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Physicochemical, Phytochemical and Toxicological Study of Hydroalcoholic Extract of *Aerva javanica* Roots

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Authors' contributions

This work was carried out in collaboration among all authors. Author PKS supervised the study, author SY managed the literature searches, author MA wrote the first draft of the manuscript and is author for correspondence, author CK proofread the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The study is aimed at determining the characters of roots of *Aerva javanica (A.javanica)* assessing acute oral toxicity of hydroalcoholic extract of roots of *Aerva javanica*.
Place and Duration of Study: the physicochemical and phytochemical evaluation was carried out at Faculty of Pharmacy, Maulana Azad University Jodhpur, Rajasthan. Acute Oral Toxicity was studied at Bilwal Medchem and Research Laboratory, Jaipur Rajasthan. The duration of study June 2021 – July 2021
Methodology: The pharmacognostical characters were evaluated in terms of organoleptic property, physico-chemical parameters, and preliminary phytochemical investigation. The acute oral toxicity was determined using the 423, OECD guideline for testing of chemical, acute toxic class method.

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Results: The physico-chemical analysis revealed total ash; water soluble ash and, acid insoluble ash to be 6.36 ± 0.26%, 0.79 ±0.23 % and, 1.23 ± 0.34%. The water, alcohol and petroleum ether soluble extractive values were found to be 17.88± 3.54 %, 15.58 ± 1.13% and, 0.3 ± 0.13 %. The percentage yield of hydroalcoholic extract of root of *A.javanica* was found to be 24%. The phytochemical screening of hydroalcoholic extract revealed the presence of carbohydrates, flavonoids, steroids, alkaloids, tannins, proteins, and fixed oil. The acute oral toxicity of hydroalcoholic extract of root of *A.javanica* revealed that the extract was found to be safe till 2000 mg/kg BW.

Conclusion: The results of the present study will furnish data helpful in the correct identification and authentication of roots of *A.javanica.* The extractive value shed light on the most suitable solvent to be chosen to obtain extract rich in phytoconstituents. The physicochemical screening furnished data on important phytoconstituents present in the hydroalcoholic extract which could be helpful in isolation and purification of desired phytoconstituents. Acute oral toxicity study revealed that the extract is safe till 2000 mg/kg BW which could be helpful in selection of dose for future pharmacological activities.

Keywords: Aervajavanica; organoleptic parameter; physico-chemical analysis; phytochemical screening; acute oral toxicity.

1. INTRODUCTION

Aervajavanica (A. javanica) belongs to family *Amaranthaceae.* It is an erect, branched perennial herb native of Africa, [1] the south-west and south of Asia [2]. It is called bui in local language and Kapok bush in English [3]. The roots of the plant has been reported to rich in flavonoids vizQuercitin, Aeryanone, Chrysin 7-galactoside [4]. The flowers are reported to contain ecdysteroids, aervecdysteroid A–D, acylated flavone glycosides. The seed oil was reported to contain pentacosane. [1] The whole plant has been reported to contain isorhamnetin, kaempferol, β-sitosterol, palmitic acid, linoleic acid, β-amyrin, betulinic acid, phytol, quercitin-3-O-rutinoside, etc [3].

Various part of the plant have been reported to have diuretic, demulcent, anti-bacterial, anti-fungal, anti-ulcer, anti-diarrheal, smooth muscle relaxant, nephroprotective activity. The aerial part decoction has been reported to be used as gargle to cure toothache and gum swelling [1], anti-urolithiasis, anti-asthmatic, and anti-fertility activity [5].

The present study deals with determination of various physico-chemical and phytochemical parameters of roots of *A.javanica* to enable the proper identification and standardization information. The study also includes the determination of toxicological profile of hydro-alcoholic extract of roots of *A. javanica*using acute oral toxicity study.

2. MATERIALS AND METHODS

2.1 Plant Material

A. javanica was identified and its roots were collected from the local area of Jodhpur, Rajasthan. The plant species were identified and authenticated Botanist of Botany department of BilwalMedchem and Research Laboratory Pvt. Ltd. Jaipur, Rajasthan. Reference no: BMRL/PA/2021-16. Roots of *A.javanica* were cleaned and reduced into small fragments. The fragments were dried at room temperature, under shade till completely dried (15-20 days). After complete drying, the roots were coarsely powdered. The powdered roots of *A. javanica* were stored in an air-tight container until used.

2.2 Chemicals and Reagent

All the chemicals and reagent used were laboratory reagent (LR) grade and were purchased from Central Drug House, New Delhi, India.

2.3 Organoleptic Characteristics of Roots of *A. javanica*

Organoleptic characteristics such as colour, odour, taste, shape and surface characteristics of root powder of *A. javanica* were determined.

2.4 Physicochemical Analysis

Physicochemical parameters such as foreign organic matter, extractive values (water soluble, alcohol soluble, and petroleum ether soluble),

ash values (total ash, water soluble ash, and acid insoluble ash), and loss on drying (LOD) of coarse root powder of *A.javanica*were performed using standard method [6,7,8]. The data was expressed as Mean ± SEM and obtained using MS Excel 2007.

2.5 Fluorescence Analysis

*A.javanica*root powder about 1 gm was put or dissolved in sufficient quantity of various reagent such as methanol, ethanol, conc. Nitric acid, conc. Hydrochloric acid, picric acid, and 5% iodine and left overnight. Afterward, the solution was filtered and the filtrate was examined under short visible light, short ultraviolet range, and long ultraviolet range under Ultraviolet lamp of range 3600 to 4200 A [9].

2.5.1 Extraction of roots of *A. javanica*

The powdered roots of *A. Javanica* (300 g) were defatted with petroleum ether at 60-80 °C using soxhlet apparatus. The process was continued till a solvent drop from the siphon tube did not leave any greasy spot when evaporated on filter paper. The extract was concentrated at 35 – 40 °C in rotary evaporator under reduced pressure and its percentage yield was calculated. The extract was used for phytochemical analysis. Afterward, the marc was taken out from extractor and dried. The obtained marc weighed around 280 gm and was further used for extraction using hydroalcoholic solvent. The defatted marc was further extracted with 70% ethanol. The extraction was continued until the solvent in the siphon tube become clear. The extract thus obtained was filtered and collected and evaporated using a rotary evaporator under reduced pressure at 40 °C. The semi-solid extract weighing 72 gm was obtained and its percentage yield was calculated. The extract was kept in a sealed container in the refrigerator until further use [1]. The hydroalcoholic extract was also used for phytochemical analysis.

2.6 Preliminary Phytochemical Screening

The hydroalcoholic extract obtained was subjected to qualitative identification tests for different phytoconstituents using standard procedures [10,11].

2.7 Experimental Animal

Wistar albino female rats (180-200 g) were purchased and kept in the animal house of Bilwal Medchem and Research Laboratory Pvt. Ltd., Reengus Industrial area (RIICO), Sikar, Rajasthan. They were fed with a standard diet (standard pellets, Hafed, Rohtak, India) and water *ad libitum*. The standard housing conditions were maintained viz, 3 animals of same-sex/cage; temperature 22°C (±3°C), humidity 45-55%, artificial lighting with sequence 12 hours light/12 hours dark. The experimental protocols were per Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and were approved by Institutional Animals Ethics Committee (IAEC) of BilwalMedchem and Research Laboratory Pvt. Ltd. (Reg No- 2005/PO/RcBT/18/ CPCSEA).

2.7.1 Acute oral toxicity

The acute oral toxicity of 70% Ethanolic extract of *A. Javanica* was performed as per guideline 423, OECD guideline for testing of chemical, Acute toxic class method. Wistar albino female rats (nulliparous, non-pregnant, 8-10 weeks) were used for the study. The overnight fasted rats were administered with single dose 2000 mg/kg BW, p.o of *A. Javanica hydroalcoholic*extract. The animals were observed individually initially for 30 minutes, then once in 4 hours , then periodically during the first 24 hours, and daily thereafter for 14 days. Observations done include changes in skin and fur, eyes, changes in behavioural pattern, somatomotor activity; respiratory, circulatory, autonomic and central nervous system functions; salivation, diarrhoea, tremors, convulsion, sleep, lethargy [1].

3. RESULTS AND DISCUSSION

3.1 Organoleptic Characteristics

The dried roots were yellowish brown in color with rough surface, camphor like odor, and bitter taste. The result is presented in Table 1.

3.2 Physico-Chemical Analysis

The results of physicochemical parameters of root powder of *A.javanica*are presented in Table 2. The foreign organic matter was found to be 0.02%; total ash, water soluble ash, and acid insoluble ash to be 6.36 ± 0.26%, 0.79 ±0.23 % and, 1.23 ± 0.34%. The water, alcohol and petroleum ether soluble extractive values were found to be 17.88± 3.54 %, 15.58 ± 1.13% and, 0.3 ± 0.13 %. Loss on drying was found to be 8.11 ± 0.34 %.

Table 1. Organoleptic characteristics of roots of *A. javanica*

S. No.	Organoleptic characters	*A. javanica*
1	Colour	Yellowish brown
2	Odour	Camphor like
3	Taste	Bitter
4	Surface characteristics	Rough
5	Shape	Long cylindrical

Table 2. Physiochemical analysis of powdered roots of *A.javanica*

S.No.	Parameters	Observed value (%w/w)
1	Foreign matter	0.02
2	Water soluble extractive value	17.88 ± 3.54
3	Alcohol soluble extractive value	15.58 ± 1.13
4	Petroleum ether soluble extractive value	0.3 ± 0.13
4	Total ash value	6.36 ± 0.261
5	Water soluble ash value	0.79 ± 0.23
6	Acid insoluble ash value	01.23 ± 0.34
8	Loss on drying /Moisture content	07.81 ± 0.90

Results are expressed as Mean ± SEM, n = 06.

3.3 Fluorescence Analysis

The characteristics fluorescent property is depicted in the Table 3. Fluorescence analysis is done to identify authentic sample and recognize adulterants. The result depicted in below table can be used to check adulteration, as the adulterated samples would show difference in emission of color when compared to authentic samples.

3.3.1 Percentage yield and characteristics of root extracts of *A. javanica*

300 gm of root powder was taken for extraction which yielded the dried hydroalcoholic extract of 72 gm. Therefore, the percentage yield of hydro-alcoholic extract of roots of *A. javanica*was found 24 %, which indicates that the hydroalcoholic extract of roots of *A. javanica* is rich in phytoconstituents. These extracts and fractions were stored in airtight container for further studies.

3.3.2 Preliminary phytochemical screening

The hydroalcoholic extract of roots of *A. javanica*indicated the presence of most of the possible phytoconstituents viz., carbohydrate, flavonoids, some glycosides, tannins, steroids and terpenoids, tannins, alkaloids and fixed oils.

3.3.3 Acute oral toxicity study of hydro-alcoholic extract of root of *A. javanica*

Animals treated with 2000 mg/kg, BW of hydroalcoholic extract of *A.javanica* does not show any sign of toxicity as no moribund status or mortality was observed, which indicates that the extract was safe even at such higher dose and thus fall under category 6 of Globally Harmonised System (GHS) classification as per OECD guideline.

Table 3. Fluorescence emitted by root powder of *A.javanica* and various treatments under visible, short UV and long UV range

S.No	Test	Color under visible light	Color under short UV 254 nm	Color under long UV 365 nm
1	Drug powder	Yellowish brown	Green	Cream
2	Drug powder + Methanol	Yellowish green	Brownish green	Brown
3	Drug powder + Ethanol	Brown	Greenish brown	Green
4	Drug powder + Conc. HNO_3	Brick red	Brown	Black
5	Drug powder + Conc. HCl	Brown	Brown	Green
6	Drug powder + 5% iodine	Black	Black	Black
7	Drug powder + picric acid	Brown	Green	Light green

Table 4. *A. javanica*

S.No	Name Of phytoconstituent	Name of the test	Hydroalcoholic extract of *A. javanica*root	Petroleum ether extract of *A. javanica*root
1	Carbohydrate	Molischs test		Negative
		Fehlings test	Positive	Negative
2	Flavonoids	Shinoda test	Positive	Negative
		Ferric chloride test	Positive	Negative
3	Tannins	Nitric acid test	Positive	Negative
		Ferric chloride test	Positive	Negative
4	Glycosides	Reduction of Fehlings A and B solution	Positive	Negative
		Keller-killani test	Negative	Negative
		Baljets test	Negative	Negative
		Borntragers test	Negative	Negative
		Sodium picrate test	Positive	Negative
5	Steroids and terpenoids	Libermannsburchard test	Positive	Positive
6	Saponins	General test	Negative	Negative
		Foam test	Negative	Negative
7	Alkaloids	Mayers test	Positive	Negative
		Wagners test	Positive	Negative
8	Proteins and Amino acids	Ninhydrin test	Positive	Negative
		Biuret test	Positive	Negative
9	Fixed oils and Fats	Spot test	Positive	Positive

Phytochemical research deals with the study of medicinal plants for desired pharmacology. Another contribution of natural product is in facilitation of mechanism-based drug development. As per modern drug development, it is now well known that the higher plants play major role in development of higher therapeutics agents [12]. However, there may be chances of adulteration in these medicinal plants due to substitution in commercial market, varied geographical locations, and different vernacular names. Therefore, it is required that an adequate standards for evaluating the quality of such drugs must be laid to facilitate the acceptance of traditional herbal medicines [13]. Standardisation of herbal medicine and their formulation is a major attribute to assure their quality and has been stressed by the world health organisation [14,15].

Physicochemical parameters of root powder of *A.javanica*were evaluated as per the methods recommended by World Health Organisation (WHO). As seen from the result that the percentage foreign matter of root powder was found to be within the limit. Loss on drying which depicts the moisture content in a drug was found to be 7.81 ± 0.90 %. Less moisture content assure less microbial degradation during storage. The ash values were calculated as these are used to estimate the quality, authenticity, and purity of the drug. The extractive values in various solvents were determined to find out which solvents provide the highest percentage of phytoconstituents. Preliminary phytochemical screening was done to identify the nature of phytoconstituents present in hydroalcoholic extract of roots of *A.javanica*. The preliminary phytochemical screening of hydro-alcoholic extract indicated the presence of alkaloids, flavonoids, steroids, tannins, proteins, carbohydrates, glycosides, and fixed oil. Further, the hydroalcoholic extract of *A.javanica*roots was found to be safe till 2000 mg/kg BW and can be used to calculate the therapeutic dose.

4. CONCLUSION

The results of the present study will furnish data helpful in the correct identification and authentication of roots of *A.javanica.* The extractive value shed light on the most suitable solvent to be chosen to obtain extract rich in phytoconstituents. The physicochemical screening furnished data on important phytoconstituents present in the hydroalcoholic extract which could be helpful in isolation and purification of desired phytoconstituents. Acute oral toxicity study revealed that the extract is safe till 2000 mg/kg BW which could be helpful in

selection of dose for future pharmacological activities.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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