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Phytochemical and Pharmacological Evaluation of *Ziziphus mauritiana, Allium sativum* and *Brassica nigra*

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Abstract

Unrefined methanolic extracts of Ziziphus mauritiana (Ber) leaves, bulbs of Allium sativum (Garlic) and seeds of Brassica nigra (Black Mustard/Sarso) generally utilized in medication were studied for their in vitro phytochemical and pharmacological screening. Phytochemical screening of Z. mauritiana showed the presence of flavonoids, flavones, phenolic compounds, saponins, terpenoids and nonattendance of tannins, steroids, and glycosides. Similarly, unrefined methanolic extract of A. sativum bulbs showed the presence of flavonoids, phenols, tannins, saponins, terpenoids and steroids. Also, B. nigra seeds showed the indicated the presence of flavonoids, phenols, tannins, terpenoids and glycosides. The antibacterial activity of plant extracts was determined by utilizing agar well diffusion technique. The plant extracts demonstrated varied levels of antibacterial activity. In this study five bacterial isolates were utilized (Escherichia coli, Staphylococcus aureus, Pesudomonas aerugenosa, Klebseila pneunomonae, and Proteus). ZM leaves and AS bulbs extracts showed good antibacterial action against P. aeruginosa and Proteus (50% and 60%), while BN seeds showed low inhibition against P. aeruginosa (28%) but showed great restraint effect against E. coli and Proteus (50%and 60%). The antifungal activity was determined by agar slant technique in contradiction of R. stolonifer, A. niger and A. flavus. AS bulbs extract showed good (14%) antifungal activity when contrasted with ZM leaves (14%) and BN seeds (13%) against R. stolonifer. The extract exhibited low activity against A. niger and A. flavus similarly methanolic extract showed low antifungal activity (10% and 11%). Phytotoxic activities show that these plants caused substantial growth inhibition of Lemna minor in all the dilutions.

Keywords: *Ziziphus mauritiana, Allium sativum, Brassica nigra*, phytochemical screening, antibacterial, antifungal, phytotoxic activities.

Introduction

In recent years, resistance of drugs to disease causing microorganisms is being commonly described from all over the

globe. Nonetheless, the situation is alarming in developing as well as developed countries due to unpredictable utilization of anti-microbial (antibiotics). Plant materials are cheap, with respect to treatment and therapeutic activity of illnesses, plants materials altogether add to the improvement of human welfare **(Adebooye and Opabode, 2004)**. Plants have numerous significant nutritious and medicinal properties **(Balunas and Kinghorn, 2005)**.

The plants contain the supplements that are required for the solid living of individuals **(Adedapo *et al.*, 2008)**. They are a part of life. It's impractical for anybody to live without them. The greater part of the people groups have confidence in unani medicines when contrasted with allopathic medicines since they don't show any side effects. Exactly when a plant has antimicrobial properties it is called therapeutic or medicinal plants. As indicated by world health organization plant contain plant contain these materials are fundamentally and optionally metabolites **(Okoko and Oruambo, 2008)**.

Since long time plants have been serving humanity as a valuable medication, foodstuffs, seasonings mediators, colorants, binders and balms **(Falodun *et al.*, 2006)**. Therapeutic plants are sources of significant medications utilized in the treatment of disorders either alone or in blend with different plants **(Ohelete, 2016)**. Biochemical constituents found in plants includes flavonoids, flavones, tannins, saponins alkaloids, steroids, terpenoids, glycosides, fundamental oils and proteins.

Different categories of dynamic compounds which are utilized in treatment of different disorders and as pain relievers are contained by medicinal plants **(Newman *et al.*, 2000; Okigbo *et al.*, 2008)**. The pharmacological information of plants is helpful in the conservation of biodiversity, customary qualities, for community health care and medication advancement **(Ajaib *et al.*, 2010)**. Many plant derivatives for example, organic product preparations, vegetable preparations, spices or their extracts have been utilized for quite a long time for the preservation and maintenance of time span of foods. The properties of restorative plants depend on the antioxidant and antimicrobial impacts of the plants phytochemicals, which are available in plants **(Ameh *et al.*, 2013)**.

Ziziphus mauritiana have a place with family *Rhamnaceae* and genus *Ziziphus*, usually known as Chinese dates or Chinese jujube, or Ber in Urdu. This plant is a dynamic grower, and it has a swiftly emerging taproot. Widely cultivated in dry region throughout the tropics and it is subtropical or tropical fruiting tree and widely distributed in many Asian countries. It is one of the plants that have various activities against different diseases. Each part of plants has various metabolites, but their quantity can differ. The pharmacological activities depend on the metabolites present in this plant **(Parmar *et al.*, 2012).**

Allium sativum commonly known as garlic, belong to family *Amaryllidaceae* and the genus *Allium*. *A. sativum* is the most widely used spice vegetable after *A. cepa* (Onion). The plant is high up to 50cm. The bulb or head of the *A. sativum* is compound or sub globular bulb of 3-5cm wide. The leaves that are emerges from the stem are not fleshy but slender and fragile, when dry. The *A. sativum* is cultivated mostly in temperate areas of the world and found only when cultivated. The plant cultivates well when the soil is loamy and rich in organic matter and humidity and 17^0C is the temperature range. When the *A. sativum* is

segmented the typical strong odour is released **(Cardelle *et al.*, 2010)**. *A. sativum* is cultured all through the world and in Central Asia, the Near East, the Mediterranean area, China, Egypt, and Southern Europe **(Singh *et al.*, 2012)**.

Brassica nigra generally known as black mustard has a place with *Cruciferae*/*Brassicaceae* family **(Chaturvedi *et al.*, 2018)** and the genus *Brassica*. The *Cruciferae* or *Brassicaceae* is important family of blooming plants and generally known as cabbage or mustard family. *B. nigra* cultured for its dark brown seeds and usually use as spices. It has stalked leaves and covered with twines or hairs at the base. It can reach up to 80-90cm or up to 2.4m in fertile soil **(Chua *et al.*, 2012)**. The *B. nigra* is mostly found in temperate regions of Europe, tropical regions of North America and some parts of the Asia, libiya, Ethopia and Egypt. In Asia it is found in Amenia, Cyprus, China India, Iran, Kazakhstan and Afghanistan **(Morales *et al.*, 2012)**.

Our aim is to screen the plant extracts for existence of a number of active compounds i.e. phytochemicals and analyze the crude methanolic extracts of selected plants for *in vitro* pharmacological activities.

Materials and Methods

Collection of Plants Materials

All phytochemical and pharmacological analysis was done at the Central LAB of Microbiology, Women University Mardan. This research work was carried out from March 2019 to June 2019.

Extraction: *Ziziphus mauritiana* leaves were collected from Mardan. The leaves are washed with clean and sterile distilled water and then shade drieded at 25±30^{0}C for seven days. The dried materials were chopped by electric grinder in to powder form, weighted 25g. The fresh bulbs of *A. sativum* were from our own farm, were desegmented. Then the peeled *A. sativum* was washed with distilled water to remove dust particles. Then crushed by sterile pestle and mortar, weighted 290g. *Brassica nigra* seeds were obtained from market and grinded by electric grinder into fine powdered form, weighted 150g.

Table 1. Parts of Plants that are used of Phytochemical and Pharmacological Screening

S.No.	Botanical name	Family	Parts used	Common Names
1	*Ziziphus mauritiana*	*Rhamnaceae/ Buckthorns*	Leaves	Chinese dates or Chinese jujube in English, or Ber in Urdu, or Bera in Pashtu.
2	*Allium sativum*	*Amaryllidaceae/ Liliaceae*	Bulbs	Garlic in English, Lehsan in Urdu, or ooga in Pashtu.
3	*Brassica nigra*	*Brassicaceae*/*Cruciferae*	Seeds	Black mustard in English, or Sarso in Urdu or Sharshum in Pashtu.

Coding: The codes given to plant samples according to their botanical name shortened forms. ZM for *Z. mauritiana* leaves, AS for *A. sativum* bulbs and BN for *B. nigra* seeds.

Phytochemicals Screening

The plants materials were identified and then the extracts were filtered and used for primary phytochemicals analysis **(Parmar *et al.*, 2012)**.

Flavonoids: For the detection of flavonoids 1ml of NaOH (dilute) was added to each extract. A profound yellow colour was formed, by the addition of few droplets of NaOH (dilute) it transforms into colourless form. This shows the presence of flavonoids **(Thomas *et al.*, 2008)**.

Flavones: To each extract 4ml of 1% aluminium chloride (in methanol) was added and yellow colour appears which represents the presence of flavones.

Phenols: To equal volume of each extract equal volume of ferric chloride was added, bluish green colour appears which represents the presence of phenols **(Gibbs, 1974; Harrison & Durance, 1989)**.

Saponins: To each extract 20ml of water (distil) was added and shake for 15 minutes, the formation of foam of 1.5cm layer demonstrates the presence of saponins **(Kokate *et al.*, 2001)**. It has antifungal properties.

Tannins: 1% lead acetate was added to each concentrate at that point if tannins were present yellow colour was created which indicating the existence of tannins **(Treare and Evans, 1985; Thite *et al.*, 2013)**.

Terpenoids: Take 2ml of chloroform in test tube and add the crude extract, then concentrated H2SO4 of 2ml was added and boil it for 2 minutes. A hazy colouration affirms the presence of terpenoids **(Savithramma *et al.*, 2011)**.

Steroids: Every extract was added in chloroform (10ml) and in equivalent amount of H_2SO_4 (conc). Yellow green light colour shows the presence of steroids **(Savithramma *et al.*, 2011)**.

Glycosides: In a test tube each extract was added with HCl, 1ml pyridine and sodium nitroprusside solutions. Occurrence of red colour affirms the presence of glycosides **(Vetter, 2000)**.

Pharmacological Screening

Pharmacological activities include antibacterial, antifungal, and phytotoxic activity. Due to evolution of new pathogens resistant to present day antibiotics various plants materials are utilized for the cure of injuries and disorders as icepacks or domestic therapies. Infectious disorders approximately cause half of the deaths particularly in the tropics of the globe **(Muhammad *et al.*, 2008; Nisar *et al.*, 2008)**.

Antibacterial Activity

***Test bacteria*:** *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsella pneumonaeae*, *Escherichia coli* and *Proteus* were used that were available at Central LAB of Women University Mardan, province KPK Pakistan.

The methanolic extracts of the particular plants were screened for potential antibacterial activity in logical inconsistency of the above said pathogenic

strains. The experimentation work was done according to agar wells technique **(Ahmad *et al.*, 2009; Bashir *et al.*, 2010)**. For this the LB agar medium was prepared and poured into sterile petri plates. Then the plates were incubated for 24 hours at 37^0C. The test cultures (72 hours old) from LB broth were transported and consistently spread over the media on the plates in the laminar air flow. Afterwards, 6mm wells were made with sterile blue tips after presenting the test culture into the plates. Tests samples at concentration of 3mg/ml were used to examine stock solutions. From the stock solutions 40µl was transferred into the wells by using micropipette gesture and vancomycin was used as a control drug. Finally, the zones of inhibitions were noted after 24 hours at temperature range of 37^0C. On the following day percent zone of inhibition was measured.

$$\text{Zone of inhibition} = \frac{\text{Zone of inhibition of sample}}{\text{Zone of inhibition of standard}} \times 100$$

Antifungal Activity

***Fungal strains*:** *Rhizohpus stolonifer*, *Aspergillus niger* and *Aspergillus flavus.* These were available at Central LAB of Women University Mardan, province KPK Pakistan.

The crude extracts were verified possible antifungal activity. The antifungal activity was performed by agar slants technique **(Braga *et al.*, 2005)**. Stock solutions of crude methanolic extracts (24mg/ml) of the test samples were taken. Saboured Dextrose Agar (SDA) was used for the growth of fungal strains. From the stock solutions of samples were transported to autoclaved test tubes.

Then the test tubes were allowed to cool in oblique position. Each fungal species fresh culture was inoculated into the crew. Then the screw tubes incubated for 72hrs at 37^0C. After 72hrs the percent inhibition was measured.

$$\%\ \text{inhibition of fungal growth} = \frac{100 - \text{linear growth in test sample (mm)}}{\text{linear growth in control (mm)}} \times 100$$

Phytotoxic Activity

***Materials required*:** Crude methanolic extracts were checked for possible phytotoxicity against the common plant, the duck-weed (*L.* minor). Test samples 20mg/ml, flasks, the *L. minor* plant leaflets and micropipettes were required in the experimental work.

The plants extracts were analyzed for possible phytotoxic activity **(Masoko and Eloff, 2005)**. The Stock solutions of 20mg/ml of samples were set and prepared in methanol for the inhibition of the growth of *L. minor* fronds. Samples at different concentrations of 10, 100, 1000 µg/ml from the stock solutions were poured into the respective flasks and were left at room temperature for about 7 days to prove that how many fronds were dead in the experimental work. Eighteen healthy *L. minor* plant with random i.e. two or three fronds were selected and put in the typical flasks. The flasks were kept at room temperature for seven days. After seven days of incubation the results were noted.

Results and Discussion

Phytochemicals Screening

The selected plants *Z. mauritiana*, *A. sativum* and *B. nigra* tested for existence of various phytochemicals. The plants contain various groups of chemical compounds such as flavonoids, flavones, phenols, tannins, saponins, steroids, terpenoids and glycosides. These compounds are directly

connected with the medicinal standards of specific plant. After sequestration and preparation, in different pharmaceutical drugs forms, these natural products are as prescription for cure of different disorders.

Phytochemical Screening of *Ziziphus Mauritiana* Leaves

The leaves of *Z. mauritiana* show the existence of flavonoids, flavones, phenols, saponins and terpenoids. The occurrence of deep yellow color observed for flavonoid confirms that the plant contains the flavonoid. Saponin form 1.5cm layer of foam. The negative result for tannins, steroids and glycosides shows the absence of these compounds in *Z. mauritiana* leaves.

Phytochemical Screening of *Allium sativum*

The bulb of *A. sativum* shows the presence of flavonoids, flavones, phenols, saponins, terpenoids and steroids. The extract shows moderate presence of tannins and more presence of flavonoids. In *A. sativum* extracts saponin form 1cm layer of foam. The negative result for glycoside shows the absence of glycoside.

Phytochemical Screening of *Brassica nigra*

The seeds of *B.* nigra show the existence of saponins flavonoids, phenols, glycosides and terpenoids. The BN seeds show more presence of terpenoids and absence of flavones, tannins and steroids.

Pharmacological Screening

Worldwide antimicrobial resistance has become a global issue. Approaches to improve the existing situations include exploration in finding new and advanced antimicrobial medications **(Leticia *et al.*, 2005)**. Antimicrobial drugs have significances in directing many infectious diseases control and depend on wise use of these antimicrobial drugs to diminish the prevalence of resistance of these microbial agents **(Danso *et al.*, 2002).**

Table 2. Phytochemical Screening of ZM Leaves, AS Bulbs and BN Seeds

S. #.	Test	ZM leaves	AS bulbs	BN seeds
1	Flavonoid	+++	+++	+
2	Flavone	++	_	_
3	Phenol	++	+	+
4	Tannin	_	++	+
5	Saponin	+++	+	_
6	Terpenoid	++	+	+++
7	Steroid	_	+	_
8	Glycoside	_	_	+

Key: _ = Absence, + = Presence, ++ = Moderate nearness, +++ = More nearness

Antibacterial Activity

Crude methanolic extracts of selected plants screened against the mentioned pathogenic strains for possible antibacterial potential.

Antibacterial Activity of *Ziziphus mauritiana* Leaves

Order of the antibacterial activity of the crude methanolic extract of the *Z. mauritiana* leaves: Against *Proteus* was 60%, *P. aeruginosa* 50%, *E. coli* 34%, *K. pneumonaeae* 26% and against *S. aureus* was 24%.

Antibacterial Activity of *Allium sativum* Bulbs

Order of the antibacterial activity of crude methanolic extract of the *A. sativum* bulbs: Against *Proteus* and *P. aeruginosa* was 50%, *E. coli* was 30% and against *S.aureus* and *K. pneumonaeae* was 24% respectively.

Antibacterial Activity of *Brassica nigra* Seeds

Order of the antibacterial activity of the crude methanolic extract of the *Brassica nigra* seeds: Against *Proteus* was 60%, *P. aeruginosa* 50%, *E. coli* 28% and against *S. aureus* and *K. pneumonaeae* was 24%.

Antifungal Activity

For possible antifungal potential the selected plants extracts were screen against *Rhizophus stolonifer*, *Aspergillus niger*, and *Aspergillus flavus.* The results were noted and compared with activity of control, isoconazole. The crude methanolic extract of all the three selected plants: *Z. mauritiana* leaves, *A. sativum* bulbs, and *B. nigra* seeds shows low antifungal activity.

Antifungal Activity of *Ziziphus mauritiana* Leaves

Z. mauritiana leaves crude extract shows low inhibitory effect as compared to control (100%). Against *R. stolonifer* the leaves crude extract shows 14%, against *A. niger* 10% and *A. flavus* 11% respectively.

Antifungal Activity of *Allium sativum* Bulbs

A. sativum crude extract also shows low antifungal activity against the selected fungal species. Against *R. stolonifer* 15%, *A. niger* 11% and *A. flavus* 13% inhibition.

Antifungal Activity of *Brassica nigra* Seeds

Crude extract of *B. nigra* seeds also shows the low antifungal activity against the selected fungal species as compared to crude extracts of *Z. mauritiana* leaves and *A. sativum* bulbs. Against *R. stolonifer* it shows 13% inhibition, *A. niger* 10% and *A. flavus* 11% inhibition.

Table 3. Antibacterial Activity (zone of inhibition in mm and percent inhibition) of Crude Extracts of ZM Leaves, AS Bulbs and BN Seeds

S.#.	Name of Bacteria	Zone of inhibition of standard Vancomycin (mm)	Crude methanolic Extract of ZM leaves		Crude methanolic Extract of AS bulbs		Crude methanolic Extract of BN seeds	
			Zone of Inhibition in (mm)	Inhibition in (%)	Zone of Inhibition in (mm)	Inhibition in (%)	Zone of Inhibition in (mm)	Inhibition in (%)
1	*P. aeruginosa*	25	12.5	50	12.5	50	12.5	50
2	*K.pneumonaeae*	25	6.5	26	6	24	6	24
3	*S. aureus*	25	6	24	6	24	6	24
4	*E. coli*	25	8.5	34	7.5	30	7	28
5	*Proteus*	25	15	60	12.5	50	15	60

Table 4. Antifungal Activity of the Crude Extract of ZM Leaves, AS Bulbs and BN Seeds

S.No.	Name of fungi	Percent Inhibition (%)			
		Control (%)	Crude Extract of ZM leaves (%)	Crude Extract of AS bulbs (%)	Crude Extract of BN seeds (%)
1	*Rhizophus stolonifer*	100	14	15	13
2	*Aspergillus niger*	100	10	11	10
3	*Aspergillus flavus*	100	11	13	11

Isoconazole was used as a control.

Phytotoxic Activity

The phytotoxic activity is the activity of bioactive compounds of plants/plants materials that retard the growth of useless or harmful herbs. *L. minor*, the common duckweed which is sensitive to active compounds (bioactive) and used for detection of phytotoxic compounds. The phytotoxic compounds are possible plants growth stimulants. All the test samples were screen for the possible phytotoxic action against the *L. minor*. The phytotoxic results of the crude methanolic extracts of all the three selected plants given below. Paraquat was utilized as standard for the inhibition of the growth.

Table 5(a). Phytotoxic activity of crude extract of ZM leaves, AS bulbs and BN seeds

Name of plant	Concentration of sample µg/ml	No. of Fronds				Concentration of standard drugs µg/ml
		Control	Crude extract of ZM leaves	Crude extract of AS bulbs	Crude extract of BN seeds	
L. minor	1000	18	15	18	17	0.015
	100	18	09	15	15	
	10	18	08	10	14	

Table 5 (b). Percent Growth Regulation of the *Lemna minor*

Concentration of sample µg/ml	Percent growth regulation (%)			
	Standard	Crude methanolic extract of ZM leaves	Crude methanolic extract of AS bulbs	Crude methanolic extract of BN seeds
1000	100	83	100	94
100	100	50	83	83
10	100	44	55	77

Paraquat: as standard drug

Conclusion

From the experimental work, it is determined that the phytochemical screening of crude methanolic extract of *Z. mauritiana* leaves revealed the presence of flavonoids, flavones, phenols, terpenoids and saponins. The crude methanolic extract also exhibited good antibacterial and moderate antifungal activities and showed moderate phytotoxic activity against the *L. minor* during this experimental work.

The qualitative screening of *A. sativum* bulbs extract has shown the existence of phenols, tannins, saponins, steroids and terpenoids. The crude methanolic extract also showed good antibacterial and antifungal activities along with considerable phytotoxic activity.

And the extract of *B. nigra* seeds shows the presence of phenols, flavonoids, terpenoids and glycosides. These contents are shown different types of activities against different pathogens. The crude extract also exhibits the moderate antibacterial activity, very low antifungal activity and good phytotoxic activity.

The antibacterial and antifungal activities of these three selected plants parts revealed that these plants have antibacterial and antifungal potentials and in future these plants extract could be utilized in making drugs or medications. And to characterize and isolate the phytochemicals that shows the antimicrobial properties. The phytotoxic activities of these plants revealed that they also have significant allopathic potential. Our present work opens an innovative frame for researchers to work further on these plant species and to systemize these extracts and seize stock shares from the world global market.

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