

Full Length Research Paper

Antifungal bioassay of selected medicinal plants of family Brassicaceae against *Colletotrichum gloeosporioides* L

Husan Ara¹, Tabassum Yaseen^{2*} and Mehwish¹

¹Department of Botany, AWKUM University of Mardan, Pakistan.

²Department of Botany Bacha Khan University of Charssada KPK.Pakistan.

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The current study evaluated and established the activity of *Allium sativum*, *Allium sepa* and *Aloe vera barbadensis* against fungal specie *Rhizopus*. The ethanol and aqueous extracts of these plants were tested to establish the antimicrobial activity against *Rhizopus* which was isolated from bread. The antifungal activity was tested by disc diffusion method. The potato dextrose agar plates were incubated with each fungal culture (10) days old by point inoculation. The filter paper discs (5mm) in diameter impregnated with 100 µg mL⁻¹ concentration of the extract were placed on the on test organisms seeded plates. Ethanol was used to dissolve the extract and was completed evaporated before application on test organisms seeded plates. The activity was determined after incubation at 28 °C for 24 hours. The Diameter of the inhibition zones were measured in mm. The plants showed considerable inhibitory effect ranges from 2.5- 12.6 mm against *Rhizopus* in case of ethanol extract and from 0.9 -14.4 mm in case of aqueous extract. The highest zone of inhibition was observed in 15% ethanolic extract of root of *Aloe vera* and the smallest zone of inhibition was detected in 15% ethanolic extract of root of *Allium sativum*. Sporulation was also observed in some of the Petri plates.

Keywords: Antifungal bioassay, Brassicaceae, *Rhizopus*, *Colletotrichum gloeosporioides*.

INTRODUCTION

Those plants which have active ingredients for the treatment of certain diseases are known as medicinal plants or herbs, in other words the plants which have healing properties are termed as medicinal plants. Man has been utilizing plants for basic preventive and curative health care since time immemorial. The most recent estimates suggest that over 9,000 plants have known medicinal application in various culture and countries, Medicinal plants in crude form are used mostly in rural

areas at the household level and by the practitioners of classical traditional systems of medicines such as Unani, Chinese medicine or the Japanese system, While the traditional medicines are derived from minerals and organic matter, the herbal drugs are prepared from medicinal plants. Pakistan has about 50000 registered practitioners of traditional medicine called Tebb-e-Unani and number of population getting health care by Tabbibs. There are many institutions for Tabb education; some of these are Hamdard medical college for Tabb, Qarshi medical college for Tabb, Falkon medical college for Tabb and so on. These institutions are registered from national consol for Tabb. A good Tabbib (Hakeem)

*Corresponding Author E-mail: sciences111@yahoo.com

completes 4 year course and get the degree F.T.J (Fazil-ul-Aljarahat) from these institutions. It is estimated that 60 % of population used herbal medicines prescribed by traditional practitioners. (Sahito *et al.*, 2003). The World Health Organization (WHO) estimated that 80 % of the population or 4.3 billion people of developing countries rely on traditional medicines, mostly plant drugs for their primary health care needs. With the passage of time the demand for medicinal plants is increasing in both developing and developed countries due to growing recognition of natural product, having less side effect, being non-narcotic and easily available for the health care of poor people at affordable price. In Pakistan there is large number of rural areas using the indigenous medicinal plants for curing their illness. Medicinal plants are widely used for treatment of diseases all over the world. According to World health organization report about 80% of the world population is taking interest in indigenous medicinal plants remedies. Herbal medicine has usually been used in the form of fruit and vegetables, drugs or their extract for the treatment of the diseases and for maintenance health. (Sahito *et al.*, 2003).

History of medicinal plants

Medicinal plants have their own evaluative history. Ancient civilizations gradually developed knowledge of plants with healing and curative properties. The knowledge transfer from generation to generation. Many authors had left important information's on the action of plants. This led to the development of various system of medicine. one of the oldest religious Hindu book of the world "Rig Veda" claimed to be written between 4500 and 1600 B.C, provides information about the use of a plant 'soma' as medicine agent and also contain a number of references about the medicinal plants. Other books like "Sumhita Samita" and "Charat Samitha" written about 1000 B.C, have also the information about the medicinal herbs. During 372-287 BC Theophrastus who is the father of botany described many plants. First time the Greeks developed "material medica" of the world, while the pharmacy began from Hippocrates (460 B.C) who is also called the father of medicine. Pliny (43-70 AD) is also the major compendium of natural history. Islam also has a great contribution to the development of separate branch of therapy based on the doctrines of Quran and Sunnah. In 500-1700 A.D the role herbal medicines were greatly extended in the Islamic era of science. The Greek (Unani) system of medicine was improved by the Muslims. One of the most important medicinal plant was introduced by the Muslims to India was Opium. Hazrat Muhammad (P.B.U.H) quoted the role of different plants like *Nigella sativa* and *Crotalaria juncea*. Later on the Muslim scientists like Zakariya Al-Razi, Ibne Sina, Al-Idris and Ibne -Al Baiter etc written

the books on the medicinal plants and their importance. Zakariya Al-Razi (864-932 A.D) has written many books, among which the most famous is "Kitab-al-Mansoori" comprised of ten volumes, Ibne-Rabban Al-Tabavi (883-970 A.D) wrote the book "Fardous al - Hikmat", Ibne sina (980-1037 A.D) wrote "Qanun fial - Tibb", Al-Idrisi (1100-1166 A.D) wrote several books on medicinal plants specially the "Kitabal-Jami-li-siffat Ashtat A l-Nabatat" and another well known botanist and pharmacist Ibne -Al Baitar wrote a known as "Kitab Al-mughni-fi-al-Adwiya-Al-Muffarada" All these book deals with the medicinal plants and drugs. The phytochemistry and pharmacognosy are the two important disciplines used in the science of the medicinal plants.

Phytochemistry in relation to medicinal plants

Medicinal plants consist of active biochemical constituents and give a definite physiological response in different diseases in animal as well as in man. So the branch of chemistry which deal with the chemical processes associated with plant life and the chemical compounds produced by plant is called phytochemistry, while the molecules characteristically in plant are called phytochemical, some of the basic phytochemicals are alkaloids, carotenoid, flavonoide, fatty acids, terpenoids, polysaccharide and aromatic compound etc. techniques commonly used in the field of phytochemistry are extraction, isolation and structural elucidation (MS, 1D and 2D NMR) of natural products, as well as various chromatography techniques (MPLC, HPLC, LC-MS) (Sahito *et al.*, 2003).

Pharmacognosy in relation to medicinal plants

Pharmacognosy is derived from Greek word pharmakon which means "drug" and gnosis mean "knowledge". The term pharmacognosy was used for the first time by the Austrian physician Schmidt in 1811 and 1815 by Crr. Anotheus seydlar in a work entitled analecta pharmacognostica, so it is the branch of medicine and biology which concerns with the study of the action of drug, in other words it is the study of the interactions that takes place between the living organisms and chemicals that affect normal or abnormal biochemical function is known as pharmacognosy (Sahito *et al.*, 2003).

MATERIALS AND METHODS

Plant material *Allium sativum*, *Allium sepa* and *Aloe Vera* specimens were collected from district Charsadda and were identified through literature flora of Pakistan (Nasir and Ali, 1990). The collected plant materials were placed in shade at room temperature for two weeks to dry.

Fungal Specie

Fungal specie namely *Rhizopus* collected from bread, were sub cultured and used throughout the research work.

Morphology of Rhizopus

Rhizopus is a genus of common saprobic fungi on plants and specialized parasites on animals. They are found on a wide variety of organic substrates, including "mature fruits and vegetables" (Kirk *et al.*, 2008) faeces, jellies, syrups, leather, bread, peanuts and tobacco. Some *Rhizopus* species are opportunistic agents of human zygomycosis (fungal infection) and can be fatal. *Rhizopus* infections may also be a complication of diabetic ketoacidosis (Chinn *et al.*, 1982). This widespread genus includes ten species (Zheng *et al.*, 2007).

Rhizopus species grow as filamentous, branching hyphae that generally lack cross-walls (i.e., they are coenocytic). They reproduce by forming asexual and sexual spores. In asexual reproduction, sporangiospores are produced inside a spherical structure, the sporangium. Sporangia are supported by a large apophysate columella atop a long stalk, the sporangiophore. Sporangiohores arise among distinctive, root-like rhizoids. In sexual reproduction, a dark zygospore is produced at the point where two compatible mycelia fuse. Upon germination, a zygospore produces colonies that are genetically different from either parent.

Preparation of Plant Extract

Different parts such as root, stem, leaves, and flower of the plant were used for preparation of extract. 2gm of powdered sample were dissolved in 20 mL of 70% ethanol. Flasks were kept in dark for two days. The solutions were filtered.

Antibiogram Analysis

Agar media was prepared by dissolving all its components in distilled water, media along with petriplates was autoclaved. Media was poured into sterile petriplates under sterile conditions and left for solidification. After solidification 50 mL fungus was spread on the different plates and wells were bored. 50 microlitre antimicrobial samples and tetracycline were loaded in the wells. Plates were incubated at 37 °C for 24 hours, and observed for zone of inhibition. The zone of inhibitions by the sample was compared with Tetracycline which is used as a standard antibiotic.

RESULTS

Antimicrobial assays can provide means to detect antibacterial as well as antifungal potential of crude plant extracts. The screening results of the plants study confirm the possible use of medicinal plants as a source of antimicrobial agent for the treatment of diseases. In the present study, antifungal activity of the three members of family Liliaceae, that is, *Allium sativum*, *Allium sepa* and *Aloe vera* was tested against *Rhizopus* given in Table 1. Potato dextrose medium was used for the culturing of fungus and also for the testing of antifungal activity of the extracts. Both the alcoholic and aqueous extracts were used. Three concentration grades of root stem and leaf extracts of each part of the plant material were prepared. Method followed was agar well diffusion method. For each treatment three replicates were maintained. All the fungal plates were incubated for 72 hours at 28 °C. The antifungal activities of the plant extracts obtained by using water and ethanol as solvents were compared with that of ethanol and distilled water as standard control respectively and the diameter of zone of inhibition was calculated, as the diameter of the zone of inhibition is an indicator of the antifungal activity. Extracts were obtained through the extracting action of the appropriate solvent on a dry plant and the active compounds are thus contained in the solvent used. Each type of extract is defined by the way it is prepared and the nature of the solvent. The following tables show the diameter of zone of inhibition in mm shown by the aqueous and ethanol extracts of the different parts of the three plant species.

Results obtained in the present study relieved that the tested three medicinal plants extracts possess potential antifungal activity against *Rhizopus*. Extracts prepared are different in the strength of their phytotoxic effects. All the crude extracts had significance antifungal activities against most of the fungi, but the activity of inhibition varied for the fungi with respect to the type of plant extract. All the concentrations of the plant extracts showed strong activity against the test pathogen on concentration dependent manner. The results showed that increase in concentration of extract increased the zone of inhibition. The plants showed considerable inhibitory effect ranges from 2.5-12.6 mm against *Rhizopus* in case of ethanol extract and from 0.9 -14.4 mm in case of aqueous extract. The highest zone of inhibition was observed in 15% ethanolic extract of root of *Aloe vera* and the smallest zone of inhibition was detected in 15% ethanolic extract of root of *Allium sativum*. Sporulation was also observed in some of the Petri plates.

Table 1. Diameter of zone of inhibition showed by the three plants i-e *Allium sativum*, *Aloe vera*. and *Allium sepa*.

Diameter of zone of inhibition (mm)									
Extract concentration (mg/mL)	<i>Allium sativum</i>			<i>Aloe vera</i>			<i>Allium sepa</i>		
	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf
Control(Water)	10.8mm	10.6mm	8.9mm	12.3mm	12.3mm	10.3mm	9.7mm	6.5mm	2.7mm
Control(Ethanol)	2.6mm	2.9mm	10.8mm	12mm	10.7mm	10.3mm	11.6mm	4.9mm	10.4mm
Aqueous extract									
5%	12mm	8.5mm	6.6mm	10.5mm	10mm	9mm	10.8mm	5.7mm	0.9mm
10%	6.8mm	13.9mm	10.3mm	13mm	12.6mm	10.3mm	7.8mm	10.2mm	4.2mm
15%	13.6mm	9.6mm	9.8mm	13.3mm	14.4mm	11.5mm	10.6mm	3.6mm	3mm
Ethanol extract									
5%	2.6mm	2.8mm	11mm	11.2mm	8.2mm	9.4mm	11mm	2.6mm	11.2mm
10%	2.6mm	2.6mm	11.7mm	12.2mm	10mm	9.5mm	12.2mm	3mm	8.1mm
15%	2.5mm	3.2mm	9.8mm	12.6mm	14mm	12mm	11.6mm	9.1mm	12mm

Ethanol extracts

The ethanolic extracts studied showed inhibition of growth of the tested fungal specie with various degrees. The highest zone of inhibition in case of ethanol extract was detected in 15% root extract of *Aloe vera* that is 12.6mm and smallest zone was observed in 15% root extract of *Allium sativum* that is, 2.5mm. Among the ethanolic leaf extracts 15% of *Aloe vera* and 15% of *Allium sepa* show highest antifungal activity that is 12mm and the lowest is shown by 10% leaf extract of *Allium sepa* that is 8.1mm. In the stem extracts, 15% stem extracts of *Aloe vera* and show the highest inhibitory action that is 14mm and the lowest is shown by 5% stem extract of *Allium sepa* that is 2.6mm. Among the root extracts, 15% *Aloe vera* has the highest antifungal activity which is 12.6mm while 15% *Allium sativum* has the lowest antifungal activity that is 2.5 mm.

Aqueous Extract

The aqueous extracts generally showed less inhibitory activity against as compared with ethanol extracts. The highest zone of inhibition in case of water extract was detected in 15% stem extract of *Aloe vera* that is 14.4mm and smallest zone was observed in 5% leaf extract of *Allium sepa* that is 0.9mm. Among the aqueous leaf extracts 10 % leaf extract of *Allium sativum* and 10% of *Aloe vera* show highest antifungal activity that is 10.3mm, the lowest inhibitory effect that is shown by 5% leaf extract of *Allium sepa* which is 0.9mm. In the stem extracts 15% stem extracts *Aloe vera* show the highest inhibitory action that is 14.4mm and the lowest is shown by 10% extract of *Allium sepa* that is 3.2mm. Among the root extracts 15% *Allium sativum* has the highest antifungal activity which is 13.6mm while 10% *Allium sativum* has the lowest antifungal activity

that is 6.8 mm. The higher antifungal activity of most of the ethanol extracts as compare to aqueous extracts might be due to the lack of solubility of active constituents in aqueous solution. Out of 27 plant extracts tested for their antifungal activity *Aloe vera* showed most promising antifungal activity and its alcoholic extract was more effective as compare to aqueous extract. Similar results were also obtained in the extracts' of the other two plants. The results showed that inhibition of microbial growth was greater in the root extract of the plant irrespective of the variety and the solvent used.

DISCUSSION

Dankert *et al* reported the inhibitory effect of *Allium sativum* against two yeast species by agar diffusion method. *Allium sativum* showed inhibited

the activity of all tested organisms and *Allium sepa* showed no effect but the present study shows that the ethanolic extract of root of *Allium sativum* shows the smallest zone of inhibition which is 2.5mm but 15 % leaf extract of *Allium sepa* shows the highest antifungal activity which is 12mm and lowest is shown by 10% of leaf extract of *Allium sepa* which is 8.1mm. Ghahfarokhi *et al* evaluated the antifungal activity of aqueous extracts of *Allium sepa* and *Allium sativum* against *candida albicans*. All the extracts shows the considerably inhibitory effect, but in the present study *Allium sepa* shows less inhibitory effect which is shown by 5% leaf extract which is 0.9mm and *Allium sativum* shows the highest antifungal activity Motesi *et al* reported the antifungal activity of *Allium sativum* bulb extract had MIC of 0.56% only at 4°C. At high temperature *Allium sativum* lost their activity, but in the present study *Allium sativum* do not lost their activity against *Rhizopus* at 37 °C. Benkeblia reported inhibitory effect of *Allium sepa* and *Allium sativum*. *Allium sativum* showed the highest inhibitory effect and *Allium sepa* showed the lowest the inhibitory effect, but in the present study *Aloe vera* shows the highest antifungal activity than *Allium sepa* against *Rhizopus*. Tongbram *et al* reported the inhibitory effect of *Allium sativum* and other plants but *Allium sativum* showed the highest antifungal activity against *Fusarium udam* at different concentrations. *Allium sativum* at 20% alone recorded 100% inhibition of mycelial growth and spore germination, but in the present study among the three concentrations *Allium sativum* shows the highest antifungal activity which is 13.6mm in 15% root extract while in 10% *Allium sativum* has the lowest antifungal activity which is 6.8mm.

CONCLUSION

It was concluded that *Aloe vera* and *Allium sativum* can be helpful in the treatment of fungal diseases caused by *Rhizopus*. And it also used as antimicrobial agents in various food products.

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