Research Article

Antibacterial, Insecticidal, Antifungal and Phytochemical Screening of *Alium sativum*, *Nigela sativa* and *Plantago ovata*

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Abstract | For the treatment and management of infectious pathogens, many antimicrobial and antifungal substances were previously found from synthetic and natural sources. Numerous investigations have demonstrated that the compounds coumarins, flavonoids, phenolics, alkaloids, terpenoids, tannins, essential oils, lectin, polypeptides, and polyacetylenes are present in medicinal plants. These bioactive chemicals serve as a foundation for the creation of antibiotics that are utilized to cure infectious illnesses. *Allium sativum* is being used traditionally since ages for many purposes. It is extensively used in numerous dishes throughout the world and due to its aromatic nature, as a fragrance and *Plantago ovata* is traditionally used for many therapeutic purposes; laxative anti-acidic stabliser, stomach soothing, diuretic; and it is also anticancerous. *Nigela sativa* is used for jaundice, paralysis, metabolic syndrome, insulin resistant syndrome, and high level of cholesterol, nerve tension problems; it stops hair fall and boost's immune system. Based on the results of this study, it can be concluded that almost all the plant extracts showed potential antibacterial, insecticidal and antifungal activities against different. The research has focused on the potential therapeutic efficacy of AliumSativum, NigelaSativa, and Plantago Ovata in the treatment of microbial infections due to their significant antibacterial activity. Additionally, Different fractions of the *A. sativum, P. ovata, N. sativa* were prepared anad analyzed for antibacterial, insecticidal and antifungal activities. Based on the results of this study, it can be concluded that almost all the plant extracts showed potential antibacterial, insecticidal and antifungal activities against different microbes.

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Introduction

Ever since the dawn of civilization, people have employed herbal remedies to improve their health, and many of the medications used today were first made with the help of natural resources. For the treatment and management of infectious pathogens, many antimicrobial and antifungal substances were previously found from synthetic and natural sources (Shriram *et al.*, 2018). The availability and price of

numerous currently recommended antibiotics have been further hampered by the rise of multidrug-resistant bacteria globally. It consequently lessens the efficacy of the treatment plans and raises morbidity, mortality, and medical expense rates. The development of ailments and the expansion of modern understanding of natural remedies as significant substitutes or supplemental treatments for diseases provide justification for the use of therapeutic substances derived from plants. Numerous investigations have demonstrated that the compounds coumarins, flavonoids, phenolics, alkaloids, terpenoids, tannins, essential oils, lectin, polypeptides, and polyacetylenes are present in medicinal plants. These bioactive chemicals serve as a foundation for the creation of antibiotics that are utilized to cure infectious illnesses (Kebede *et al*., 2021).

*Allium sativum*is composed of numerous fleshy cloves. It is either soft or hard neck garlic which can be grown near equator or the cooler regions (Aly *et al*., 2012). Alliumsativum has 500 types differing in colour, odours and taste. *Allium sativum* is being used traditionally since ages for many purposes. It is the main component of many dishes throughout the world and used as source of several aromatic, as a fragrance (Mohamed, 2008). *Allium sativum* reduces cholesterol accumulation on the vascular walls; therefore, it is useful in cardiovascular disease. It is also used against some other diseases, such as chest infections and digestive disorders (Dorant *et al*., 1996).

Allium sativum enhances thiamine absorption and reduces the beriberi disease. This herb is used in acquired immune deficiency syndrome (AIDS) to control toxoplasmosis (Samaranayake *et al*., 2000).

It can boost level of testosterone in the body. In 1985, Weishberger and Pensky reported its anticancer properties, due to the presence of allixin, which has anti-tumor property. It is effective for cancer of several types such as those of lungs, liver, prostate and breast (Powolny and Singh, 2008).

Plantago ovata is bushy herbs grow in sandy land. The plants are 60cm tall; and leaves are sessile. The flowers are tiny, borne on stalks (5-40cm) and wind pollinated. It is cultivated in cool and dry weather from October to March. Crop gets ready between 119 and 130 days (Anderson *et al*., 1991). Traditionally used for many therapeutic purposes; laxative anti-acidic stabliser, stomach soothing, diuretic; and it is also anticancerous. The plant is mostly used for constipation problems, help in digestive system functioning, reduce fatigue and low density lipoproteine LDL in the blood. (Mark *et al*., 2013). A study conducted on 60 million Americans successfully reported lowering of high level of (LDL) in the blood, while it was also used upon 26 million people of all ages in USA with type 2 diabetes in 2011. The results indicated an improvement in the glucose fasting levels after using *P. ovata* seeds (Liangli and Jonathan, 2002).

It is more effective under the age of 60 years and is safe even for pregnant and lactating mothers. Food and drug administration (FDA) recommends consumption of 1.7g of *P. ovata* seeds per serving in daily food. It is also used to control appetite because its, water absorbing and gelling capacity gives a feeling of satiety (Song *et al*., 2000).

Nigella sativa is a flowering annual herb. It grows 20-30 cm tall, has fine thread like leaves, pale flowers with, 5-10 blue and white petals, and large capsular fruits and having 3-7 united follicles with numerous seeds. It has many names, like black seed, fennel flower, Habbat al Barakah (blessed seed) (Warner and Ramankutty, 2004). Ibn-e-Sina in his famous book Cannon of medicine wrote about the benefits of *N. sativa* due to its multi healing properties (Meddah *et al*., 2009). This plant has 100 healing properties, it has bronchodilactory and anti spasmodic effects and also useful in asthma and whooping cough (Kanter and Demir, 2003). *N. sativa* is also known as "miracle cure". It regulates female sexual hormone (Entela *et al*., 2012) helps in pregnancy, used in menopause problems (Salem, 2005). Useful for *Helicobacter pylori* infection reduces elevated blood sugar level and useful for different heart problems and allergies (Dadgar *et al*., 2005).

N. sativa is used for jaundice, paralysis, metabolic syndrome, insulin resistant syndrome, and high level of cholesterol, nerve tension problems; it stops hair fall and boost's immune system. *N. sativa* is an expectorant and stimulates body to recover fatigue. The seed oil is used as a soothing agent for stomach (Mashhadian and Rakhshandeh, 2005).

N. sativa is useful in controlling erythrocytes deformity and shows antibacterial activities Plant seed extract is used against methicillin resistant

Staphylococcus aureus (MRSA) A study conducted on rats and guinea pigs, having pancreatic cancer reported antitumor activity of, crude methanolic extract, it is, useful for gastric ulcers and decreases the pus cells in urinary tract infection (Suboh and Aburjai, 2004). In recent Scenario there is a dire need to gain knowledge prior the utilization of medicinal plants in any kind of herbal formulations, which are widely practiced worldwide, as well as in Pakistan. Therefore, the current study intended to evaluate and assess the potential medicinal properties of *Allium sativum, Nigella sativa* and *Plantago ovata* and also to compare the results with published data. Morever this study will contribute significanly in future plants based formualations in the field of food science and future prospects in the field of medicinal plants utilizations.It is imperative to investigate different plant species for their medicinal and curative prospectus in order to reach to the conclusions regarding their safety,efficacy and utilization of these herbs.As per estimates of World Health Orginization, about 80% of the World's population residing in underdeveloped and Developing Countries depends on herbal Formulations as a medicine for their primary health care. Pakistan is among one of them and hence it have created avenues for reseachers to work on Etheno-Medicinal aspects of various herbs with the help of their antibacterial, insecticidal, antifungal and phytochemical Screening and this study is a distinctivecontribution in the field of food science and will provide knowledge about herbal attributes of *Allium sativum, Nigella Sativa* and *Plantago ovata*.

Materials and Methods

This research aimed to design the antibacterial, antifungal and insecticidal activity of *Allium sativum, Nigella sativa* and *Plantago ovata*.

Chemicals and experimental conditions
The materials required for insecticidal assay included test insect (*T. castaneum*).

Plant material
The herbs *A. sativum, P. ovata, N. sativa*were purchased from the local herbal market in Peshawar and identified by Prof. Dr. Farrukh Hussain, Department of Botany, UOP, Pakistan. The herbs were shade-dried and ground to powder using an electric grinder.

Extraction
Each herb (10kg) was soaked in methanol with occasional shaking at room temperature for 15 days. The prepared suspensions were then filtered followed by heating at 40°C temperature in a rotary evaporator for making it concentrated by evaporating the methanolic extract (Siddiqui and Ali, 1997).

Fractionation of Allium sativum
The crude MeOH extract of *A. sativum* (860g) was suspended in 500ml distilled water which was then partitioned with 1500mL CHCl3, *n*-hexane and EtOAc to their respective fractions as follows: aqueous @ 210g, $CHCl_3$ @ 160g, *n*-hexane @ 170g and EtOAc @ 150g fractions (Oloyede and Adebooye, 2005). About 90g of the crude. MeOH extract Ext. were stored for biological/ pharmacological investigations.

Fractionation of Nigella sativa
The Cr. MeOH Ext of *N. sativa* (725g) was suspended in 500 mL distilled water which was then partitioned with 1500mL of $CHCl_3$, *n*-hexane and EtOAc to their respective fractions as follows: aqueous @ 190 g, $CHCl_3$ @ 135g, *n*-hexane @ 140g and EtOAc @ 130g fractions. About 90g of the Cr. MeOH Ext. were stored for biological/pharmacological investigations (Ebi and Ofoefula, 1997) (Figure 1).

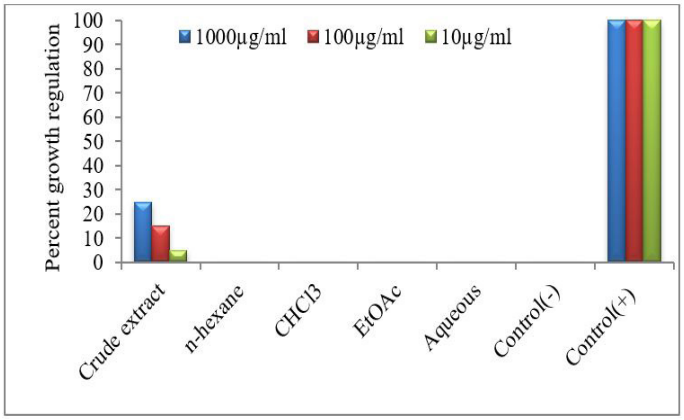
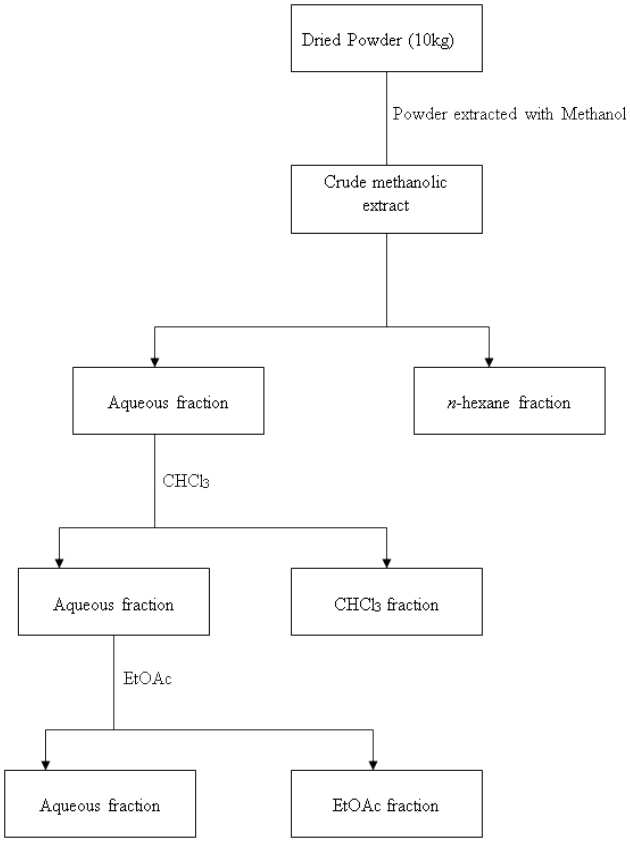


Figure 1: *Cr. Phytotoxic activity of Meth. Ext. and various fractions of A. sativum.*

Fractionation of Plantago ovata
The Cr. MeOH Ext. of*P. ovata* (550g) was suspended in 500 ml distilled water which was then partitioned with 1500 mL CHCl3, *n*-hexane and EtOAc to their respective fractions as follows: $CHCl_3$ @ 150g, *n*-hexane @ 160g and EtOAc @ 140g fractions. About 90g of the crude Cr. MeOHextractwasstored for biological investigations (Flow Chart 1).



Flow Chart 1: *Fractionation of crude methanolic extracts of different herbs.*

Phytochemical investigations

Tannin: 500mg of sample was suspended in a 250mL plastic bottle, and 50 mL of distilled water was addedto increase up to 100 mL. The filtrate (5mL) was pipetted out and mixed in a test tube containing 3 mL of 0.1M $FeCl_3$, 0.1N HCl and 0.008 M potassium ferrocyanide. The absorbance of the sample was measured at a wavelength of 120 nm using a spectrophotometer within 10 minutes time interval. The change in absorbance of the sample was compared with prepared standard; using tannic acid at 100 ppm to calculate of the quantity of tannin in the sample (Obadoni and Ohuko, 2001).

Alkaloid: Five grams of sample was added to 200mL of 20% acetic acid in ethanol in a 250mL beaker and kept for 4 hr. It was then filtered and concentrated using a water bath to a quarter of the original solution. Ammonium hydroxide was added dropwise, until a precipitate was formed, settled, filtered and weighed to calculate the amount of alkaloid (Boham and Kocipai, 1994).

Flavonoid: Ten grams of the sample were suspended in 100mL of 80% aqueous MeOH at room temperature. The suspension was extracted several times, and the solution was filtered through Whatman filter paper no. 42 (125 mm). The filtrate was transferred to a crucible for drying with the help of a water bath and weighed to calculate the amount of total flavonoid content present in the sample (Barakat *et al.*, 1973).

Ascorbic acid (Vitamin C): Five grams of the sample were taken in an extraction tube and 100mL of ethylene diamine tetraacetic acid (EDTA)/ Trichloroacetic Acid (TCA) (2:1) was added to it. It was shaken for 30 min, centrifuged at 3000 rpm for 20 min and transferred to a 100 mL volumetric flask 20 mL of the extract was pipetted out in to a flask, mixed with 1% starch indicator, and titrated against 20% $CuSO_4$ until the dark colour was obtained (Kim *et al.*, 2000).

Biological evaluation

Phytotoxic activity : The screening of the phytotoxicity of the plant extracts was according to a reported protocol (Bashir *et al.*, 2011). Healthy 16 *L. minor* plants with three fronds rosette were placed in each flask. These flasks were then incubated at 27 °C for 7 days in growthchamber. The results were obtained by counting damaged number plants after seven days of incubation.

The results were obtained by counting the damaged number of plants after seven days of incubation.

Antibacterial activity

The plant crude methanolic extract and various fractions were screened for pathogens according to the method reported in the literature (Bashir *et al.*, 2011). The nutrientbroth was first prepared and then autoclaved. The broth was then poured into sterile test tubes and then stored for one day in an incubator at 37 °C. The broth medium was analyzed for any occurrenceof contaminations. Aseptically the nutrient broth was inoculatedwith the tested culture by spreading equally the sample on an agar medium in a laminar airflow cabinet. Wells of 6mm were made on the nutrient agar with a sterile borer. The zones of inhibitions were recorded after 24 hr of incubation at 37 °C.

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

Antifungal activity

The antifungal activity was performed with stock

solutions (24mg/mL) of Cr. Met.Ext and fractions in DMSO. Aliquots of 4 mL of SDA were taken in test tubes and autoclaved. After sterilization as the media temperature reach around 50°C from the stock solution 66.6 µL of solution was transferred in each tube. The solution was left to cool down and then each fungal species that was culturedfor 7 days was inoculated in these test tubes.

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

Insecticidal activity

The pests were reared with sterile plastic bottles with uniform age and size of insects .

The contact toxicity assay was adapted for insecticidal bioassay. On the first day, the filter paper was placed in Petri dishes of 9 cm. The crude methanolicextract and fraction from the samplesolution were loaded in petridishes separately with help of a sterile micropipette. The sample plates were left overnight for the organic solvents to evaporate. This was followed by 10 healthy insects transferred to the plate with a clean brush. The percent mortality of insects treated with the test samples wascalculated according to the formula:

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

A standardinsecticidal drug (Permethrin) was used as positive control.

Statistical analysis

All data obtained from different activities were analyzed using SPSSstatistical package version 16. Two-way and, one-way analysis of variance (ANOVA), chi-square, and least significance difference test (LSD) was used for analysis.

Results and Discussion

Phytotoxic activity of A. sativum

The phytotoxicity of the test samples was determined against *L. minor*. The results of phytotoxic activity of Crd. MeOH Ext. and various fractions of *A. sativum* against *L. minor* are summarised in Table 1 and presented in Figure 1. The Crd. MeOH Ext. showed (25, 15 and 5%) growth regulation at 1000, 100 and 10µg/mL respectively, while no phototoxic activity was observed in *n*-hexane, $CHCl_3$, EtOAc and aqueous fractions.

Phytotoxic activityof N. sativa

Herbicides of plant origin are environment friendly. *Lemna minor* is sensitive to bioactive compounds containing aquatic monocot small plants. The phytotoxicity assay results of crude. MeOH extract and various fractions of *N. sativa* against- *L. minor* is summarised in Table 2 and presented in Figure 2. The crude MeOHextract (75, 50 and 40%), $CHCl_3$(10 and 5 %) and aqueous (60, 50 and 30%) extract showed growth regulation at 1000, 100 and 10µg/mL, respectively, while no activity was observed in *n*-hexane and EtOAc fractions.

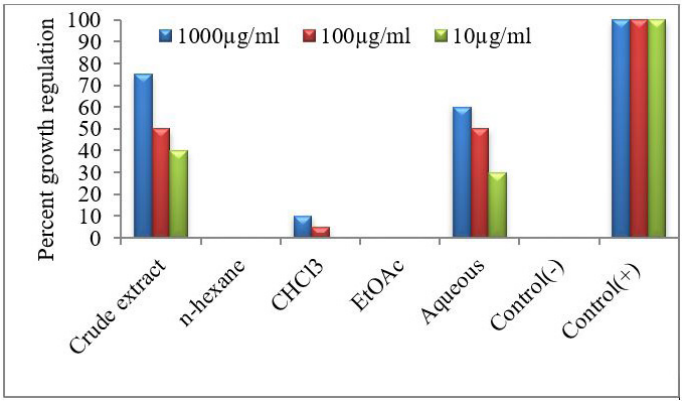


Figure 2: *Phytotoxic activity of Cr. Meth. Ext. and various fractions of N. sativa.*

Table 1: *Phytotoxic activity of Cr. Meth. Ext. and various fractions of A. sativum.*

Name of Plant	Conc. of sample (µg/mL)	No. of fronds killed						Conc. of Stnd. drug * (µg/mL)
		Crd. MeOH Ext.	n-hexane	$CHCl_3$	EtOAc	Aqueous	Control	
Lemna minor	1000	5	0	0	0	0	20	0.015
	100	3	0	0	0	0	20	
	10	1	0	0	0	0	20	
		Percent growth regulation					**Standard**	
	1000	25	0	0	0	0	100	
	100	15	0	0	0	0	100	
	10	5	0	0	0	0	100	

**Paraquat was used as standard drug.*

Table 2: *Phytotoxic activityofCr. Meth. Ext. and various fractions of N. sativa.*

Name of plant	Concentration of sample (µg/mL)	No. of fronds killed						Concentration of standard drug * (µg/mL)
		Crd. MeOH Ext.	n-hexane	$CHCl_3$	EtOAc	Aqueous	Control	
Lemna minor	1000	15	0	2	0	12	20	0.015
	100	10	0	1	0	10	20	
	10	8	0	0	0	6	20	
		Percent growth regulation					Standard	
	1000	75	0	10	0	60	100	
	100	50	0	5	0	50	100	
	10	40	0	0	0	30	100	

Table 3: *Phytotoxic activity of Cr. Meth. Ext. and various fractions of P. ovata.*

Name of plant	Conc. of sample (µg/mL)	No. of fronds killed						Conc. of Stand drug * (µg/mL)
		Crd. MeOH Ext.	n-hexane	$CHCl_3$	EtOAc	Aqueous	Control	
Lemna minor	1000	4	2	0	0	0	20	0.015
	100	2	1	0	0	0	20	
	10	1	0	0	0	0	20	
		Percent growth regulation					Standard	
	1000	20	10	0	0	0	100	
	100	10	5	0	0	0	100	
	10	5	0	0	0	0	100	

**Paraquat was used as standard drug.*

Phytotoxic activity of P. ovata

The results of phytotoxic activity of crude methanolic extract and various fractions of *P. ovata* against *L. minor* are summarised in Table 3 and presented in Figure 3. The crude methanolic extract (20, 10 and 5%) and *n*-hexane (10, 5 and 0%) showed growth regulation at 1000, 100 and 10µg/mL, respectively while no phototoxic activity was observed in $CHCl_3$, EtOAc and aqueous fractions (Patel *et al.*, 2015).

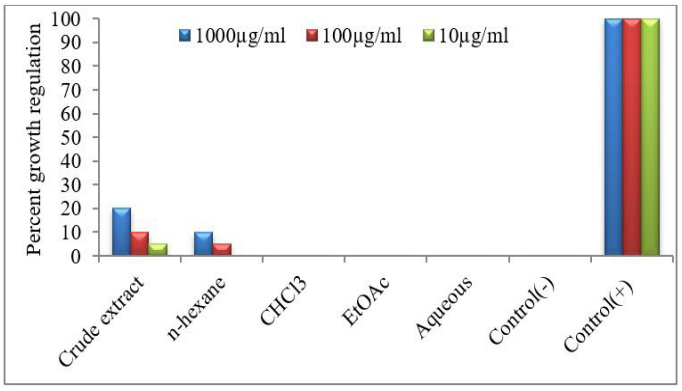


Figure 3: *Phytotoxic activity of Cr. Meth. Ext. and various fractions of P. ovata.*

Anti-bacterial activity

Anti-bacterial activity of *A. sativum:* The Crd. MeOHExt.and various fractions of *A. sativum* were screened against test pathogens for antibacterial activity. The results are summarised in Table 4 and presented in Figure 4.

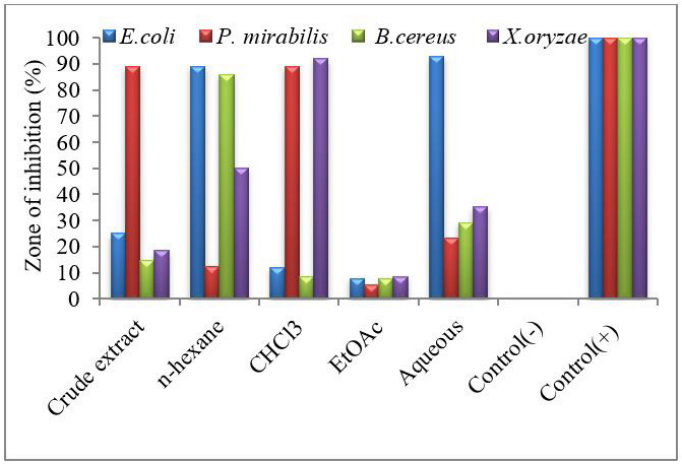


Figure 4: *Anti-bacterial activity of Cr. Meth. Ext. and various fractions of A. sativum.*

The crude MeOH extract. exhibited significant antibacterial activity against *P. mirabilis* (89%) low activity against *E. coli* (25%), *B. cereus* (14%) and *X. oryzae* (18%), *n*-hexane fraction exhibited significant antibacterial activity against- *B. cereus* (86%) and *E. coli* (89%), moderate activity against *X. oryzae* (50%) and low activity against *P. mirabilis* (12%).

The$CHCl_3$ fraction showed significant antibacterial activity against *X. oryzae* (92%) and *P. mirabilis* (89%) and low activity against *E. coli* (12%) and *B. cereus* (8%). Similar findimgs were reported by Safithri *et al.* (2012). TheEtOAc fraction showed low activity against *E. coli* (7%), *P. mirabilis* (5%), *B. cereus* (7%) and *X. oryzae* (8%). The aqueous fractions showed significant activity against *E. coli* (93%) low activity against *P. mirabilis* (23%), *B. cereus* (29%) and *X. oryzae* (35%). Microbial cultures of *X. oryzae*, *P. mirabilis*, *E. coli* and *B. cereus* were obtained from the Centre of Biotechnology and Microbiology, University of Peshawar (COBAM, UOP).

Fungalculturesincluding*Verticilliumlecanii*,*P.oxalicum*, *Acremoniumstrictum* and *Trichodermaharzianum* species were obtained from the COBAM, UOP. These fungal were tested for antifungal activities of the herb extracts.

The Two-way ANOVA test showed ($P > 0.05$) insignificant difference for different bacterial pathogens but a significant difference for ($P < 0.05$) different fractions (Supplementary Table 1).

Anti-bacterial activity of N. sativa

The Crd. MeOH Ext. and various fractions of *N. sativa* were screened against the-test pathogens for antibacterial activity. The results aresummarised in Table 5 and presented in Figure 5. The Crd. MeOH Ext. showed significant activity against *E. coli* (89%), *P. mirabilis* (96%), *B. cereus* (89%) and *X. oryzae* (88%). The *n*-hexane fraction exhibited significant antibacterial activity against *X. oryzae* (92%), *B. cereus* (82%), *P. mirabilis* (85%) and low activity against *E. coli* (7%). $CHCl_3$ fractions showed significant activity against *X. oryzae* (96%), *E. coli* (93%) and *P. mirabilis* (89%) and low activity against *B. cereus* (17%). The EtOAc fractions showed moderate activity against *X. oryzae* (50%), *B. cereus* (93%), *E. coli* (89%) and low activity against *P. mirabilis* (12%). The aqueous fractionshowed significant activity against *B. cereus* (93%), *X. oryzae* (92%), *E. coli* (86%) and *P. mirabilis* (85%). The Two-way ANOVA test showed ($P>0.05$) insignificant difference for different bacterial pathogens but significant difference for ($P <0.05$) different fractions (Supplementary Table 2). LSD results showed significant difference between different fractions compared with the negative control.

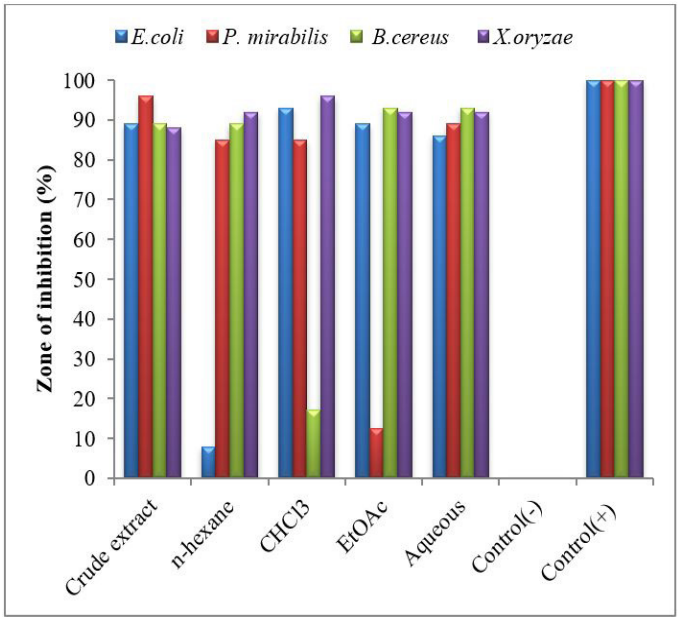


Figure 5: *Anti-bacterial activity of Cr. Meth. Ext. and various fractions of N. sativa.*

The Crd. MeOH Ext. and various fractions of *P. ovata* were screened against test pathogens for antibacterial activity similar findings about *N. sativa* was affirmed earlier by (Ali and Blunden, 2003). The two-way ANOVA test calculation showed highly significant difference between the fractions ($P= 0.000$) and insignificant difference between bacterial test samples ($P = 0.091$) (Supplementary Table 3).

Table 4: *Cr. Antibacterial activities of Meth. Ext. and various fractions of A. sativum.*

Name of Bacteria	Zone of inhibition of standard (amoxicillin) 10µg	Crd. MeOH Ext.		n-hexane		CHCl3		EtOAc		Aqueous	
		Zone of Inhibition (mm)	Inhibition (%)	Zone of Inhibition (mm)	Inhibition (%)	Zone of Inhibition (mm)	Inhibition (%)	Zone of Inhibition (mm)	Inhibition (%)	Zone of Inhibition (mm)	Inhibition (%)
E. coli	29	7.3	25	26	89	3.5	12	2.3	7	27	93
P. mirabilis	28	25	89	3.5	12	26	89	1.5	5	6.5	23
B. cereus	29	4.3	14	25	86	2.5	8	2.3	7	8.5	29
X. oryzae	27	5	18	13.5	50	25	92	2.3	8	9.5	*35*

Table 5: *Antibacterial activities of Cr. Meth. Ext. and various fractions of N. sativa.*

Name of Bacteria	Zone of inhibition of standard (amoxicillin) 10µg	Crd. MeOH Ext.		n-hexane		CHCl3		EtOAc		Aqueous	
		Zone of Inhibition (mm)	Inhibition (%)	Zone of Inhibition (mm)	Inhibition (%)	Zone of Inhibition (mm)	Inhibition (%)	Zone of Inhibition (mm)	Inhibition (%)	Zone of Inhibition (mm)	Inhibition (%)
E. coli	29	26	89	2.3	7	27	93	25	89	25	86
P. mirabilis	28	27	96	24	85	25	89	3.5	12	24	85
B. cereus	29	26	89	24	82	5	17	27	93	27	93
X. oryzae	27	24	88	25	92	26	96	13.5	50	25	92

Table 6: *Antibacterial activities of Cr. Meth. Ext. and various fractions of P. ovata.*

Name of Bacteria	Zone of inhibition of standard (amoxicillin) 10µg	Crd. MeOH Ext.		n-hexane		CHCl3		EtOAc		Aqueous	
		Zone of Inhibition (mm)	Inhibition (%)	Zone of Inhibition (mm)	Inhibition (%)	Zone of Inhibition (mm)	Inhibition (%)	Zone of Inhibition (mm)	Inhibition (%)	Zone of Inhibition (mm)	Inhibition (%)
E. coli	29	13.3	45	26	89	0	0	0	0	0	0
P. mirabilis	28	26	92	25	89	0	0	0	0	0	0
B. cereus	29	25	86	25	86	0	0	0	0	0	0
X. oryzae	27	0	0	0	0	0	0	0	0	0	0

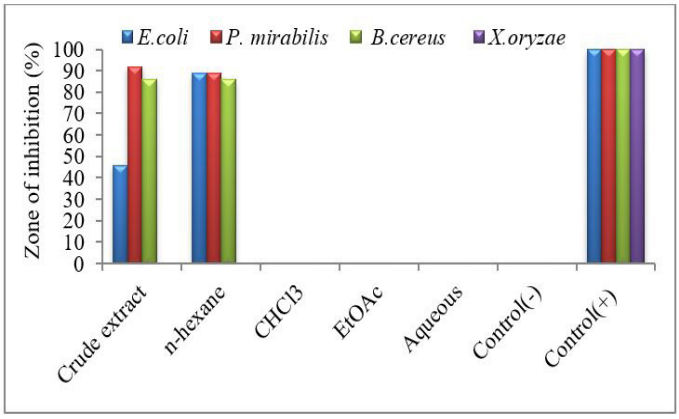


Figure 6: *Anti-bacterial activity of Cr. Meth. Ext. and various fractions of P. ovata.*

Antifungal activity

Antifungal activity of *A. sativum:* The Crd. MeOH Ext. and various fractions of *A. sativum* were screened against test pathogens for antifungal activity andresults are summarised in Table 7 and presented in Figure 7. The Crd. MeOH Ext. showed good antifungal activity against *P. oxalicum* (62%), *A. strictum* (61%) and moderate activity against *V. lecanii* (53%) and *T. harzianum* (48%). The *n*-hexane fraction showed moderate activity against *P. oxalicum* (58%), *A. strictum* (56%) and *V. lecanii* (43%) and low activity against *T. harzianum* (36%). The $CHCl_3$ fraction showed significant activity against *V. lecanii* (87%), good activity against *P. oxalicum* (69%) and *A. strictum* (64%) and moderate activity against *T. harzianum* (42%). The EtOAc showed good activity against *A. strictum* (69%) and *P. oxalicum* (65%) and moderate activity against *V. lecanii* (50%) and low activity against- *T. harzianum* (30%). The aqueous fraction showed good activity against *A. strictum* (64%), moderate activity against *V. lecanii* (47%) and low activity against *T. harzianum* (18%) and *P. oxalicum* (15%).

Table 7: *Antifungal activity of Cr. Meth. Ext. and various fractions of A. sativum.*

Fractions	*V.lecanii*	*P. oxalicum*	*A. strictum*	*T. harizinum*
Crude extract	53	62	61	48
n-hexane	43	58	56	36
$CHCl_3$	87	69	64	42
EtOAc	50	65	69	30
Aqueous	47	15	64	18
Control (-)	0	0	0	0
Control (+)	100	100	100	100

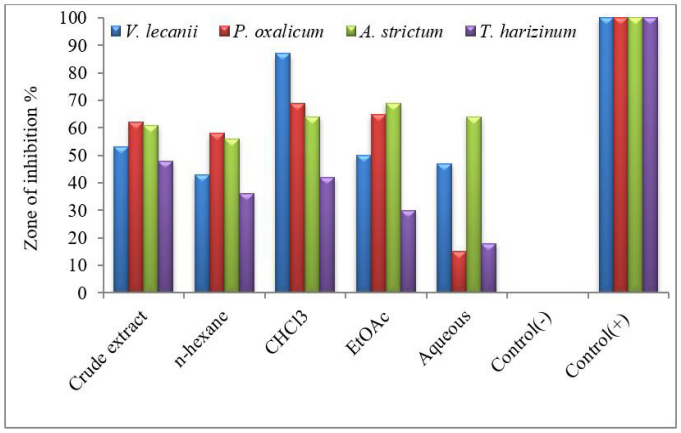


Figure 7: *Anti-fungal activity of Cr. Meth. Ext. and various fractions ofA. Sativum.*

Two-way ANOVA test showed highly significant difference between the fractions (P= 0.000) and between fungal strains (P= 0.03) (Supplementary Table 4). The LSD between *A. sativum, T. harzianum* and *V. lecanii* showed highly significance difference (P = 0.026). The LSD comparison between the fractions of *A. sativum* showed highly significance difference due to control.

Antifungal activity of P. ovata

The Crd. MeOH Ext. and various fractions of *P. ovata* were screened against test pathogens for antifungal activity and results aresummarised in Table 8 and presented in Figure 8.

Table 8: *Antifungal activity of Cr. Meth. Ext. and various fractions of P. ovata.*

Fractions	*V. lecanii*	*P. oxalicum*	*A. strictum*	*T. harizinum*
Crude extract	43	19	86	55
n-hexane	50	46	69	58
$CHCl_3$	0	0	0	0
EtOAc	0	0	0	0
Aqueous	0	0	0	0
Control (-)	0	0	0	0
Control (+)	100	100	100	100

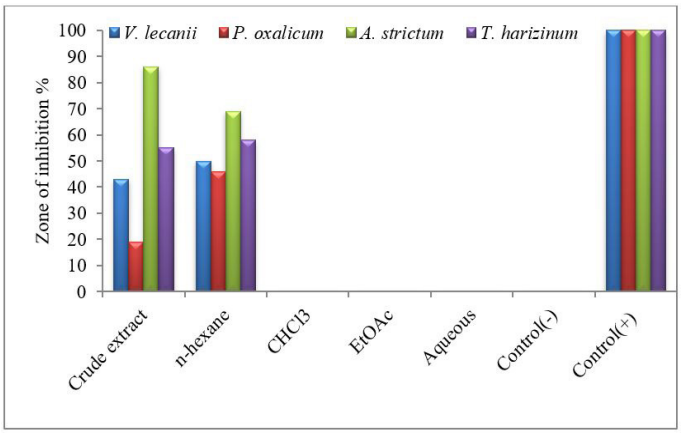


Figure 8: *Anti-fungal activity of Cr. Meth. Ext. and various fractions of P. ovata*

The Crd. MeOH Ext. showed significant activity against *A. strictum* (86%), moderate activity against *T. harzianum* (55%) and *V. lecanii* (43%) and low activity against *P. oxalicum* (19%). The *n*-hexanefractions showed good activity against *A. strictum* (69%), moderate activity against *T. harzianum* (58%), *V. lecanii* (50%) and *P. oxalicum* (46%). The Two-way ANOVA test showed insignificant difference between the different strains of fungi (P = 0.179) and highly significant difference showed for fractions (P = 0.000) (Supplementary Table 5). The LSD showed significant difference between the different fractions compare with control.

Antifungal activities of N. sativa

The Crd. MeOH Ext. and various fractions of *N. sativa* were screenedagainst- different strains of fungi for antifungal activity and results are summarised in Table 9 and presented in Figure 9. The Crd. MeOH Ext. showed significant activity against *P. oxalicum* (92%), good against *A. strictum* (64%), moderate activity against *V. lecanii* (56%) and *T. harzianum* (42%). The *n*-hexanefractions showed good activity against *A. strictum* (69%), *T. harzianum* (61%), and moderate activity against- *P. oxalicum* (54%) and *V. lecanii* (53%). The $CHCl_3$ fraction showed significant activity against *P. Oxalicum* (100%), *T. harzianum* (100%), *V. lecanii* (100%) and *A. strictum* (100%). The EtOAc fraction showed significant activity against *A. strictum* (80%), good activity against- *V. lecanii* (67%), *T. harzianum* (61%) and low activity against *P. oxalicum* (26%). The aqueous fraction showed significant activity against *A. strictum* (86%), good activity against- *V. lecanii* (60%), moderate activity against *T. harzianum* (58%) and low activity against *P. oxalicum* (19%).

Table 9: *Antifungal activity of Cr. Meth. Ext. and various fractions of N. sativa.*

Fractions	*V. lecanii*	*P. oxalicum*	*A. strictum*	*T. harizinum*
Crude extract	56	92	64	42
n-hexane	53	54	69	61
$CHCl_3$	100	100	100	100
EtOAc	67	26	80	61
Aqueous	60	19	86	58
Control (-)	0	0	0	0
Control (+)	100	100	100	100

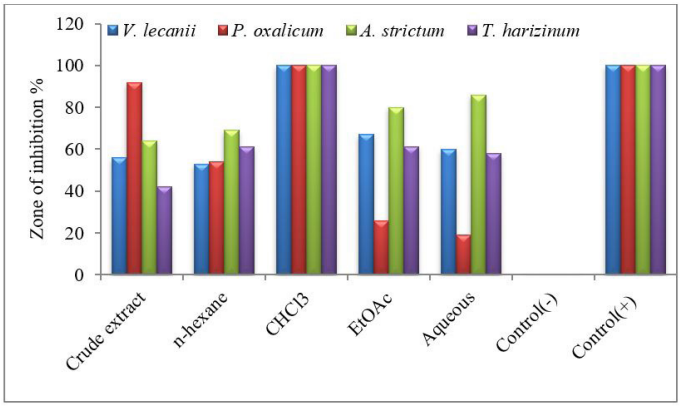


Figure 9: *Anti-fungal activity of Cr. Meth. Ext. and various fractions of N. sativa.*

The two-way ANOVA test showed null hypothesis is rejected for different fractions of *N. sativa* (P = 0.000) but accepted for different test pathogens of fungi (P = 0.349) (Supplementary Table 6). The LSD (Comparison between the fractions) showed highly significant difference between fractions compare with control (P = 0.000).

Insecticidal activity of A. sativum

The Crd. MeOH Ext. and various fractions of *A. sativum* were screened for insecticidal activity against *T. castaneum*. The results are summarised in Table 10 and presented in Figure 10. The Crd. MeOH Ext. showed significant insecticidal activity (90%), while low activity was found in the $CHCl_3$ (40%), EtOAc and aqueous (20%) fractions, the similar results were generated by Waller (1987). The *n*-hexane fraction showed no activity against *T. castaneum*. Pearson's chi-Square test provided significant association between row and column variables; Value = 27.989 and P = 0 .000 (Supplementary Table 6).

Table 10: *Insecticidal activity (% Mortality) of Cr. Meth. Ext. and various fractions of the selected plants.*

Fractions	*A. sativum*	*N. sativa*	*P. ovate*
Crude extract	90	20	20
n-hexane	0	0	0
CHCl3	40	70	0
EtOAc	20	0	0
Aqueous	20	30	0
Control (-)	0	0	0
Control (+)	100	100	100

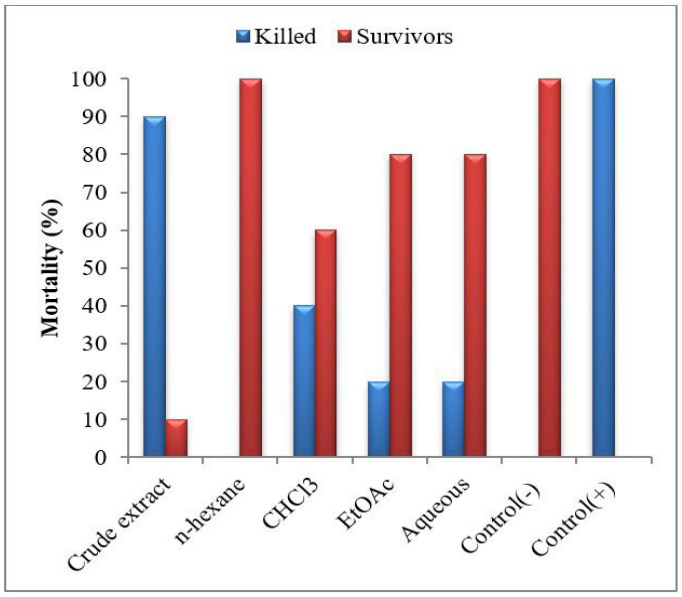


Figure 10: *Percent insecticidal activity of Cr. Meth. Ext. and various fractions of A. sativum.*

Insecticidal activity of N. sativa

The Crd. MeOH Ext. and various fractions of *N. sativa* were screened for insecticidal activity against *T. castaneum*. The results are summarised in Table 10 and presented in Figure 11.

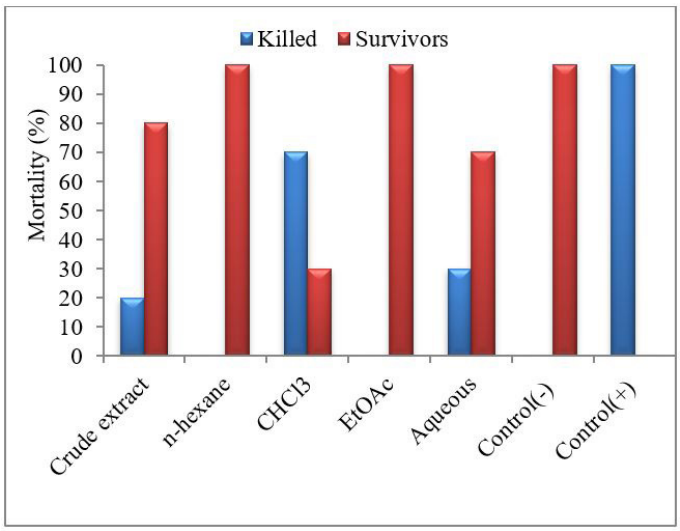


Figure 11: *Percent insecticidal activity of Cr. Meth. Ext. and various fractions of N. sativa.*

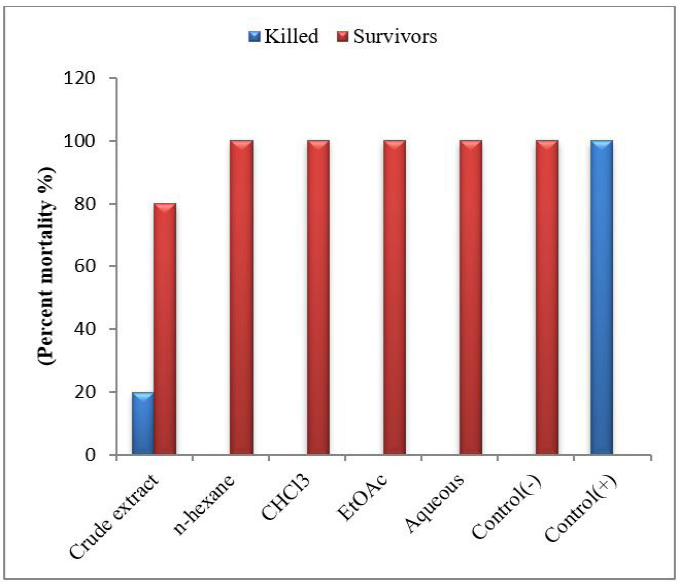


Figure 12: *Percent insecticidal activity of Cr. Meth. Ext. and various fractions of P. ovata.*

The $CHCl_3$ showed good insecticidal activity (70%), while low activity was found in Crd. MeOH Ext. (20%) and aqueous (30%) fraction. The *n*-hexane and EtOAc fractions showed no activity against *T. castaneum*. Pearson's chi-Square test provided significant association between row and column variables; Value is 23.750 and P = 0.000 (Supplementary Table 7).

Insecticidal activity of P. ovata

The Crd. MeOH Ext. and various fractions of *P. ovata* were screened for insecticidal activity against *T.*

castaneum. The results are summarised in Table 10 and presented in Figure 12.

The Crd. MeOH Ext. showed low activity (20%) while *n*-hexane, $CHCl_3$, EtOAc and aqueous fractions showed no activity against *T. castaneum*. Pearson's chi-square test provided insignificant association between row and column variables; Value = 4.286 and P = 0.117 (Supplementary Table 8). *P. Ovata* have important insecticidal properties due to the presence of varios compounds such as flavonoids, alkaloids, terpenoids, phenolic compounds (caffeic acid derivatives), antioxidants, vitamin C, and anti-inflammatory agents (Saghir *et al*., 2008).

Conclusions and Recommendations

Based on the results of this study, it can be concluded that almost all the plant extracts showed significant activities against different bacterial, fungal and insect species. In conclusion, the research has contributed to the potential therapeutic efficacy of *Alium sativum, Nigela sativa, and Plantago ovata* in the treatment of microbial infections due to their significant antibacterial activitives. Additionally, the findings showed that *Alium sativum, Nigela sativa*, and *Plantago ovata* have very powerful antifungal and insecticidal activities, supporting their promising usage as possible antifungal and bio-insecticides in agricultural production and preservation of leguminous plants.

It is imperative to investigate different plant species for their medicinal and curative prospectus in order to reach to the conclusions regarding their safety, efficacy and utilization of thsese herbs. As per estimates of World Health Orginization, about 80% of world's population residing in underdeveloped and developing countries depends on herbal formulations as a medicine for their primary health care. Pakistan is among one of them and hence it have created avenues for reseachers to work on Etheno-Medicinal aspects of various herbs with the help of their antibacterial, insecticidal, antifungal and phytochemical screening.

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Novelty Statement

This research study intended to evaluate and assess the potential medicinal properties of *Allium sativum, Nigella sativa* and *Plantago ovata* in the field of medicinal plants utilizations.

Author's Contribution

Amina Rahat: Data collection, lab work and analysis.
Zahin Anjum: Writing of the manuscript and analysis.
Sehlina Zehra: Helped in manuscript literature.
Fatima Umal Baneen: Helped in samples treatments.
Rabia Chishti: Helped in statistical analysis and tabulation.

Supplementary material
There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.sja/2023/39.2.531.544

Conflict of interest
The authors have declared no conflict of interest.

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