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RESEARCH ARTICLE

PHYTOCHEMICAL AND ANTIOXIDANT ANALYSIS IN THE AERIAL PART OF *AERVA JAVANICA* (BURM. F.) SCHULT. (AMARANTHACEAE)

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ABSTRACT

Plants are the important source for the discovery of new products of medicinal value for drug development. Secondary plant metabolites are chemical compounds produced by the plant cell, defending themselves from biotic and abiotic stress. The study aims about the qualitative and quantitative analysis of various phytochemicals present in the plant *Aerva javanica* (Burm.f.) Shult. (Amaranthaceae). The aerial part of the plant was extracted using hexane, chloroform and ethanol and screened for the presence of the secondary metabolites and *in vitro* antioxidant properties by standard procedures. The present study indicates the presence of alkaloids, flavonoids, phenols, coumarins, glycosides, carbohydrates, proteins and amino acids. Further findings revealed that the ethanolic extract found to have more constituents in the terms of quantitative analysis and antioxidant activity showed highest radical scavenging activity when compared with other solvent extracts.

Keywords: *Aerva javanica*, Phytochemical, Phenolic content, Antioxidants

1. INTRODUCTION

India is endowed with a rich wealth of medicinal plants. India recognizes more than 2500 plant species which have medicinal values (1). Plants are the source of many bioactive compounds. India is the largest producer of medicinal herbs and is rightly called the botanical garden of the world. There are very few medicinal herbs of commercial importance, which are not found in this country. India officially recognizes over 3000 plants for their medicinal value. It is generally estimated that over 6000 plants in India are in use in traditional, folk, and herbal medicine, representing about 75% of the medicinal needs of the third world countries (2). Secondary metabolites are not involved directly and of plants and are potential sources of drugs. The most important secondary metabolites they have been worked as biocatalysts which are synthesized during secondary metabolism are saponins, alkaloids, tannins, flavonoids, and cardiac glycosides (3). Free radicals are produced by our body and to stabilize the body's natural function, but the excess amount could cause the cell and tissue damage (4). *Aerva javanica* is an erect, much-branched perennial herb that is a native of Africa and also distributed in various parts of the world. In traditional medicine it has been used against skin infections, inflammation, abdominal worms, fever, intestinal gases and rheumatism (5,6). In the present study the aerial part of the plant *A. javanica* was studied exhaustively for its potential phytochemical constituents for its medicinal values.

2. PLANT DESCRIPTION

Fig. 1. Habit of *Aerva javanica*

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Systematic position
Class : Dicotyledons
Order : Caryophyllales
Family: Amaranthaceae
Genus : *Aerva*
Species: *A. avanica* (Burm. F.) Shult.
Vernacular name: Desert cotton

3. MATERIALS AND METHODS

3.1. Collection of the plant samples

The plant *A. javanica* was collected in Coimbatore District, Tamil Nadu. The authenticity of the plant was confirmed in the Botanical survey of India, Southern Circle, Coimbatore by referring the deposited specimen. The voucher number of the specimen is BSI/SRC/5/23/2021/Tech-746. The aerial part was shade dried and powdered coarsely. The powder was stored in air tight container and used for further successive extraction.

3.2. Solvent extraction

The powdered plant sample was extracted excessively with hexane, chloroform and ethanol using Soxhlet apparatus at 55- 85^{0}C for 8 to 10 hours in order to extract the nonpolar and polar compounds. The solvent of respective extracts were reduced at room temperature and stored under 4^{0}C for further use.

3.3. Extractive yield

The extracted plant samples of Hexane, chloroform and ethanol were concentrated to dryness under reduced pressure using rotary vacuum evaporator to remove traces of water molecules and lyophilized powder were stored at -20^{0}C for further studies.

3.4. Phytochemical screening

Secondary metabolites are substances manufactured by plants that make them competitive in their own environment. The screening of phytochemicals suggests both physiological and medicinal activities of the plant. Chemical tests were carried out by using standard procedures to identify the preliminary phytochemical screening by following the methodology of Sofowara (7), Trease and Evans (8), Savithramma *et al.* (9), Kokate (10) and Harborne (11).

3.5. Quantitative determination of secondary metabolites

3.5.1. Estimation flavonoids

Total flaonoid content was estimated by the method of Zhishen *et al*. (12). 0.5 ml of extract solution was mixed with 0.2ml of diluted water and 0.15ml of 5% sodium nitrate solution and maintained for 6 mins. 0.15 ml of 10% aluminium chloride solution was added and allowed to stand for 6min. To that 2.0 ml of 4% sodium hydroxide solution was added. Finally the volume was made upto 5.0ml with distilled water and mixed thoroughly. After 15 mins of incubation the absorbance was determined at 510nm. Appearance of pink colour shows the presence of flavonoid content. The total flavonoid content was expressed as Rutin equivalent mg RE/g extract on a dry weight basis using the standard curve.

3.5.2. Total phenol content

The total phenolic content was estimated using Folin ciocalteau reagent by Sidduraju and Becker (13). 20µg of the extract was taken and made up to 1ml using distilled water. To that 500µl of Folin-Phenol reagent (1:1) was added and 2.5ml of sodium carbonate was added. The mixture was shaken well and incubated in dark for 40 mins to develop colour. The absorbance was measured at 725 nm. A calibration curve of Gallic acid was constructed and linearity was obtained in the range of 10-50 µg/ml. The total phenol content in the plant extracts were expressed as mg of Gallic acid equivalent (mg GAE/g extract) by using the standard curve.

3.5.3. Total tannin content

Tannin content of *A. javanica* was estimated by the method of Siddhuraju and Manian (14). 500 µL of the extract were taken in the test tube separately and treated with100mg of polyvinyl polypyrrolidone and 500 µL of distilled water. This solution was incubated at 4^{0}C for 4 h. Then the sample was centrifuged at 5000rpm for 5 mins and 20 µL of the supernatant was taken. The phenolic content of the supernatant was measured at 725nm and expressed as the content of the free phenolics on a dry matter basis. From the above results, the tannins content of the extract were calculated as follows:

Tannins (mg GAE/g extract) = Total phenolics (mg GAE/g) – Free phenolics (mg GAE/g)

3.6. Determination of invitro antioxidant activity

3.6.1. DPPH radical scavenging activity

The hydrogen donating capacity was assessed by using stable DPPH method (15). Briefly, a solution of 0.1Mm DPPH was prepared using methanol. The sample (50-250μg/mL) was mixed with 5.0 mL of DPPH solution. Reaction mixture was shaken incubated at 30^0 c for 20 minutes and the absorbance was measured at 517nm. Results were compared with the activity of rutin. Antioxidant activity of the extract were expressed as IC_{50}, the values were calculated from the linear regression of the percentage antioxidant activity versus concentration of the extracts. A lower IC_{50}, values indicate greater antioxidant activity. DPPH dis-colouration of the sample was calculated using the formula.

DPPH radical scavenging activity (%) = [(control OD- sample OD)/control OD] x100.

3.6.2. $ABTS^+$ free radical scavenging assay

Antioxidant activity was performed using an improved $ABTS^+$ method proposed by Siddhuraju and Manian (14). The ABTS radical cation was $ABTS^+$ was generated by a reaction of 7mm $ABTS^+$ and 2.45 mm potassium persulphate and the mixture was incubated for 12 – 16 hrs at room temperature in dark. Prior to assay, the solution was diluted in ethanol (about 1:89 v/v) and equilibrate to obtain an absorbance of 0.700± 0.02 at 734 nm. 10 μL/ml of the sample was added to 1.0ml of diluted $ABTS^+$ solution. After 30 min of incubation, absorbance was read at 734nm. Trolox was used as reference material.

4. RESULTS AND DISCUSSION

4.1. Extractive yield

The extractive yield is a significant method which solubilizes the biologically active ingredients. In the current study the extractive yield percentage of *Aerva javanica* aerial part with their successive solvent system were represented in (Fig. 2) fluctuated within the ranges from 0.3 to 4.9, while the minimum percentage yield was found in hexane with the value of (0.3%). Th**is** extraction technique is also important for the standardization of herbal products as it is utilized in the removal of desirable soluble constituents, leaving out those not required with the aid of the solvent.

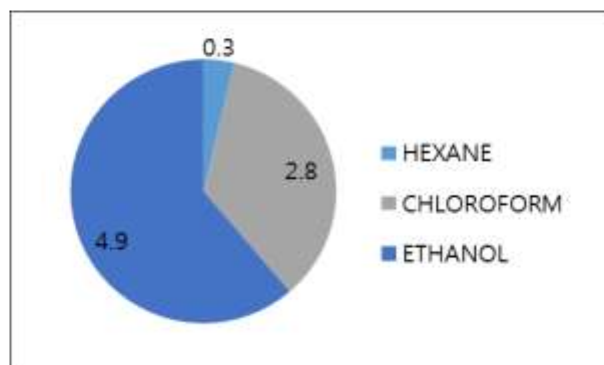

Fig. 2. Extractive yield

4.2. Qualitative estimation of secondary metabolites

The screening of phytochemicals suggests both physiological and medicinal activities of the plant. In present investigation secondary metabolites were analyzed qualitative and quantitatively. The phytochemical tests would reveal some medicinal and biological activities of the studied natural substances. The present analysis of phytochemical screening (Table 1) revealed that *A. javanica* aerial part contained alkaloids, flavonoids, terpenoids, tannins, carbohydrates, proteins and amino acids. Similarly the inflorescence of *A. javanica* revealed the presence of alkaloids, flavonoids, terpenoids, tannins, carbohydrates, proteins and amino acids (16).

Table 1. Qualitative phytochemical estimation of secondary metabolites

Tests	Hexane	Chloroform	Ethanol
Alkaloids	++	+	+++
Flavonoids	++	++	++
Steroids	++	–	–
Terpenoids	–	+	+
Tannins	–	+	+
Phenols	+	+	+
Courmarins	+	+	++
Glycosides	+++	–	+++
Saponin	+	+	+
Gums & Mucilage	+	+	++
Carbohydrates	++	+	++
Amino acid	+	+	+++
Protein	+	+	+++
Volatile oil	–	–	–
Resin	–	++	–

4.3. Quantitative estimation of secondary metabolite

Plant produce a vast and diverse assortment of organic compounds the great majority of which do not appear to participate directly in growth and development. Plant secondary metabolites are unique sources for pharmaceuticals, food additives, flavors, and other industrial materials and the use of plant cell cultures has overcome several inconveniences for the production of these secondary metabolites (17). Secondary metabolites are the useful natural products that are synthesized through secondary metabolism in the plants (18).

4.3.1. Estimation of flavonoids

Flavonoids are an important class of natural products; particularly, they belong to a class of plant secondary metabolites having a polyphenolic structure (19). The biological functions of flavonoids are linked to their potential cytotoxicity and their capacity to interact with enzymes through protein complexation. Some flavonoids provide stress protection, for example, acting as scavengers of free radicals such as reactive oxygen species (ROS), as well as chelating metals that generate ROS via the Fenton reaction (20). The total flavonoid content of the extract was found to be 1.24±0.6mg RE /g in hexane, 1.05±0.2mg RE /g in chloroform and 1.89±0.3mg RE /g in ethanolic extract (Table.2) and was expressed in Rutin equivalent. Flavonoids have antioxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties (21).

4.3.2. Total phenolic content

Phenols are the largest group of secondary metabolites. Phenolic acids, known for diverse biological applications, are the main polyphenols produced by plants and work as ancestor for bioactive molecules regularly used in therapeutics (22). The phenolic content in the aerial part extract of *A. javanica* were expressed in gallic acid equivalence and were found to be 1.45±0.3mg GAE /g in hexane, 1.32±0.4mg GAE /g in chloroform and 1.66±0.1mg GAE /g in ethanol (Table.2). They carry strong natural antioxidants having key role in wide range of biological and pharmacological properties such as anti-inflammatory, anticancer, antimicrobial, antiallergic, antiviral, antithrombotic, hepatoprotective (23).

4.3.3. Total tannin content

Tannins are amorphous, astringent substances occurring widely in the bark, wood, leaves, and resinous exudations of plants. The antioxidant properties of tannins are widely utilized in the food and medical fields. It has been reported that the amount of hydroxyl groups in tannins and the release of hydrogen peroxide are important indicators to evaluate the antibacterial properties, which are positively correlated with antioxidant properties (24). The natural polyphenolic compounds, tannins display antibacterial effects. The total tannin content of the extract were expressed in Tannic acid equivalents and was found to be 0.35±0.2mgGAE/g in hexane extract, 0.43±0.4mg GAE/g in chloroform extract and 0.24±0.3 mg GAE/g in ethanolic extract (Table 2). In the past few years tannins have also been studied for their potential effects against cancer through different mechanisms.

Table 2. Estimation of total flavonoids, phenols and tannins

Constituents	Flavonoid mg RE/g	Tannin mg GAE/g	Phenol mg GAE/g
Hexane	1.24±0.6	0.35±0.2	1.45±0.3
Chloroform	1.05±0.2	0.43±0.4	1.32±0.4
Ethanol	1.89±0.3	0.24±0.3	1.66±0.1

4.4. In -vitro antioxidant activity

4.4.1. DPPH reducing power assay

Free radicals are inevitably produced in biological systems and also encountered exogenously, and are known to cause various degenerative disorders, like mutagenesis, carcinogenesis, cardiovascular disturbances and ageing (25). Antioxidants are the compounds, which combat the free radicals by intervening at any one of the three major steps of the free radical mediated oxidative process, viz., initiation, propagation and termination. The present study was conducted to determine the antioxidant properties of two well- known memory enhancer medicinal plant *A. javanica* aerial plant part. The plant parts were evaluated by measuring reducing ability by DPPH model. The antioxidants compound of rutin was also evaluated of studied plant. The ethanolic leaf extract (3.97±0.3 μg/ml) exhibited significantly higher antioxidant activity than the other solvent systems. Therefore the present result

can be concluded from the study that regular use of *A. javanica* as a supplement could be more helpful to the treatment of neurological disorders caused by free radical damage.

4.4.2 ABTS+ free radical scavenging assay

This study reports the antioxidant properties of three wild medicinal plants from various solvent systems of *A. javanica* aerial part materials of this plant were investigated using ABTS assays. This was ranked by the assays as the ethanolic extract possessing the highest antioxidant with value of (3.21±0.1µmol/g) Despite the fact that these methods have different reaction mechanisms and do not necessarily measure the same activity (26) revealed this method clearly indicated that the studied plants possess variable but considerable antioxidant and antiradical activities. The studied medicinal plants possess considerable antioxidant activities and may contribute to the well-being of individuals who consume them.

Table 3. DPPH and ABTS+ radical scavenging activity of *A. javanica* aerial part

Solvent	DPPH IC_{50} mg/mL	ABTS+ Scavenging activity
Hexane	9.24±0.2	6.21±0.3
Chloroform	5.40±0.5	4.45±0.4
Ethanol	3.97±0.3	3.21±0.1

5. CONCLUSION

The medicinal plants are known to have highest therapeutic value in pharmaceutical field. Qualitative phytochemical analysis confirmed the presence of carbohydrates, alkaloids, flavonoids, etc., in all the extracts. Flavonoids, phenols and tannins were quantitatively analyzed. Antioxidant activity revealed the maximum inhibitory concentration in the ethanolic extract. Besides the above findings, it would be more appropriate to enhance further research on clinical application for improving the plant based drug industry and the development of the new drugs of herbal origin.

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