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THE PHYTOCHEMICAL ANALYSIS OF THE ETHANOLIC EXTRACT OF SUDANESE *AERVA JAVANICA* (BURM.F.) JUSS. EX J.A. SCHULTES

Nada Mohammed Osman Abbas[1*], Yahia Mohamed Ahmed El Imam[2], Mona A M Abdelmageed[3]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Khartoum College of Medical Sciences, Khartoum, Sudan.

[2]Faculty of Pharmacy, The National Ribat University, Khartoum, Sudan.

[3]Department of Pharmacognosy, Faculty of Pharmacy, Omdurman Islamic University, Omdurman, Sudan.

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***Correspondence for Author**
Dr. Nada Mohammed Osman Abbas
Department of Pharmacognosy, Faculty of Pharmacy, Khartoum College of Medical Sciences, Khartoum, Sudan.

ABSTRACT

This study deals with the analysis of the phytochemical constituents present in the whole dried plant of *Aerva javanica*(Burm.f.) Juss. ex J.A. Schultes. The percentage yield was calculated which was found to be 40.7%. The investigation revealed the presence of the following constituent's anthraquoinones, courmain, flavonoids, diterpenes, triterpenes, saponins, alkaloids, carbohydrates and condensed tannins. Then the extract was fractionated using solvent fractionation starting with hexane, chloroform, ethyl acetate, acetone the acetone fraction was centrifuged and the precipitated was dissolved in 70% ethanol. Each fraction was then analyzed using TLC(thin layer chromatography) with different mobile phases this lead to discovering that in the hexane fraction there were a number of flavonoids, coumarins, terpenes and alkaloids present. The chloroform fraction showed the presence of coumarins, terpenes and alkaloids, the ethyl acetate fraction indicated the presences of flavonoids, saponins, anthraquinones, terpenes and alkaloids, the acetone fraction established the presence of flavonoids, saponins, anthraquinones, terpenes and alkaloids. Finally the 70%ethanolic fraction illustrated the presence of anthraquinones, terpenes and alkaloids. This plant is indigenous to Sudan and is traditionally used for a number of aliments some of its uses for wound healing purposes, removal of swelling,

relieving inflammation, and the flowers and roots are used to relieve kidney problems and rheumatism.

KEYWORDS: *Aerva javanica*, phytochemical constituents, 70% ethanolic extract, fractionation

INTRODUCTION

Aerva javanica is a shrubby perennial herb that stands 50 cm to 1.6m high often forming clusters, it's widespread in the drier parts of tropical and sub-tropical Africa and Asia, commonly found at roadsides, stony rough valley's and hill slopes. It is found throughout much of mainland Africa -in Algeria, Egypt, Libya, Morocco, Chad, Somalia, Sudan, Kenya, Tanzania, Uganda, Cameroon, Mali, Mauritania, Niger, Nigeria, Senegal, Malawi, Mozambique and Botswana- and is also present in the Cape Verde islands and in Madagascar. Its natural range also includes the Arabian Peninsula- in Saudi Arabia, Iran, Israel and Jordan- and areas further eastwards in southern Asia and the Indian sub-continent - in Afghanistan, India, Pakistan, Myanmar and Sri Lanka.[1] In Sudan and Pakistan the paste and decoction of *Aerva javanica* is used traditionally for wound healing, jaundice, diabetes and cough.[2, 3] In Ayurveda the leaves, seeds and roots are used for the treatment of kidney stones, and as an astringent. Also in India this herbs decoction is traditionally used as a demulcent for diabetics, in the improvement of swellings and in urinary disorders. The powder is applied externally to treat ulcers in domestic animals. The seeds are used to relieve headaches and in rheumatism.[4] Reddy and Reddy concluded that the ethanolic extract of Aerva javanica was found to have hypoglycemic activity at a dose of 400mg/kg and also prevented any loss in the body weight of the experimental mice.[5] Another study made in 2012 in India also indicated that different solvent extraction of different part of *A. javanica* showed significant antibacterial activity when compared to the standard- streptomycin.[4] Khan et al established that A. javanica possess significant anti-ulcer activity. They deduced that the activity is due to the bioactive constituents found in the plant.[6] Therefore traditionally medicinal plants and their products are potential agents for healing aliments, their availability and effectiveness as crude preparations make them largely more preferable to use. Hence the use of herbal medicines is a cheaper and an affordable alternative especially in developing countries.[7]

MATERIALS AND METHODS

Collection and Preparation of the sample

In October 2014 the plant was collected locally from the state of Khartoum in Sudan and it was taxonomically authenticated at the herbarium of the Medicinal and Aromatic Plants Research Institute (MAPRI), National Center of research, Khartoum, Sudan, by Dr. Haidar Abd Algadir and a voucher herbarium samples was deposited there for further future reference. Then the whole plant was washed carefully and then air dried in the shade. After drying it was pulverized by using a mechanical grinder. 500 gm of the coarse powder plant was weighed and packed into the soxhlet. The plant was first defatted using petroleum ether (60-80^{o}C) then it was left to dry. After it was completely dried the marc was extracted using 70% ethanol for 12 hours once the extraction was complete the extract was dried using a rotary evaporator at a temperature of 50-60^{o}C. After the completion of the drying of the extract the yield in percentage was calculated and the extract was kept in an air-tight container.[8] Then the crude extract was divided into 2 portions the first portion underwent the phytochemical screening and the second portion was fractionated for TLC

Phytochemical Screening[8-11]

Phytochemical screening was carried out according to standard procedures.

Alkaloid

Mayer's Test

A few grams of the extract were dissolved in dilute hydrochloric acid, and then a few drops of the Mayer's reagent were added. Formation of a creamy precipitate indicated the presence of alkaloids.

Dragendorff's Test

Few grams of the extract were dissolved in dilute hydrochloric acid, and then a few drops of the Dragendorff's reagent were added. Formation of orange brown precipitate indicated the presence of alkaloids.

Carbohydrates

Molisch's Test

In a test tube 2ml of 95% ethanol were used to dissolve the extract, then 2 drops of freshly prepared 10 percent alcoholic solution of α- naphthol was added. Then it was shaken and 2

ml of conc. sulfuric acid was added from sides of the test tube. A violet ring was formed at the junction of two liquids, indicating the presence of carbohydrates.

Fehling Test

2 ml of 95% ethanol was used to dissolve the extract, then 2ml of Fehling A and B were added and heated a brick red precipitate indicates the presence of reducing sugar.

Glycosides

Anthraquinones

Borntrager Test

5ml of dilute sulfuric acid was added to the extract after it was dissolved it was then boil and filtered. Cold filtrate was treated with chloroform (equal volume) and shaken for some time. The organic layer is separated and treated with dilute ammonia. A pinkish color of the ammonia layer indicated the presence of anthraquinones.

Saponins

Foam Test

The extract was shaken vigorously with water in a test tube for 15 seconds. The formation of persistent foam indicated the presence of saponins.

Sterols and Triterpenes

Liebermann-Burchard test

The extract was dissolved in 2ml glacial acetic acid, then 1 ml of concentrated sulfuric acid was added from the sides of the test tube. The presence of a brownish-red ring and/or greenish color at the contact zone of the 2 liquids indicates the presence of sterols and/or triterpenes.

Salwoski's test

2 ml of chloroform was used to dissolve the extract, and then a few drops of sulfuric acid were added, a reddish brown color indicated the presence of a steroidal ring.

Tannins

Ferric Chloride Test

The extract was dissolved in distilled water it was then treated with 5% ferric chloride solution. Formation of blue color indicated the presence of hydrolysable tannins and formation of a green color indicated the presence of condensed tannins.

Bromine water

Ethanol 50% was added to dissolve the extract, to the 2ml a few drops of bromine water was added, a buff color indicated the presence of condensed tannins.

Flavonoids

Sodium hydroxide test

95% ethanol was used to dissolve the extract then 2ml of 10% sodium hydroxide was added to of the extract a yellow coloration was observed indicating a positive test for flavonoids.

Shinoda's test

To 3ml distilled water was added to dissolve the extract to it a few grams of magnesium were added then 2 drops of dilute hydrochloric acid was added and then warmed, a red color indicated the presence of flavonoids.

Diterpene

The extract was dissolved in water and treated with 10 drops of copper acetate solution. An emerald green color indicates the presence of diterpenes.

Coumarin

2ml of distilled water was used to dissolve the extract it was then divided into 2 test tubes –A and B. Test tube A had 1ml of the extract dissolved in distilled water and in test tube B to the extract and distilled water 1ml dilute sulfuric acid was added. When observed under the microscope test tube gave a faint bluish-green fluorescence which intensified in test tube B this indicates the presence of coumarin.

Fraction preparation

The dried 70% ethanolic extract was first dissolved in distilled water then a sequential partition starting with hexane (3x100ml), chloroform (3x 100ml), ethyl acetate (3x 100ml), acetone (3x 100ml). The acetone fraction was centrifuge the precipitate obtained was collected and dissolved in 70% ethanol. Each fraction was then concentrated so it could be applied for TLC.[12]

Thin Layer Chromatography[13]

From each fraction prepared about 20-100 µl are applied using capillary tubes to a precoated silica gel 60 F_{254} plates (5cmx10cm), Merck, Germany. Six such plates are prepared to cover

each of the main classes of constituents. Each having a different solvent system and detection method:

Plant constituent	Solvent system	Detection method
Anthraquinones:	Ethyl acetate-methanol-water (100:13.5:10)	10% ethanolic KOH reagent
Alkaloids	Toluene-ethyl acetate-diethylamine (70:20:10)	Dragendorff reagent
Flavonoids	Ethyl acetate-formic acid-glacial acetic acid-water (100:11:11:26)	UV-365 nm
Saponins	Chloroform-glacial acetic acid-methanol-water (64:32:12:8)	Vanillin sulfuric acid reagent
Terpenes	Toluene-ethyl acetate (93:7)	Vanillin sulfuric acid reagent
Coumarins	Diethyl ether-toluene (1:1; saturated with 10% acetic acid)	10% ethanolic KOH reagent then UV-365 nm

RESULTS

Using the soxhlet 500 gm of Aerva javanica herb were defatted with petroleum ether and then it was extracted with 70% ethanol using soxhlet and the percentage yield was found to be 40.7%.

Phytochemical screening

The results obtained from the phytochemical screening of the herb was summarized in table 1.

Table 1: Results of the phytochemical screening of Aerva javanica

Phytochemical constituents	70% ethanolic extract
Alkaloids	+
Anthraquinones	+
Carbohydrates	+
Coumarins	+
Diterpenes	+
Flavonoids	+
Reducing sugars	+
Saponins	+
Steriods	+
Tannins	+
Triterpenes	+

Table 2: The chromatographic analysis of the anthraquinones from the different fractions

Anthraquniones: Ethyl acetate-methanol-water (100:13.5:10)		**Detection: 10% ethanolic KOH reagent**
Fractions	R_f value	Color
Ethyl acetate	Spot 1(0.40 cm) Spot 2(0.58 cm) Spot 3(0.69 cm) Spot 4(0.81 cm)	Yellow Yellow Yellow Yellow
Acetone	Spot 1(0.08 cm) Spot 2(0.15 cm)	Red Yellow
70% ethanol	Spot 1(0.08 cm) Spot 2(0.15 cm)	Red Yellow

Table 3: The chromatographic analysis of the alkaloids from the different fractions

Alkaloid: Toluene-ethyl acetate-diethylamine (70:20:10)		**Detection: Dragendorff reagent**
Fractions	**R_f value**	**Color**
Hexane	Spot 1(0.27 cm) Spot 2(0.34 cm) Spot 3(0.60 cm) Spot 4(0.69 cm) Spot 5(0.78 cm) Spot 6(0.87 cm) Spot 7(0.94 cm) Spot 8(0.97 cm)	Orange Orange Orange Orange-green brown Orange-green brown Dark green brown Dark green-brown Orange
Ethyl acetate	Spot 1(0.34 cm) Spot 2(0.78 cm) Spot 3(0.87 cm) Spot 4(0.94 cm) Spot 5(0.97 cm)	Orange Orange-green brown Dark green brown Dark green-brown Orange
Chloroform	Spot 1(0.34 cm) Spot 2(0.78 cm) Spot 3(0.87 cm) Spot 4(0.94 cm) Spot 5(0.97 cm)	Orange Orange-green brown Dark green brown Dark green-brown Orange
Acetone	Spot 1(0.34 cm) Spot 2(0.78 cm) Spot 3(0.87 cm) Spot 4(0.94 cm) Spot 5(0.97 cm)	Orange Orange-green brown Dark green brown Dark green-brown Orange
70% ethanol	Spot 1(0.34 cm) Spot 2(0.78 cm) Spot 3(0.87 cm) Spot 4(0.94 cm) Spot 5(0.97 cm)	Orange Orange-green brown Dark green brown Dark green-brown Orange

Table 4: The chromatographic analysis of the flavonoids from the different fractions

Flavonoids: Ethyl acetate-formic acid-glacial acetic acid-water (100:11:11:26)		**Detection:UV-365 nm**
Fractions	R_f value	Fluorescence
Ethyl acetate	Spot 1(0.28 cm) Spot 2(0.33 cm) Spot 3(0.57 cm)	Blue Bluish green Bluish green
Acetone	Spot 1(0.55 cm) Spot 2(0.79 cm) Spot 3(0.88 cm)	Blue Blue Bluish green

Table 5: The chromatographic analysis of the saponins from the different fractions

Saponin: Chloroform-glacial acetic acid-methanol water (64:32:12:8)		**Detection: Vanillin sulfuric acid reagent**
Fractions	**R_f value**	**Color**
Ethyl acetate	Spot 1(0.91 cm)	Orange
Acetone	Spot 1(0.41 cm) Spot 2(0.49 cm) Spot 3(0.57 cm) Spot 4(0.64 cm) Spot 5(0.75 cm) Spot 6(0.85 cm) Spot 7(0.95 cm)	Blue Brown Blue Brown Brown Violet Orange

Table 6: The chromatographic analysis of the terpenes from the different fractions

Terpene: Toluene-ethyl acetate (93:7)		**Detection: Vanillin sulfuric acid reagent**
Fractions	**R_f value**	**Color**
Hexane	Spot 1(0.17 cm) Spot 2(0.23 cm) Spot 3(0.27 cm) Spot 4(0.34 cm) Spot 5(0.39 cm) Spot 6(0.51 cm) Spot 7(0.74 cm) Spot 8(0.88 cm) Spot 9(0.97 cm)	Green Blue violet Light green Dark green Dark green Blue violet Violet Violet Violet
Chloroform	Spot 1(0.34 cm) Spot 2(0.39 cm) Spot 3(0.97 cm)	Green Dark green Violet
Acetone	Spot 1(0.08 cm)	Red
70% ethanol	Spot 1(0.23 cm) Spot 2(0.34 cm) Spot 3(0.39 cm) Spot 4(0.97 cm)	Blue violet Dark green Dark green Violet

Table 7: The chromatographic analysis of the coumarins from the different fractions

Coumarins: Diethyl ether-toluene (1:1; saturated with 10% acetic acid)		**Detection: 10% ethanolic KOH reagent then UV-365 nm**
Fractions	**R_f value**	**Fluorescence**
Hexane	Spot 1(0.24 cm) Spot 2(0.32 cm) Spot 3(0.44 cm) Spot 4(0.56 cm) Spot 5(0.76 cm) Spot 6(0.97 cm)	Blue green Blue green Blue green Blue Blue Green
Ethyl acetate	Spot 1(0.09 cm) Spot 2(0.76 cm)	Blue Blue
Chloroform	Spot 1(0.09 cm) Spot 2(0.76 cm)	Blue Bue

DISCUSSION

The plant material was extracted using 70% ethanol which gave a percentage yield of 40.7 %. Then in the phytochemical screening of the whole dried plant of *Aerva javanica* it revealed the presence of flavonoids, condensed tannins, diterpenes, alkaloids, anthraquinones, saponins, triterpenes, steroids, coumarins, reducing sugars and carbohydrates [this could be seen in Table 1]. This might indicate that the 70% ethanol was able to extract more constituents or the same constituents but in a higher concentration. The cause of this could be due to the fact that alcohol extracts numerous plant constituents although some remain difficult to be extracted by it, for example fixed oils.[8] Then the fractionation of the extract showed the presences of various constituents in each fraction when it was analyzed using TLC with different solvent systems. When examining the anthraquinones after spraying with 10% ethanolic KOH a red zone indicates the presence of anthraquinones and a yellow zone indicates the presence of anthrones, the ethyl acetate gave 4 spots that were all yellow this indicates that this fraction contains anthrones while the acetone and 70% ethanol fraction had a red and yellow spot this reveals that they have both anthraquinones and anthrones[Table 2]. All of the fractions showed the presence of alkaloids due to the orange to green brown color obtained when spread with Dragendorff reagent with the largest number of alkaloids present in the hexane fraction[Table 3]. A green to blue fluorescence indicates the presence of flavonoids when observed untreated under the UV 365nm the ethyl acetate and acetone fractions shoed the presence of flavonoids with the possibility that the 0.55 cm could be chlorogenic acid as indicated in Plant Drug Analysis[Table 4]. The saponins are indicated by mainly blue, violet, yellow, orange and reddish brown zones the ethyl acetae fraction contained an orange spot while the acetone fraction had blue, violet, orange and brown spots

all which indicate the presence of saponins[Table 5]. Terpenes that are present give red, blue, green, violet and orange zones all the fractions had terpenes present except for the ethyl acetate fraction[Table 6]. A bright blue fluorescence indicates the presence of coumarins and a blue-green fluorescence indicates the presence of furano- and pyranocoumarins this means that in the hexane fraction there were coumarins, furano- and pyranocoumarins due to the presence of blue, blue green anf green spots while in the chloroform and ethylacetate fractions there were only coumarins[Table 7].[13]

CONCLUSION

This study identified the presence of different constituents that all play an important role in therapeutics. It could be used for further investigation of the constituents present also this plant has various uses that could be further investigated using these findings. Previous studies were carried out on different parts of the plant**.**

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REFERENCES

1. Ghazanfar, S.A., *Handbook of Arabian Medicinal Plants*. Taylor & Francis., 1994; 17-19.
2. El Ghazali, G.E.B., M.S. El Tohami & A.A.B. El Egami, *Medicinal plants of the Sudan.Part 3 : Medicinal plants of the White Nile Provinces. Medicinal and aromatic plants institute.National council for research*. Khartoum: Augustus., 1994; 116.
3. Qureshi, R., G.R. Bhatti, and R.A. Memon, *Ethnomedicinal uses of herbs from northern part of Nara desert, Pakistan.* Pak. J. Bot., 42(2): 839-851.
4. Srinivas, P., Reddy, S. Ram, *Screening for antibacterial principle and activity of Aerva javanica (Burm. f) Juss. ex Schult.* Asian Pacific Journal of Tropical Biomedicine, 2012; 2(2): S838-S845.
5. Reddy, K.S. and V.M. Reddy, *Antihyperglycaemic activity of ethanol extract of Aerva javanica leaves in alloxan-induced diabetic mice.* Journal of Pharmacy Research, 2009; 2(7): 1259-1261.

6. Khan, A.W., et al., *Phytochemical analysis and Enzyme Inhibition Assay of Aerva javanica for Ulcer.* Chemistry Central Journal, 2012; 6(1): 1-6.
7. Kumari, P., Yadav, P., Verma, P.R., Kumar, S., Arya, A., *A Review on Wound Healing Properties of Indian Medicinal Plants.* Indian Journal of Fundamental and Applied Life Sciences, 2013; 3(1): p. 220-232.
8. Evans, W.C., G.E. Trease, and D. Evans, *Trease and Evans Pharmacognosy*., 2002; W.B. Saunders.
9. Harborne, J., *Phytochemical methods, a guide to modern techniques of plant analysis, JB Harborne*. 13 ed. 1973, London: Chapman and Hall Ltd. 5-15.
10. Sofowora, A., *Medicinal Plants and Traditional Medicine in Africa*, ed. S.B. Limited. 1993; Ibadan.
11. Godghate, A., R. Sawant, and A. Sutar, *Phytochemical Analysis of Ethanolic Extract of Roots Of Carrisa carandus Linn.* Rasayan J Chem, 2012; 5(4): 456-459.
12. Sharif, A., et al., *Lipoxygenase inhibitory constituents from Indigofera oblongifolia.* Archives of pharmacal research, 2005; 28(7): 761-764.
13. Wagner, H. and S. Bladt, *Plant Drug Analysis: A Thin Layer Chromatography Atlas*. 2 ed. 2001: Springer Science & Business Media.