

Pharmacological activities of *Justicia adhatoda*

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Abstract: The current study focused on the pharmacological activities of *Justicia adhatoda*; including antibacterial, antifungal, phytotoxic, cytotoxic, haemagglutination, insecticidal, *in vitro* antiglycation, DPPH antioxidant and anti-termite. The crude methanolic extract (Crd. Met. Ext) showed 46.4 % antibacterial activity against *M. morganii* while the *n*-hexane fraction showed good (71.4%) and moderate (55.1%) activity against *M. morganii* and *A. baumannii* respectively. The EtOAc and aqueous fractions, in most of the cases, showed low to no activity against the selected bacterial pathogens, against *A. niger*, *T. harzianum*, *A. parasiticus* and *V. dahliae*. The Crd. Met. Ext and fractions showed low activity, against *P. notatum* and *P. digitatum*, Crd. Met. Ext. and all fractions were inactive. The percent growth regulation, in case of phytotoxic activity, by Crd. Met. Ext was 25 and 16.6, *n*-hexane fraction 16.6, 16.6 and 0, CHCl₃ 25, 8.33 and 0 % and EtOAc fractions 8.33, 8.33 and 0% at 1000 and 100 and 10µg/ml respectively. The aqueous fraction was inactive at all the test concentrations. The results of brine shrimp cytotoxic activity for Crd. Met. Ext was 13.33% and *n*-hexane fraction 20% at 1000, µg/ml respectively. All of the other fractions showed low to no activity at different test concentrations. All of the test samples were inactive against RBC's of the blood groups at all concentration indicating that the selected plant lack phytolectins and haemagglutination activity. The Crd. Met. Ext and various fraction showed low activity against the test insects i.e. *C. pulicaria*, *C. chinensis* and *T. castaneum*. The absorbance value of plant extract for anti-glycation activity at various concentration were: 0.08, 0.067, 0.053 and 0.04 in comparison with Aminoguanidine 0.04, 0.035, 0.03 and 0.02 respectively at 10, 50, 90 and 130µl. The DPPH radical scavenging activities were proportional to the concentration of the fractions, as the concentration of these increased, the percent scavenging activity also increased. The CHCl₃ and EtOAc fractions killed all the termites in 24 hours while Crd. Met. Ext, *n*-hexane and aqueous fractions took 2-3 days.

Keywords: Antimicrobial, phytotoxic, haemagglutination, *in vitro* antiglycation, antioxidant.

INTRODUCTION

Allah has created this Universe with a great diversity. In the Holy Quran, Allah says "I have created all the things in the Universe for the welfare of mankind and have created mankind for my worship (Al Quran, 12). Keeping in view the first part of the saying, we need to explore the universe for our benefits. One of the main purpose of man, was and is, to discover ways to treat ailments especially diseases and every civilization developed its own ways for it. Most herbalists are of the opinion that pharmaceuticals are good only when there is emergency e.g. heart attack, however it exhibit side effects. Herbal medicines on the other hand give fewer side effects. The most important point is that in most cases you need synergistic effect of many compounds to treat a disease. In plants this combination occurs naturally making them more effective to treat a disease (Tapsell *et al.*, 2006).

Justicia adhatoda, locally called as Baikar and Vasaka, belongs to family *Acanthaceae*. It is present in Pakistan, India, Panama, Indonesia, Malaya and South East Asia (Kamal and Ghafoor, 1978). The leaves and roots are used for rheumatism, pneumonia, cough, snake-bites, eye and

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ear ailments, asthma and tuberculosis (Haider *et al.*, 2011; Sharma *et al.*, 1992). The famous ancient Indian saying, "No man suffering from phthisis needs despair as long as the Vasaka plant exists" shed light on the importance of this plant to treat respiratory diseases (Dymock *et al.*, 1890). "The Use of Traditional Medicine in Primary Health Care" published by World Health Organization (WHO), contains *J. adhatoda*, which is focusing on the restorative utility of the surrounding flora of South East Asia (WHO, 1990). The fruit of *J. adhatoda* are used for curing cold, jaundice, bronchitis, diarrhea, dysentery and fever and as laxative (Kirtikar and Basu, 1975; Roberts, 1931; Rahman *et al.*, 1986). Keeping in view the importance of the selected plant, the current study was designed to screen the Crd. Met. Ext and various fractions of plant for different biological activities including antibacterial, antifungal, phytotoxic, cytotoxic, haemagglutination, Insecticidal, *in vitro* antiglycation, DPPH antioxidant and anti-termite.

MATERIALS AND METHODS

Plant material

J. adhatoda (aerial parts) was collected from Mardan, Khyber Pakhtunkhwa, Pakistan, exposed to shade drying

and grounded to powder using an electric grinder. Soaking of the powdered material was performed in methanol for 15 days with occasional shaking at room temperature, and filtrates were concentrated below 40°C under vacuum using rotary evaporator which gave Crd. Met. Ext of *J. adhatoda*.

Fractionation

The Crd. Met. Ext was suspended in distilled water and fractionated with *n*-hexane, CHCl₃ and EtOAc yielding *n*-hexane, CHCl₃, EtOAc and aqueous fractions. Some of the Crd. Met. Ext was reserved for biological activities.

Antibacterial activity

The bacterial species used in the current research were; *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Morganella morganii*, Methicillin resistant *Staphylococcus aureus* and *Acinetobacter baumannii*.

The antibacterial activity was performed following Ahmad *et al.*, 2009. Sterile Nutrient agar and broth media used for antibacterial activity. Wells were made with help of sterile borer (6mm) in the plates. Stock solutions, 3mg/ml in DMSO (<1%) were prepared. From stock solutions 100µl was introduced in the respective well and incubated for 24hours at 37°C. DMSO and Amoxicillin were taken as negative and positive control, respectively. Zone of inhibition was measured after incubation in comparison with standard.

$$\% \text{ Zone of inhibition} = \frac{\text{Zone of inhibition of Sample}}{\text{Zone of inhibition of Standard}} \times 100$$

Antifungal activity

The test fungi used in the current research were; *Aspergillus niger*, *Penicillium notatum*, *Aspergillus parasiticus*, *Verticillium dahillae*, *Penicillium digitatum* and *Trichoderma harzianum*.

Ahmad *et al.*, 2009 was followed for the determination of antifungal activity. The sterilized Sabouraud Dextrose Agar (SDA) plates were used to refresh the above mentioned fungal species. 4 ml of SDA was introduced into the test tubes, autoclaved and when the temperature dropped to 50°C, 67 µl of test samples were added from each stock solution (24 mg/DMSO) into respective test tube and slants were made. After incubation, the fungal culture, 5-7 days old, was introduced into labeled test tubes and incubated at 25±1°C for seven days. DMSO and Miconazole were used for negative and positive controls, respectively. The linear growth of fungal species was measured on day 7th on the slanted test tubes in comparison with negative control.

$$\% \text{ Linear growth inhibition} = \frac{\text{Linear growth in test (mm)}}{\text{Linear growth in ive standard (mm)}} \times 100$$

Phytotoxic activity

Khan *et al.*, 2013 was followed to perform the phytotoxic bioassay of the test samples against *Lemna minor* L. 10, 100 and 1000µl from the stock solutions (20mg/ml of methanol) were introduced into the respective flask. After evaporation of methanol, E-media (20 ml) and 12 healthy plants were introduced into each flask. Finally, the flasks were incubated in growth chamber at 28±1°C for 5-7 days. The number of damaged plants was counted after incubation and results were noted.

Brine shrimp lethality bioassay

As per reported procedure of Alluri *et al.*, 2005, the cytotoxic effect of the test samples was carried out against *Artemia salina* (brine-shrimp eggs). Artificial sea water, prepared in double distilled water with a commercial salt mixture; was used for eggs to hatch and mature.

From the stock solution (20 mg/ml) 5, 50 and 500µl were transferred to vials (3 vials/concentration). Each vial was added with larvae (10) and sea water (1ml) after evaporation of methanol. The final volume of each vial was settled to 5ml with artificial sea water. The incubation of vials was done under illumination at 26±1°C for 24 hours. Etoposide (7.4625µg/ml) was used as positive control. Brine shrimps that survived after incubation period were counted using a magnifying glass.

Haemagglutination activity

According to reported procedure (Khan *et al.*, 2013), haemagglutination activity of test samples was performed. From stock solutions (1mg/ml of DMSO) different dilutions; 1:2, 1:4, 1:8 and 1:16 were made in phosphate buffer (Na₂HPO₄ and KH₂PO₄ in 3:7). 2% RBC's suspension was prepared in phosphate buffer. 1ml of sample was taken in a test tube from each dilution and then adds 1ml of the RBC's suspension to the sample. The tubes were incubated at 37°C for 30 minutes. The tubes were centrifuged and looked for agglutination after period of incubation. Rough granules and smooth button formation indicated positive and negative results, respectively. The intensity of positive result was determined by the extent of deposition.

Insecticidal activity

Test insects (*Chaetocnema pulicaria*, *Callosobruchus chinensis* and *Tribolium castaneum*) were provided by the NIFA (Nuclear Institute for Food and Agriculture), Peshawar, Pakistan. 200mg of the test samples were dissolved in 3ml of methanol for stock solution preparation.

Insects of uniform size and age were chosen for experimental work. The protocol of the contact toxicity assay was used for insecticidal activity (Ahn *et al.*, 1995). The filter papers were kept in the Petri plates and stock solutions of test samples were introduced. After

evaporation of methanol, 10 healthy insects of same size from each species were chosen and transferred to the labeled plates using a brush. Further, incubation of the plates was done for 24 hours at 27°C with 50% relative humidity in growth chamber. After incubation, results were noted by counting the number of survivals in each plate as following formula.

$$\text{Percent Mortality} = \frac{\text{No. of insects alive in test}}{\text{No. of insects alive in control}} \times 100$$

Permethrin (235.9 µg / cm²) served as positive control.

***In vitro* anti-glycation assay**

The method of Matsuura *et al.*, 2002 was followed for *in-vitro* anti-glycation assay. From the stock solution (3mg/1ml of alkaline PBS), 10, 50, 90 and 130µL solutions were mixed with a solution containing 200 mM glucose and 400µg Bovine Serum Albumin (BSA) and were kept in a water bath at 55°C for 48 hours. Glucose and BSA without any inhibitor was used as control. After incubation time, 10µL of 100% w/v Tri-Chloro Acetic Acid (TCA) was added to reaction mixture in separate Eppendorf tubes, centrifuged at 14500 rpm at 4°C for 4 minutes and pellet was re-dissolved in 400µL alkaline PBS. The degree of absorbance for both the control and the test reaction mixtures were taken at 350 nm using automated UV double beam spectrophotometer. Percent inhibition was calculated using the following formula:

$$\text{Percent inhibition} = ((A_s - A_o) / A_o) \times 100$$

Where A_s is absorbance of test samples, A_b is absorbance of reaction mixture without plant extract and A_o is absorbance of blank control.

DPPH antioxidant activity

The scavenging of free radical of the extracts were determined using 1,1-diphenyl-2-picryl hydroxyl (DPPH) as reported (Kanatt and Sharma, 2007). For stock solution 10 mg of extract was dissolved in 1ml of methanol and further diluted to five different concentrations (100-500 µg/ml). Same dilutions were also made for ascorbic acid standard. One ml of each concentration was mixed thoroughly with freshly prepared DPPH solution and incubated for 10 minutes in dark at room temperature. After that, absorbance of each sample was determined at 517nm wavelength for its antioxidant activity by the scavenging of free radical of DPPH. The scavenging capacity of the sample was compared to that of control (1ml methanol+2ml DPPH). The percent scavenging activity of the free radical of each sample was determined using the given equation.

$$\text{Percent (\%) inhibition of DPPH activity} = ((A_b - A_s) / A_b) \times 100$$

Where A_b ; represents the absorbance of the blank sample or control reaction and A_s ; the absorbance of the test sample. A curve of percent inhibition or percent

scavenging effect against samples concentrations was plotted and the on the concentration where the scavenging reaches to 50% is its EC₅₀.

Anti-termite activity

The procedure of Salihah *et al.*, 1993 was employed for determining the anti-termite activity against *Heterotermes indicola*. The blotting papers were dipped in the respective test sample (2 mg / ml of methanol), held for some time to remove the excess test sample and were then kept in the Petri plates. *H. indicola* (10 in number) were then transferred to each Petri plate after evaporation of methanol and observed with the help of magnifying glass after 24 hours, till all the termites were dead. All the experiments were performed three times and the average number of termites killed each day was noted.

RESULTS

Antibacterial activity

Antibiotic resistance is a great dilemma faced by the world today. To overcome this problem, innovative antimicrobials present naturally in plants will help to tackle it. The example of *S. aureus* reveals the fact that it has become resistant to several antibiotics; Tetracyclines, Tenuicillin G, Macrolides, Gentamicin and Lincosamides to which it was previously susceptible (Chatterjee and Chakroborti, 1980). The test samples (Cr. Met. Ext. and fractions) were tested against the selected bacterial species and results are presented in fig. 1. The results of the antibacterial activity in terms of percent zone of inhibition against *P. aeruginosa* were; Cr. Met. Ext (0), *n*-hexane (7.4), CHCl₃ (44.4), EtOAc (66.6) and aqueous fraction (62.9). Against *S. aureus*, the percent zone of inhibition was; Cr. Met. Ext (0), *n*-hexane (22.2), CHCl₃ (55.5), EtOAc (44.4) and aqueous fraction (0). The percent zone of inhibition against *M. morgani* by the test samples were; Cr. Met. Ext (46.4), *n*-hexane (71.4), CHCl₃ (60.7), EtOAc (39.2) and aqueous fraction (0). The percent zone of inhibition against *A. baumannii* by the test samples were; Cr. Met. Ext (0), *n*-hexane (55.1), CHCl₃ (72.4), EtOAc (41.3) and aqueous fraction (55.1). The results of the antibacterial activity in terms of percent zone of inhibition against *MRSA* were; Cr. Met. Ext (0), *n*-hexane (20), CHCl₃ (56), EtOAc (52) and aqueous fraction (0).

Antifungal activity

The results of antifungal activity of the test samples are summarized in fig. 2. Against *A. niger* all the fractions showed low activity; the Cr. Met. Ext (6%), *n*-hexane (6%), CHCl₃ (5%), EtOAc (4%), and aqueous fraction showed no activity against it. Against *T. harzianum*, the activities were in the order; Cr. Met. Ext (7%), *n*-hexane (7%), CHCl₃ (7%), EtOAc (3%) and aqueous fraction (6%). The Cr. Met. Ext, *n*-hexane, CHCl₃, EtOAc and aqueous fraction showed low activity in the range of 6-

8% against *A. parasiticus*. Low activity was recorded against *V. dahlia* by Crd. Met. Ext (6%), CHCl_3 (6%), EtOAc (5%) and aqueous fraction (18%). The *n*-hexane fraction was inactive against it. The Crd. Met. Ext and all fractions were inactive against *P. notatum* and *P. digitatum*.

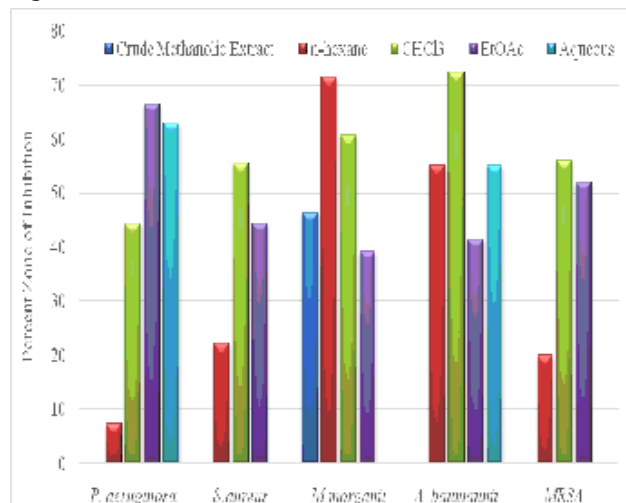


Fig 1. Antibacterial activity of Crd. Met. Ext. and fractions of *J. adhatoda*.

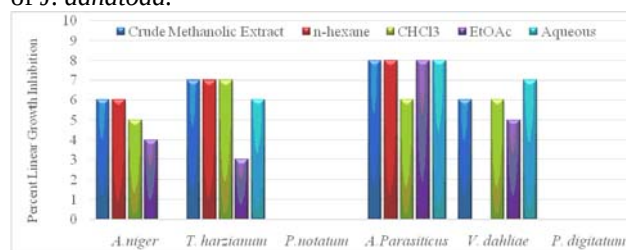


Fig. 2: Antifungal activity of the Crd. Met. Ext and fractions of *J. adhatoda*.

Phytotoxic activity

The results of phytotoxic activity of *J. adhatoda* are shown in fig. 3. The percent growth regulation of Crd. Met. Ext was 25 and 16.6 at 1000 and 100 $\mu\text{g/ml}$, respectively, while it was inactive at 10 $\mu\text{g/ml}$. The *n*-hexane fraction showed percent growth regulation of 16.6, 16.6 and 0% at 1000 and 100 and 10 $\mu\text{g/ml}$, respectively. For CHCl_3 fraction, percent growth regulation was 25, 8.33 and 0% at 1000, 100 and 10 $\mu\text{g/ml}$, respectively. The EtOAc fraction exhibited percent growth regulation of 8.33, 8.33 and 0% at 1000, 100 and 10 $\mu\text{g/ml}$, respectively. Finally, the aqueous fraction was inactive at all the test concentrations.

Brine shrimp cytotoxicity

In the brine shrimp cytotoxicity test, if higher the mortality of the test sample(s) the lower will be the safety of the drug and *vice versa*. Fig. 4 indicates the results of brine shrimp cytotoxicity. The results showed that Crd. Met. Ext. of *J. adhatoda* showed 13.33% cytotoxicity at 1000 $\mu\text{g/ml}$ while at lower concentration of 100 and 10

$\mu\text{g/ml}$, the cytotoxic effect were 3.33 and 0%, respectively. The *n*-hexane fraction exhibited 20, 10 and 0% cytotoxic effect at 1000, 100 and 10 $\mu\text{g/ml}$, respectively. The CHCl_3 fraction at higher concentration (1000 $\mu\text{g/ml}$) showed 10% cytotoxicity and low activity at low doses; 100 $\mu\text{g/ml}$ (6.66%) and 10 $\mu\text{g/ml}$ (3.33%). EtOAc fraction showed 23.33% cytotoxicity at higher concentration while low activity was observed at low doses; 100 $\mu\text{g/ml}$ (13.33%) and 10 $\mu\text{g/ml}$ (3.33%). The aqueous fraction of *J. adhatoda* showed 16.66, 6.66 and 0% cytotoxic activity at concentration 1000, 100 and 10 $\mu\text{g/ml}$, respectively.

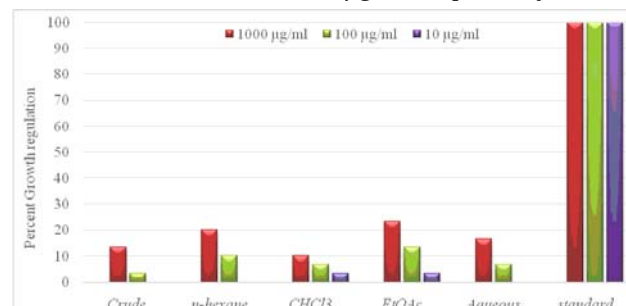


Fig 3: Phytotoxic activity of Crd. Met. Ext and fractions of *J. adhatoda*.

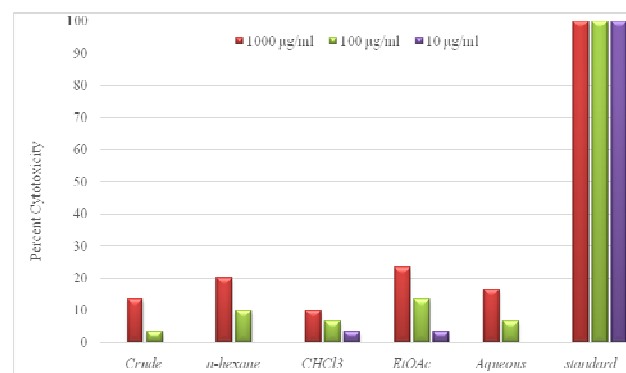


Fig. 4: Brine shrimps cytotoxicity of Crd. Met. Ext. and various fractions of *J. adhatoda*.

Haemagglutination activity

To study the sugar components on cancerous and normal cell surfaces as well as structural and functional roles of cell surface carbohydrates, lectin specificities has been used. It is already known that agglutinin from the plant sources is more fruitful over animal sources due to their easy availability and abundance (Lis and Sharon, 1986; Sharon and Lis, 1972).

Haemagglutination activity of the test samples was carried out against human RBC's of all blood groups and results were noted in Table 1. All of the test samples were inactive against RBC's of the blood groups at all concentration indicating that the selected plant lack phytolectins.

Insecticidal activity

The toxic effect of synthetic insecticides indicates that they are harmful both for humans and environment. To

overcome this toxicity, these harmful insecticides should be replaced by environment friendly insecticides obtained from the natural resources (Shala by *et al.*, 1998). The insecticidal activity of test samples was practiced against *C. pulicaria*, *C. chinensis* and *T. castaneum*. Fig.5 indicates the results. It was observed that CHCl_3 fraction showed low activity of 20% against *C. pulicaria* while Crd. Met. Ext and other fractions were inactive against it. Low activity of 20 and 40% has been shown by *n*-hexane and CHCl_3 fractions against *C. chinensis* and other fractions were inactive against it. Against *T. castaneum*, Crd. Met. Ext, CHCl_3 and EtOAc fractions showed 20, 20 and 40 % activity while *n*-hexane and aqueous fractions were inactive against it.

Table 1: Haemagglutination activity of Crd. Met. Ext and fractions of *J. adhatoda*

Blood group	A+ive, A-ive, B+ive, B-ive, O+ive, O-ive, AB+ive, AB-ive,			
Dilutions	1:2	1:4	1:8	1:16
Crd. Met. Ext	-	-	-	-
<i>n</i> -hexane	-	-	-	-
CHCl_3	-	-	-	-
EtOAc	-	-	-	-
Aqueous	-	-	-	-

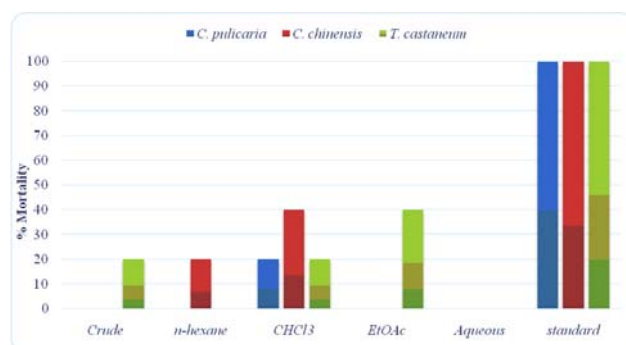


Fig. 5: Insecticidal activity of Crd. Met. Ext and fractions of *J. adhatoda*.

In vitro anti-glycation assay

The UV double beam spectrophotometric analyses of both the reaction and test mixtures for *J. adhatoda* is shown in Fig. 6. the absorbance value of plant extract at various concentration were: 0.08, 0.067, 0.053 and 0.04 at 10, 50, 90 and 130 μ l respectively. The absorbance values of Aminoguanidine at 10, 50, 90 and 130 μ l were 0.04, 0.035, 0.03 and 0.02 respectively.

DISCUSSION

Medicinal plants are getting attention once again because of the high cost of allopathic medications and recent antimicrobial resistance of pathogens to the available antibiotics.

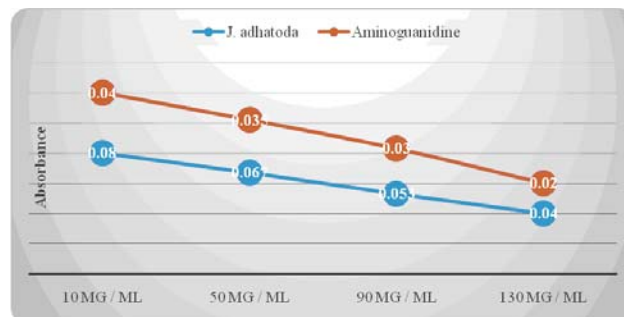


Fig. 6: Antiglycation activity of Crd. Met. Ext of *J. adhatoda*

DPPH antioxidant activity

The *n*-hexane, CHCl_3 , EtOAc and aqueous fractions of *J. adhatoda* were screened for their scavenging activity of DPPH free radical at different concentrations (100, 200, 300, 400 and 500 μ g/ml). The activity in percent of test samples and control (vitamin C standard) are presented in fig. 7. These scavenging activities were proportional to the concentration of the fractions, as the concentration of these increased, the percent scavenging activity also increased, when the scavenging reached to 50% was its EC_{50} value. This EC_{50} value inversely related to percent scavenging. The sample with lower EC_{50} value showed higher antioxidant activity (Gheld of and Engeseth, 2002).

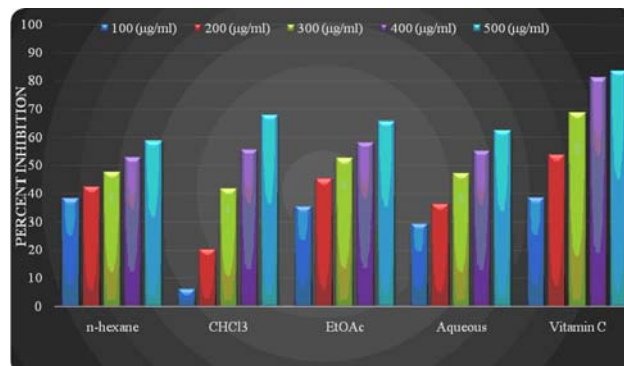


Fig. 7: DPPH radical scavenging activity of different fractions of *J. adhatoda*

Anti-termite activity

Termite has an important property of recycling woody and other plant materials. Soil aeration is the result of the tunneling efforts of termites. Tropical and subtropical regions are the common and suitable habitat for termites. On the other hand they also cause a great economic damage while destroying wooden products and infrastructure (Suszkiw, 1998).

The anti-termite activity of Crd. Met. Ext, *n*-hexane, CHCl_3 , EtOAc and aqueous fractions of *J. adhatoda* were carried out against the *H.indicola*. Table 2 illustrates the results. Low activity of Crd. Met. Ext was observed against *H. indicola*. The duration of experiment was extended for two days. On day 1, 9 termites were found dead on an average while on the second day no termite

remained alive. The *n*-hexane fraction took two days to kill all the termites. on the 1st day, 7 termites were dead and on 2nd day all were dead. The CHCl₃ and EtOAc fractions killed all the termites in 24 hours. For the aqueous fraction the experiment extended for three days. It was noted that on average 6 termites were killed on day 1. On the second day, 9 termites were found dead on an average and on the third day, no termite survived. All the experiments were conducted in triplicate to ensure the results.

Table 2: Anti-termite activity of the Crd. Met. Ext and fractions of *J. adhatoda*

Sample	No. of termites	Day	Average Termites killed
Crd. Met. Ext	10	1	9
		2	10
<i>n</i> -hexane		1	7
		2	10
CHCl ₃		1	10
EtOAc		1	10
Aqueous		1	6
		2	9
		3	10

A lot of studies have been conducted in the context of the quest for antibacterial activities. Recent study of Ezequiel *et al.*, 2015 reported some good antibacterial activity against *S. aureus* and *Enterococcus faecalis* (Ezequiel *et al.*, 2015). The CHCl₃ fraction in our study showed moderate activity against *S. aureus* while good activity against *A. baumannii* indicating that selected plant could be used for active compound isolation against these pathogens. The antifungal activity of our selected plant was low which is in accordance of many of the previously published work (Ahmad *et al.*, 2010). A recent publication showed significant phytotoxic activity of *Fagonia cretica*, *Peganum harmala*, *Tribulus terrestris*, *Chrozophora tinctoria* and *Ricinus communis* against *L. minor* L (Dastagir and Hussain, 2013) which is in contrast of results of the present study. In our activity, the Crd. Met. Ext and various fractions of *J. adhatoda* showed low activity against *L. minor* L. Alluri *et al.*, 2005 has reported an extensive screening of medicinal plants for Brine shrimp cytotoxicity in which *Aristolochia indica*, *Boswellia serrata*, *Ginkgo biloba*, *Garcinia cambogia* and *Semecarpus anacardium* have showed significant cytotoxicity (Alluri *et al.*, 2005). In our study, the cytotoxicity of the selected plant, was low, at all test concentration. No haemagglutination activity was observed in the Crd. Met. Ext and various fraction of *J. adhatoda*, which is in line with the previously published work (Ahmad *et al.*, 2010). Our results showed good, concentration dependent, antioxidant activity in comparison with Vitamin C which is supported by the previously published work that medicinal plants could be

a good source of antioxidant compounds (Pourmorad *et al.*, 2006). Accumulation of advanced glycation end products (AGE's) in different parts of the body like heart, muscles and large blood vessels, results in the promotion and progression of diabetic complication like nephropathy, neuropathy, cardiovascular disease and atherosclerosis (Sugimoto *et al.*, 2008). Therefore agents that have antiglycation abilities (stop the formation of AGE's) could be used to prevent these complications. The results of the current study showed that *J. adhatoda* could be a good source for antiglycation agents.

CONCLUSION

The *n*-hexane fraction showed good (71.4%) antibacterial activity against *M. morganii* while the Cr. Met. Ext showed moderate (46.4%) antibacterial activity against it. The CHCl₃ fraction showed good activity against *A. baumannii* while EtOAc and aqueous fractions, in most of the cases showed low to no activity against the selected pathogens. Against *A. niger*, *T. harzianum*, *A. parasiticus* and *V. dahliae*, the Cr. Met. Ext and various fractions showed low activity. The percent growth regulation, in case of phytotoxic activity, by Cr. Met. Ext and various fractions at 1000, 100 and 10µg/ml, was low against *L. minor*. All of the test samples were inactive against RBC's of the blood groups at all concentration indicating that the selected plant lack phytolectins. The insecticidal activity of test samples was low against the test insects showing lack of anti-insecticidal components in the selected plant. The in-vitro antiglycation activity of test samples showed concentrated dependent activity and was comparable with the standard (Aminoguanidine). The DPPH free radical scavenging activities were proportional to the concentration of the fractions, as the concentration of these increased, the percent scavenging activity also increased. The anti-termite and brine shrimp cytotoxic activity of test samples was low against the test organisms.

REFERENCES

- Ahmad B, Ali N, Bashir S, Choudhary MI, Azam A and Khan I (2009). Parasitocidal, antifungal and antibacterial activities of *Onosma griffithii* Vatke. *Afr. J. Biotechnol.*, **8**(19): 5084-5087.
- Ahn YJ, Kim GH and Cho KY (1995). Bioassay system for insecticidal compounds. In: Proceedings of the third symposium on the biochemical methodology for the research and development of the bioactive substances, held at Seoul, Republic of Korea, pp.495-506.
- Ahmad B, Sadia, Azam S, Bashir S and Khan I (2010). Biological screening of the aerial parts of the *Sarcococca saligna*. *J. Med. Plants Res.*, **4**(22): 2404-2410.
- Ahmad B, Azam S, Bashir S, Khan I, Achyut A and Choudhary MI (2010). Anti-inflammatory and enzyme

- inhibitory activities of a crude extract and a pterocarpan isolated from the aerial parts of *Vitex agnus-castus*. *J. Biotechnol.*, **5**: 1207-1215.
- Atta-Ur-Rahman, Said HM, Ahmad VU (1986). Pakistan Encyclopaedia Planta Medica. Hamdard Foundation Press, Karachi, **1**: 181-187.
- Alluri VK, Tayi VNR, Dodda S, Mulabagal V, Hsin-Sheng T, Gottumukkala VS (2005). Assessment of Bioactivity of Indian Medicinal Plants Using Brine Shrimp (*Artemia salina*) Lethality Assay. *IJASER*, **3**(2): 125-134.
- Chatterjee S, Chakroborti SK (1980). Antimicrobial activities of some antineoplastic and other withanolides. *Antonie Van Leeuwenhoc*. **46**(1): 59-63.
- Dymock W, Waeden CJH and Hooper D (1890). *Pharmacographia Indica*, A history of the principal drugs of vegetable origin. Paul, Trech, Trubner and Co. Ltd, London. pp.50-54.
- Dastagir G and Hussain F (2013). Phytotoxic and insecticidal activity of family Zygophyllaceae and Euphorbiaceae. *Sarhad J. Agric.*, **29**(1): 83-91.
- Ezequiel VV, Azucena OC, Catalina RM, María JVS and Pilar CR (2015). Biological activities of *Morus celtidifolia* leaf extracts. *Pak. J. Pharm. Sci.*, **28** (4): 1177-1180.
- Gheldof NF, Engeseth NJ (2002). Antioxidant capacity of honeys from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of *in vitro* lipoprotein oxidation in human serum samples. *J. Agric. Food Chem.*, **50** (3): 3050-3055.
- Haider A, Junaid S, Hassan S and Abdul R (2011). Ethnobotanical profile of some plant resources in Malam Jabba valley of Swat, Pakistan. *J. Med. Plants Res.*, **5** (18): 4676-4687.
- Kamal AM, Abdul Ghafoor (1978). *Justicia adhatoda*, Flora of Pakistan, p.39.
- Kirtikar KR, Basu BD (1975). Indian Medicinal plants (second Ed.) Bishen Singh Mahendra Pal Singh, Delhi, India. **3**: 1899-1902.
- Khan I, Ahmad H, Ali N, Ahmad B and Tanoli H (2013). Screening of *Bunium bulbocastanum* for antibacterial, antifungal, phytotoxic and haemagglutination activities. *Pak. J. Pharm. Sci.*, **26**(4): 787-791.
- Kanatt SR and Sharma A (2007). Antioxidant potential of mint (*Mentha spicata* L.) in radiation-processed lamb meat. *Food Chem.*, **100**(2): 451-458.
- Lis H, Sharon NP (1986). The lectins: Properties, functions and applications. Liener IE, Sharon N, Goldstein IJ (eds). Academic Press, New York, pp.294-327.
- Matsuura N, Aradate T, Sasaki C, Kojima H, Ohara M, Hasegawa J and Ubukata M (2002). Screening system for the maillard reaction inhibitor from natural product extracts. *J. Health Sci.*, **48** (6): 520-526.
- Pourmorad F, Hosseinimehr SJ and Shahabimajd N (2006). Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. *Afr. J. Biotechnol.*, **5** (11): 1142-1145.
- Roberts E (1931). Vegetable materia medica of India and Ceylon. Plate Limited, Colombo. pp.16-17.
- Sharma MP, Ahmad J, Hussain A and Khan S (1992). Folklore medicinal plants of Mewat (Gurgaon district), Haryana, India. *IJP*, **30**: 129-134.
- Salihah Z, Khatoon R, Khan A, Alamzeb and Sattar AA (1993). Termite trap, NIFA, TERMAP, for capturing large number of field population of *Heterotermes indicola*. Proc. Pak. Congr. Zool., **13**: 395-400.
- Sharon N and Lis H (1972). Lectins: Cell agglutinating and sugar-specific proteins. *Science*, **177**: 949-959.
- Shalaby AA, Allam KAM, Mostafa AA and Fahmy SME (1998). Insecticidal properties of citrus oil against *Culex pipens* and *Musca domestica*. *J. Egypt Soc. Parasitol.*, **28**: 595-606.
- Suszkiew J (1998). The formosan termite: A formidable foe. *Agric. Res.*, **46**: 4-9.
- Sugimoto, K. M. Yasujima and S Yagihashi (2008). Role of advanced glycation end products in diabetic neuropathy. *Curr. Pharm. Des.*, **14**: 953-961.
- The Holy Quran, Surah Nahl, Ayat Tapsell LC, Hemphill I, Cobiack L, Patch CS, Sullivan DR, Fenech M, Roodenrys S, Keogh JB, Clifton PM, Williams PG, Fazio VA and Inge KE (2006). Health benefits of herbs and spices, the past, the present, the future. *Med. J. Aust.*, **21**(185): S4-S24.
- WHO (1990). The use of traditional medicine in primary health care. A manual for health workers in South-East Asia, SEARO Regional Health Papers, No. 19. New Delhi, India, pp.1-2.