International Journal of Phytomedicine 9 (2017) 10-14
http://www.arjournals.org/index.php/ijpm/index

Short Communication

ISSN: 0975-0185

Phytochemical and Biological Evaluation of *Justica adhatoda*

Shahid Aziz [1*] , Hidayat Hussain[2*], Anisa Younis[1], Habib-ur-Rehman[1], Muhammad Shahid[3], Arshad Farooq Butt[1], Ivan R Green[4]

***Corresponding authors:**

Shahid Aziz, Hidayat Hussain

[1]Department of Chemistry, Mirpur University of Science & Technology (MUST), Mirpur (AJK), Pakistan
[2]UoN Chair of Oman's Medicinal Plants and Marine Natural Products, University of Nizwa, Birkat Al-Mouz, Nizwa 616, Sultanate of Oman
[3]Department of Chemistry and Biochemistry, University of Agriculture Faisalabad, Pakistan.
[4]Department of Chemistry and Polymer Science, University of Stellenbosch, P/Bag X1 Matieland 7602,South Africa

Abstract

Phytochemical and biological investigations were undertaken on ethanolic extracts of the leaves and root bark of *Justica adhatoda* L. Alkaloids, cardiac glycosides, tannins, steroids and saponins were detected in extracts from both parts of the title plant. Reducing sugars were not detected in either extract whereas flavonoids were detected in ethanolic extract of *J. adhatota* leaves only. Significant antibacterial and antifungal activities against *Escherichia coli*, *Staphylococcus aureus* and *Aspergillus niger* were demonstrated by both the leaf and root bark extracts. Additionally, both extracts displayed good antioxidant and heomolytic potential. Interestingly, eight metal ions were detected in both extracts in different concentrations.

Keywords: *Justice adhatoda* L*,* anti-microbial, antifungal, antioxidant and heomolytic activity.

Introduction

Justica adhatoda L. (Syn. *Adhatoda vasica* Nees, *Adhatodazeylanica* Medicus) is grown in Indonesia, Malayasia, Southeast Asia, India and Pakistan [1] and has been an integral component in Unani medicine in India for some 200 years [2]. Moreover the title plant is used as an antispasmodic, anti-diabetic, expectorant, mitigating, fever reducer, hostile to dying, bronchial dilating, disinfectant, to treat dysentery, Bronchitis, whooping cough, glandular tumor, jaundice, diabetes, uterine constrictions, diarrhea including some liver relevant disorders [3-5]. Furthermore the alkaloids present in *J. Adhatoda* L. are reported to have antimicrobial effects towards *Staphylococcus aureus, Pseudomonas aeruginosa* and exceedingly pathogenic microscopic organisms like *Salmonella typhi* [3].

Over the years a large number of natural products have been detected from the genus *Justicia* and include: alkaloids, lignans, steroids, flavonoids, vitamins, iridods, coumarin, diterpenoids, triterpenoids and triterpenoidal glycosides [5]. What is of interest currently is that these natural products demonstrate anti-inflammatory, anti-hypertensive, antinociceptive, bronchodilator, antifertility, antiplatelet aggregation, antiviral, anticancer effects and quite remarkably, to treat HIV/AIDS [5]. Taking account of these medicinal applications, we have decided to extend the phytochemical investigations and biological potential further by including antibacterial, antifungal, antioxidant and hemolytic activities of *J. adhatoda* L extracts.

Materials and methods

Plant Collection

Leaves and root bark of *J. adhatoda* were carefully selected for this investigation and collected from the hills of the district Kotli in the State of Jammu & Kashmir. The plant was identified by a plant taxonomist at the Department of Botany, Mirpur University of Science & Technology (MUST), Mirpur, Azad Jammu and Kashmir. The voucher specimen of the plant is available in the herbarium of the department.

Sample preparation

Dry powdered plant material (60g) from the leaves and root bark was soaked in 250 mL of 90 % ethanol for 15 days and then filtered. Concentration of the filtrate under vacuum gave a semi-solid residue which was allowed to dry at room temperature for a further 7 days. The crude extracts were stored in desiccators for

DOI:10.5138/09750185.1875

maximum of 3 days and later preserved in a deep freezer (-20 ◦C) for further use.

Phytochemical screening

Screening for different phytochemical classes viz. alkaloids, flavonoids, glycosides, steroids, tannins and terpenoids was conducted in the laboratory according to standard protocols on both extracts with little modification [6,7].

Detection of Alkaloids

The availability of alkaloid is detected by taking 2 g of leaf and root bark extract of *J. adhatoda* in a china dish and administering them with successive additions of 1mL of Dragondroff's reagent. Formation of a ruddy tan or pinkish purple shade demonstrated the presence of alkaloids.

Detection of terpenoids

Salkowski'sstrategy was employed to detect the presence of terpenoids in the plant material. Both extracts (2.5g each) were blended together with 1mL of chloroform ($CHCl_3$) after which 1.5mL of concentrated sulphuric acid (H_2SO_4) was added. A rosy tan coloration at the interface shows the presence of terpenoids in given example [8].

Detection of cardiac glycosides

In this case the Keller-Kiliani test is was used to detect cardiovascular glycosides [8]. According to this protocol, the presence of a tan ring at the interface beneath the greenish layer is a positive indication of cardiac glycosides in a given sample. . Accordingly, 2.5g of each of the ethanolic concentrates were placed in a china dish followed by the addition of 1mL of glacial acetic acid and 1mL of a freshly prepared solution of ferric chloride ($FeCl_3$). After mixing for a few minutes, 0.5mL of concentrated sulphuric acid (H_2SO_4) was added drop wise to the sample.

Detection of tannins

Tests for tannin were performed using the published approach [8]. According to this protocol the presence of a caramel green or blue black color demonstrated the presence of tannins in the plant specimen. Ethanol extracts (3g) of each plant part along with 10 mL of water was heated to boiling point in a 100 mL measuring beaker and allowed to cool to room temperature and then filtered. To this solution was added a few drops of 0.1mL freshly prepared ferric chloride ($FeCl_3$).

Detection of flavonoids

Extracts (1g) of both plant parts of *J. adhatoda*were separately placed in a 100 mL glass beaker and warmed on a water bath along with 10mL of ethyl acetic acid for 3 minutes. The warmed mixtures were filtrated and samples of 4ml of the filtrates were each shaken well with 1mL of aqueous smelling salts to allow the liberated ammonia to react.The appearance of a yellow shade demonstrated the presence of flavonoids in the plant specimen.

Detection of steroids

Ethanol extracts (0.5g) of each part of *J. adhatoda* were placed in a china dish and 2mL of acidic anhydride was added and the mixture then blended with a glass rod. After the blending, 2 mL of concentrated sulphuric acid (H_2SO_4) was carefully added to the stirring mixture. A shade change from violet to green or blue indicated the presence of steroids.

Detection of saponins

Ethanol extracts (0.5g) of each part in a china dish with 2mL of distilled water were warmed to 100 ^oC and then filtered. The aggregate, (1mL) was filtered and the subsequent filtrate vigorously shaken with refined water (2 mL). The formation of an emulsion indicated the presence of saponins.

Detection of anthraquinone

According to this technique, ethanol extracts (1 g) of each of the leaves and root bark of *J. adhatoda* in a china dish were well blended with 0.5 mL of ether after which water was added and vigorously shaken. The presence of a pink, red or violet color indicated the presence of anthraquinones or their analogues in the plant specimen.

Detection of reducing sugar

According to the protocol used the presence of any shade of a red or violet colour demonstrated the presence of a reducing sugar in plant specimen. Thus an ethanol extract of each plant part (0.5g)in a 100 mL glass beaker was treated with refined water (5.0 mL) followed by a couple of drops of freshly prepared Fehling's solution. After warming for up to 30 min colour changes would indicate the presence of reducing sugars in the specimens.

Pharmacological studies of *J.adhatoda*

Biological studies conducted include antibacterial, antifungal, antioxidant and heamolytic activity.

Antibacterial activity

Sample preparation: The ethanol extract (10mg) of leaves or bark is taken and dissolved in DMSO (0.5 mL).
Media preparation: Agar (28g) was mixed with distilled water (1.0 litre) and heated to 100 ºC to dissolve completely. After that it was sterilized by autoclaving at 121ºC for 15 minutes.
Culture used: Antibacterial activity of *J. adhatoda* was evaluated on the following cultures:
Escherichia coli (Gram negative bacteria)
Staphylococcus aureus (Gram positive bacteria)

Antifungal activity

Sample preparation: Identical as for antibacterial activity.
Media preparation:. PDA(39g)in distilled water (1 litre) was heated to 100 ºC to dissolve it completely. After that it was sterilized by autoclaving at 121ºC for 15 minute and mixed well before pouring.
Use of culture: Aspergillus was used as a culture.
Method: Same method was applied for antifungal activity as for antibacterial activity as mentioned above except for the culture used.

Antioxidant activity employing the DPPH method

Stable DPPH (2, 2 Diphenyl, 1-picrylhadrazylhydrate) was utilized to measure the antioxidant activity of different fractions of *J. adhatoda.* When DPPH reacts with an antioxidant, the reaction progress is followed by a change in color of the extract from deep violet to light yellow) and is measured at 518nm. The different extracts of *J. adhatoda* were tested for the DPPH free radical test by modified method described in literature [9]. The ethanol extracts of *J. adhatoda* were dissolved in DMSO and made up to 1mL of each separately. This was followed by taking 80*u*L of DPPH and adding to it 20*u* L of each sample to give a total of 100*u*L of each test sample. This was repeated for all the samples and fractions which were then put in a 96 well plate and placed in a UV spectrophotometer to measure the antioxidant activity of given sample.

Hemolytic Activity

Hemolytic activity of the leaf and root bark extracts (ethanol) was studied by the published method [10]. Freshly obtained heparinized human blood (3 mL) was collected from volunteers after consent and counseling. The blood was centrifuged for 5 min at 1000x*g* and the plasma was discarded and the cells were washed with 5 mL of chilled (4ºC) sterile isotonic phosphate-buffered saline (PBS) pH 7.4. Erythrocytes were maintained at 10^8 cells per mL for each assay. Each compound (100 µL) was mixed with human (10^8cells/mL) separately. Samples were incubated for 35 min at 37ºC and agitated after each 10 min. period. Immediately after incubation the samples were placed on ice for 5 min then centrifuged for 5 min at 1000xg. Supernatant (100 µL) was taken from each tube and diluted 10 time with chilled (4ºC) PBS. Triton X-100 (0.1% v/v) was taken as a positive control and phosphate buffer saline (PBS) was taken as a negative control and each treated via the same process. The absorbance was noted at 576 nm using μQuant. The% RBCs lysis for each sample was calculated.

Metal Ion Detection

Digestion of sample: The following acid digestion procedure was used for sample preparation to be used in the AAS protocol for the determination of different metal ions in *J. adhatoda* leaves and root bark as described by Zheljazkov and War man [11]. The basic advantage of this strategy is that it eliminates elemental loss by volatilization because the digestion takes place at a low temperature.
Dried leaf powder (I g) from *J. adhatoda* ina 250 mL assimilation tube was treated with concentrated HNO3(10 mL). The sample tube was kept for 45 min at 9ºC after which the temperature was raised to 150ºC for 8 h. Concentrated HNO3 was added to the specimen (3x5 mL) during the digestion and finally the volume was reduced to 1mL. The inside dividers of the tube were washed down with distilled water and the tube was swirled all the while to keep the divider clean and reduce any loss of the specimen. To the cool solution was added 5 mL of 1% $HNO_3$was added to the specimen. The solution was filtered with Whatman No. 42 filter paper and <0.45 Millipore channel paper. The filtrate was then transferred quantitatively to a 25 mL volumetric flask and made up to the mark with distilled water. A analogous procedure was followed for the root bark powder.

Results and Discussion

Phytochemical screening of leaves and root bark of *J. Adhatoda*

The qualitative screening of phytoconstituents showed the presence of alkaloids, terpenoids, cardic glycosides, tanins, steroids, saponins and anthraquinones in both the ethanol extracts of leaves and root bark of *J. adhatoda* (Table 1). Flavonides are present only in the ethanolic leaf extract of *J.adhatoda* (JAL) whereas the reducing sugars were not detected in either of the extracts of *J. adhatoda.* The results for the phytochemical investigations of *J. adhatoda* leaves(JAL) *and J. Adhatoda* root bark (JARB) are illustrated in Table 1.

Table-1: Phytochemical constituents analysis of *J.adhatoda* leaves (JAL) and root bark (JARB).

Phytochemical constituents	Leaves extract	Root bark extract
Alkaloids	+	+
Terpenoids	+	+
Cardiac glycosides	+	+
Tannins	+	+
Flavonoids	+	-
Steroids	+	+
Saponins	+	+
Anthraquinone	+	+
Reducing sugar	-	-

Biological activities

Antibacterial activity

Ethanolic extracts of *J.adhatoda* leaves (JAL) and root bark (JARB) were evaluated for their antibacterial activity against pathogenic microorganisms. The results for antibacterial activity of JAL and JARB are presented in Table 2. Ethanolic extract of *J.adhatoda* leaves showed good antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* 56.57% and 58.66% respectively while only the ethanolic extract of the root bark of *J.adhatoda* showed 88.15% activity against *Staphylococcus aureus*.

Table-2: Antibacterial activity of *J.adhatoda* leaves (JAL) and root bark (JARB)

Plant extracts	Bacterial culture	Inhibition zone (mm)	%age Inhibition
Leaves extract(JAL)	*Escherichia coli*	21.5	56.6
Root bark extract(JARB)	*Escherichia coli*	33.5	88.1
Leaves extract (JAL)	*Staphylococcus aureus*	22	58.7
Root bark extract(JARB)	*Staphylococcus aureus*	26	69.3
Control	*Escherichia coli*	38	100
Control	*Staphylococcus aureus*	37.5	100

Antifungal activity

Ethanolic extract of *J. adhatoda* leaves(JAL) *and J. adhatoda* root bark (JARB) were evaluated fortheir antifungal activity against the pathogenic microorganism, *Aspergillus niger*. JAL showed 54.32% activity while JARB showed 81.48 % activity against *Aspergillus niger* (Table 3).

Table 3: Antifungal activity of *J. adhatoda* leaves (JAL) and root barks (JARB)

Plant extracts	Bacterial culture	Inhibition zone (mm)	% age Inhibition
Leaves extract (JAL)	*Aspergillus niger*	22	54.3
Root bark extract(JARB)	*Aspergillus niger*	33	88.5
Control	*Aspergillus niger*	40.5	100

Antioxidant activity

The antioxidant activities of the ethanolic extract of *J. adhatoda* leaves (JAL) *and J. adhatoda* root bark (JARB) were studied by using the DPPH method and gallic acid was used as standard. Both extracts illustrated very good antioxidant activity with 70.8 and 73.4 percent inhibition respectively (Table 4).

Table 4: Antioxidant activity of *J. adhatoda* leaves (JAL) and root bark (JARB)

Plant extracts	DPPH inhibition	DPPH% age Inhibition
Leaves extract (JAL)	3.5	70.8
Root bark extract(JARB)	3.7	73.4
Gallic acid	5	100

Hemolytic activity

The hemolytic activities of ethanolic extracts of *J. adhatoda* leaves(JAL)*and J. adhatoda* root bark (JARB)were evaluated using Triton-X-100 as a positive control and by using PBS as negative control. Results are illustrated in Table 5. Table5: Hemolytic activity of *J. adhatoda* leaves (JAL) and root bark (JARB) extracts

Table 5: Hemolytic activity of *J. adhatoda* leaves (JAL) and root bark (JARB) extracts

Plant extracts	Cytotoxicity
Leaves extract (JAL)	7.08
Root bark extract(JARB)	7.39
Triton-X-100(Positive control)	100

Metal Ion detection

Metal ions of the ethanolic extracts of *J. adhatoda* leaves(JAL) *and J. adhatoda* root bark (JARB) samples were on the Zeeman atomic absorption spectrometer. Metal ions detected in ppm are given below in Table 6.

Table 6: Metal ion detection in *J. adhatoda* leaves (JAL) and root bark (JARB) extracts.

Names of Metal ions detected.	Permissible limit by WHO (ppm).	Concentration (ppm) in JAL	Concentration (ppm) in JARB
Manganese Mn	2.00	18.36	2.06
Cobalt Co	3.41	1.50	0.06
Cadmium Cd	0.21	0.50	0.01
Nickel Ni	1.63	1.47	0.45
Ferrum Fe	20	39.09	16.65
Copper Cu	3.00	3.58	1.45
Lead Pb	0.43	2.05	1.40
Zinc Zn	27.4	7.84	2.00

The data shows that most of the metals found in *Justica adhatoda* leaves and root bark are within the range of permissible limit of the WHO. In *Justica adhatoda* leaves Mn and Fe are above the permissible limit while in the root bark Pb is present above the permissible limit.

Conclusion

The overall result of this research allows the authors to conclude that the leave and root bark extracts of *J. adhatoda* contain a number of classes of active natural compounds most of which demonstrate a significant antibacterial, antifungal, antioxidant and heomolytic activity. It is thus imperative that further quantitative investigations are pursued to isolate and elaborate the structures of the active components in *Justica adhatoda*.

References

[1]. Sunita M, and Dhananjay S. Quantitative analysis of total phenolic content in *Adhatodavasica*Nees Extracts. Int J Pharm Tech Res. 2010;2:2403-2406.

[2]. Atal CK. Chemistry and Pharmacology of Vasicine. A New Oxytocic and Abortifacient Regional Resarch Laboratory, Jammu Tawi (1980).

[3]. Sawant CS, Save SS. and Bhagwat AM. Antimicrobial activity of alkaloids extract from *Adhatodavasica*. Int J Pharm Bio Sci. 2013;4:803-807.

[4]. Gulfraz M, Ahmad A, Asad MJ, Sadiq A, Afzal U, Imran M, Anwar P, Zeenat A, Abbasi KS, Maqsood S, Qureshi RU. Antidiabetic activities of leaves and root extracts of *Justiciaadhatoda* Linn against alloxan induced diabetes in rats. Afr. J. Biotechnol. 2010;10:6101-6106.

[5]. Corrêa GM, Alcântara AFC. Chemical constituents and biological activities of species of *Justicia*-a review. Braz. J. Pharmacog. 2012;22:220-238.

[6]. Jamuna KS, Ramesh CK, Srinivasa TR and Raghu KL. *In vitro* antioxidant studies in Raaman N. Phytochemical Techniques. New India Publishing Agency, ISBN, 9798189422300. 2006.

[7]. Harbone JB. Phytochemical methods: A guide to modern techniques of plant analysis. (3rd ed). Chapman and Hill, London, 279, 1998.

[8]. Edeoga HO, Okwu DE, and Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr J Biotechnol. 2005;4:685-688.

[9]. Bandoniene D, Markovic M, Pfannhauser W, Venskutonis PR, and Gruzdiene D. Detection and activity evaluation of radical scavenging compounds by using DPPH free radical and on-line HPLC-DPPH methods. Eur Food. 2002;214:143-147.

[10]. Anjum F, Shazia AB, Shahid M, Tanveer HB, and Munsif AT. Exploration of nutraceutical potential of herbal oil formulated fromparasitic plant. Afr J Tradit Complement Altern Med. 2012;11:78-86.

[11]. Zheljazkov VD, Warman PR. Comparison of three digestionmethods for the recovery of 17 plant essential nutrients and trace elements from six composts. Compost SciUtiliz. 2002;10:197–203.