tests of extracts showed significant indication about the presence



of metabolites. Preliminary phytochemical investigations tests are useful to isolate the pharmacologically active principles present in the plant. The research work done in *Aerva javanica* plant were very few and hence the plant has to be explored more to reveal more pharmacological activities out of it. Isolation of the active compounds from the plant should be done further in order to discover more drugs out of it. The pharmacological industry could be benefitted by utilizing the above properties in order to produce the new drug compound.

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Table 1. Qualitative phytochemical screening of *Aerva javanica*

Phytochemicals	Aqueous	Ethanol	Methanol
Alkaloids	+	+	+
Flavanoids	+	+	+
Steroids	-	-	+
Cardiac glycosides	+	+	+
Terpenoids	-	+	-
Triterpenoids & Steroids	-	-	-
Phenols	+	+	+
Tannins	+	+	+
Sopans	+	+	+
Phlobatannins	-	-	-
Reducing sugar	-	-	-
Anthroquinones	-	-	-
Gum & Mucilages	-	-	-

Table 2. Total Flavonoid content of *Aerva javanica*

S.No.	Extracts	Total phenol content (mg/g)
1	Ethanol	49
2	Methanol	135
3	Aqueous	38

Table 3. Total Phenolic content of *Aerva javanica*

S.No.	Extracts	Total phenol content (mg/g)
1	Ethanol	32
2	Methanol	56
3	Aqueous	62.4

Table 4. Total Tannin content of *Aerva javanica*

S.No.	Extracts	Tannin content (mg/g)
1	Ethanol	31
2	Methanol	36
3	Aqueous	38

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