Research Article

Phytochemical analysis, antifungal bioassay and folklore uses of selected medicinal plants of family Rosaceae

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Abstract

Keeping in view the traditional uses of medicinal plants, this study was conducted to evaluate the antifungal activity of selected members of family *Rosaceae* for the first time. Activity of *Rosa indica* L. *Prunus amygdalus* L. and *Prunus armeniaca* L was evaluated against the *Penicillium digitatum* L. Antifungal activity of the aforementioned plant extracts were indicated by the measuring zone of inhibition on agar plate. Highest activity against the test strain of fungus was recorded in ethanol extracts of the selected plants. Antifungal activity of extracts from the selected plants was dependent on the concentration of the extracts with highest activity being recorded when its strength was 15%. Maximum zone of inhibition (19.6 mm) was formed against the test fungus exposed to 15% root extract of *R. indica* L. taken in ethanol. However, aqueous root extract of the same plant was less effective indicating that the bioactive compound(s) in the root were of relatively low polarity. Bioactive compounds of the other two plants were also of low polarity as their activity was relatively greater when extracted in ethanol than that in aqueous extract. Contrary to *R. indica*, Leave of *P. armeniaca* were seat of high amount of bioactive compounds. While flavonoids and tannin were recorded in all samples, alkaloids and carbohydrates were found only in *R. indica* L. Similarly, fixed oil and fats were present in *P. amygdalus* L. and *P. armeniaca* L only. According to antifungal activity the aforementioned plants can be arranged as *R. indica* L.>*P. armeniaca* L.>*P. amygdalus* L. It may be concluded that these plants may be of great medicinal value and may provide a source of valuable novel compounds.

Key words: Medicinal plants; antifungal activity; *Penicillium digitatum* L.

Introduction

Medicinal uses of the plants have been explored through the observations and experimentations of the different communities of the world. The Holy Quran

is the best reference book describing the significance of plants used for different ailments in various Surah's. Honey, the sweetest and tasty, which is the product of different plant species, is mentioned in the

Holy Quran to evaluate its medicinal importance [1]. The first manuscript about medicinal plants dates back to 3000 BC and was written by the Sumerians [2]. The earliest drug of the traditional folklore that became a modern drug by the end of the 18th century was *Digitalis purpurea* L. This great achievement means the inauguration of the modern pharmacology [3]. The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannin, flavonoid and phenolic compound [4]. Higher plants for antifungal activity has declared that plant extracts have the potential to reduce or completely stop the growth of fungi. These extracts can prove to be effective and safe antifungal agents than synthetic fungicides [5] (Albiero *et al.*, 2002). Valinton *et al.* [6] evaluated the antimicrobial activity of *Euphorbia hirta* against, *Staphylococcus aureus*, and *Candida albicans* by agar disc diffusion method. Ethanol and aqueous extracts of the plant material were prepared. Medium used for fungi was potato dextrose agar medium. Ethanolic extracts showed good results. Bobbarala *et al.* [7] reported that medicinal plants form the basis of traditional medicines. These medicinal plants are readily available in rural areas. They produce secondary metabolites which act as strong antimicrobial agents. In this research the antifungal activity of 49 different medicinal plants used as traditional

medicines was evaluated against *Aspergillus niger*. Agar well diffusion method was used for this purpose against fungal strains and can be used for the control of that fungal strain [8] assayed the antifungal activity of alcoholic and aqueous extracts of 19 medicinal plants including *Curcuma longa*, *Cassia fistula*, *Solanum nigrum*, *Calotropis procera* etc against two fungi *Candida albicans* and *Aspergillus niger*. The method used was agar well diffusion method. Fluconazole was used as standard drug for antifungal activity. The alcoholic extracts showed significant antifungal activity with *Curcuma longa* showing the highest degree of antifungal activity. Shaik [9] investigated the antifungal activity of aqueous, acetone, methanol and ethanol leaf and stem extracts of *Argyrea involucre* against three fungi including *Aspergillus niger*, *Aspergillus flavus* and *Candida albicans*. Acetone, methanol and ethanol extracts were found effective against *Aspergillus niger*. Co-trimoxazole and Fluconazole were used as standard antibiotics. These results suggest that they can be used in treating infections caused by the test organisms.

Pakistan has a diverse flora containing about 6000 plant species. About 80% of the people belonging to the rural areas still depend upon the local herbal medicines [10] (Ahmad, 1999). It is an old practice to extract and process the medicinal plants for daily human use, local economic uplift and for animal treatment ([10] Ahmad, 1999; [11]. The local uses of these selected plants are listed in the Table 1.

Table 1. The local uses of the selected plants

S. No.	Plant	Part used	Local Use
1	<i>Rosa indica</i> L	Flower	Emolient, Demulcent, water rose use in stomach problems in sore eyes
2	<i>Prunus amygdalus</i> L	Fruit	As a tonic to increase mental power use in constipation
3	<i>Prunus armeniaca</i> L	Fresh and Dry fruit	As appetizer in constipation

Materials and methods

Collection of plant material

Three different members of family *Rosaceae* were selected for evaluation of antifungal activity. These include the *R. indica* L., *P. armeniaca* L., *P. amygdalus* L. Plants were collected from different areas of district Mardan, Khyber-Pakhtunkhwa, Pakistan. These plant specimens were taxonomically identified through literature using Flora of Pakistan [12]. After identification, fresh plant parts (root, stem and leaves) were collected in bulk. They were washed thoroughly and separately under running tap water to remove adhering dirt followed by rinsing with distilled water for 2-3 times. Then the plant materials were placed in sunlight for two hours. After this, the surface was sterilized with 95% ethanol for twenty minutes. Again the plant parts were washed thoroughly with distilled water (three times) and then placed in sunlight for 15 minutes. The plant materials were air dried at room temperature.

Ethanol extract

The Ethanol Extract was made by soaking 10 g of each powdered plant material in a solution of 40 mL of 95% ethanol (C_2H_5OH). The mixture was allowed to stay for 48 hours at room temperature. The resulting liquid was filtered using Whatman No.1 filter paper. The residue left was further extracted using the same solvent and procedure. Then the solvent from the filtrate was evaporated to dryness so that organic compounds remain in semi-dry form. After evaporation the dried residue was weighed and stored in sterile containers [13-15].

Aqueous extract

For extraction with water, 10g of powdered plant material was dissolved in 40 mL sterilized distilled water to make aqueous extract (25% w/v) and boiled it for 10 minutes. Then, the mixture was subjected to filtration through sterilized Whatman no.1 filter paper. After filtration, the water was

evaporated in water bath until semi-dry fluid was left in the container [16, 17].

Antifungal activity using agar well diffusion method

The extracts (alcoholic and aqueous) of 3 plants were subjected to antifungal screening procedure by agar well diffusion method [18]. Three concentration grades of the 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.

Phytochemical tests

Test for Alkaloids

The alcoholic extract was evaporated to dryness and the residue was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Mayer's reagent. The samples were then observed for the presence of turbidity or yellow precipitation [19].

Test for Flavonoids

0.5 g of the ethanolic plant extract was shaken with petroleum ether to remove the fatty materials (lipid layer). The defatted residue was dissolved in 20 ml of 80% ethanol and filtered. 3 ml of the filtrate was mixed with 4 ml of 1% potassium hydroxide in a test tube and the colour was observed. A dark yellow colour indicated the presence of flavonoids [20].

Test for Tannin

To 0.5 ml of extract solution 1 ml of water and 1 - 2 drops of ferric chloride solution was added. Blue color was observed for Gallic tannins and green black for catholic tannins [21].

Test for fixed oil and fats

Spot test-A small quantity of extract was pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oils [22].

Test for Carbohydrates

Few drops of Molisch's reagent was added to each of the portion dissolved in distilled water, this was then followed by addition of 1 ml of conc. H_2SO_4 by the side of the test tube. The mixture was then allowed to stand for two minutes and then diluted with 5 ml of distilled water. Formation of a red or dull violet color at the interphase of the two layers was a positive test [20].

Results

Phytochemical test were carried out for alkaloids, flavonoids, tannin, carbohydrates, fixed oils and fats. Phytochemical analysis showed the presence of flavonoids and

tannin in all samples, alkaloids and carbohydrates were found only in *R. indica* L. whereas fixed oil and fats were present in *P. amygdalus* L. and *P. armeniaca* L.

Results obtained in the present study revealed that the tested three medicinal plants extract possessed potential antifungal activity against *P. digitatum*. Extracts prepared were different in the strength of their phytotoxic effects. All the concentrations of the plant extracts showed strong activity against the test pathogen in concentration dependent manner (Fig. 1, 2, 3, 4, 5 and 6).

Figure 1. Zone of inhibition shown by the ethanolic leaf extracts of the three plants

Figure 2. Zone of inhibition shown by the aqueous leaf extracts of the three plants

Figure 3. Zone of inhibition shown by the ethanolic stem extracts of the three plants

Figure 4. Zone of inhibition shown by the aqueous leaf extracts of the three plants

Figure 5. Zone of inhibition shown by the ethanolic root extracts of the three plants

The plant extracts, taken in ethanol, showed considerable inhibitory effect, ranged from 4-19.6 mm against the test fungus. On the other hand, activity of the aqueous extract was relatively lower, ranged from 4.2 -14.6 mm in well diffusion assay. The highest zone of inhibition was observed in 15% ethanol extract of root of *Rosa indica* L. (Table 2) and the smallest zone of inhibition was detected in 5% ethanol extract of stem of the same plant (Table 2). Sporulation was also observed in some of the Petri plates.

Ethanol extracts

Extract taken in ethanol was the most toxic for the test pathogen reducing its growth by varying degrees. Greater the concentration of the plant extract high was the activity against the pathogen. We test three different concentrations of extracts from root, stem and leave i.e. 5 %, 10 % and 15 %. Roots of *R. indica* were the most potent source of antifungal compounds resulting in growth inhibition of the pathogen in a sphere of 19.6 mm around the well on agar plate. Compared to stem, concentration of bioactive compounds was greater in leaves of this plant. Zone of inhibition produced by leaf extract of *R. indica* was broader (14 mm) than that of the stem extract (Table 2). In case of *P. armeniaca*, highest activity against the test strain was recorded in 15 %

ethanolic extract from stem as compared to that in other parts. Similarly, activity of 15 % extract of root, stem and leaves was almost similar (Table 2b).

Aqueous extracts

The aqueous extracts generally showed less inhibitory activity against the fungus as compared with that of the ethanol extracts. The highest zone of inhibition (14.6 mm) in case of water extract was detected in 15% stem extract of *P. armeniaca* L. (Table 2). Similarly, 10% leaf extract of *P. amygdalus* L. showed the least activity of all (Table.2a). Among the aqueous leaf extracts, 15 % extract of *P. armeniaca* L. produced 12 mm wider zone of inhibition against the test strain. Activity of 10% leaf extract of *P. amygdalus* L. (Table 2) was lowest as indicated by a zone of 4.2 mm. In case of stem extracts, 15% stem extracts of *P. armeniaca* L. produced widest zone of inhibition (14.6 mm) against the pathogen. Similarly, an inhibitory zone of 14 mm diameter was recorded on agar plate containing 15 % aqueous extract taken from roots of *R. indica* L. (Table 2). Out of 27 plant extracts tested for their antifungal activity, *Rosa indica* L. showed most promising antifungal activity and its ethanol extract was more effective as compared to aqueous extract. Similar results were also

obtained in the extracts' of the other two plants. Both the ethanol and aqueous extracts of *Prunus amygdalus* L. did not show any inhibitory activity against *Penicillium digitatum* L. The results showed that inhibition of microbial growth was

greater in the root extract of the plants irrespective of the type and the solvent used. Stem and leaf extracts comparatively showed lesser inhibitory activity irrespective of the type of solvent and plant part used (Figures 1-6).

Table 2. Antifungal activity of extracts of *Rosa indica* L., *Prunus amygdalus* L. and *Prunus armeniaca* L. showed considerable inhibitory effect against the tested

Diameter of zone of inhibition (mm)									
Extract conc.(g/mL)	<i>Rosa indica</i>			<i>Prunus armeniaca</i>			<i>Prunus amygdalus</i>		
	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf
Control(Water)	10	11	14	11	11	13	11	13	14
Control(Ethanol)	15	14.6	12	13.3	14	13.6	16	15	14.5
Table 2a Aqueous extract									
5%	11.8	8	9.6	11	10	9	7.8	0	0
10%	12.3	10.6	10.5	12.3	13.3	10.5	10	6	4.2
15%	14	14	10	13	14.6	12	11.5	13	8
Table 2b Ethanol extract									
5%	15	4	11.6	9	4.33	10	11	0	11.5
10%	18	6.33	12	8.6	8.3	11	11.5	4.33	12
15%	19.6	8.3	14	12.6	15	12.5	13	12	14

Discussion

The present study was carried out to study the phytochemical analysis and antifungal activity of the ethanol and aqueous extracts of three plants of family *Rosaceae* (*R. indica* L., *P. amygdalus* L. and *P. armeniaca* L.) against *P. digitatum* Lin view of local uses of these plants. Three concentration grades of the root, stem and leaves of each plant extract were checked against *P. digitatum* L. Results obtained in the present study revealed that the tested three medicinal plants extracts possessed potential antifungal activity against *P. digitatum* L. All the crude extracts had noteworthy antifungal activities against the fungus but the activity of inhibition varied for the fungus 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. Naturally occurring water-soluble components in most

plant materials include thiocyanate, nitrate, chlorides and sulphates, starches and tannins, saponins, terpenoids, polypeptides and lectins [23]. Phytochemicals having solubility in ethanol include tannins, polyphenols, polyacetylenes, flavonol, sterols and alkaloids [24]. In the present study, ethanol and water were used as extraction solvents for preparation of all the extracts. The antifungal activity of weed species including *Chenopodium murale*, *Crepisa spera* and *Ranunculus asiaticus* against *Penicillium digitatum*. *Erodium cruciatum*, *Euphorbia helioscopia* showed varied degree of inhibition and inhibited their growth and sporulation at any period of incubation [25]. Shinde *et al.* [26] worked on the antifungal activity of five *Terminalia* species (*Terminalia alata*, *Terminalia aarjuna*, *Terminalia bellerica*, *Terminalia catappa* and *Terminalia chebula*) against five plant pathogenic fungi like *Aspergillus*

flavus, *Aspergillus niger*, *Alternaria brassicicola*, *Alternaria alternata* and *Helminthosporium tetramera*. Aqueous, alcoholic and ethyl acetate extracts of leaves of these species were assayed by paper disc diffusion method. Most of the extracts showed significant inhibitory zones. From the results it is clear that all the ethanol extracts proved to be more effective against the fungus as compared to the aqueous extracts. Among the ethanol extracts 15% ethanolic extract of root of *Rosa indica* L. (Table 1) showed the highest inhibitory activity that is, 19.6 mm while the lowest inhibitory was observed in 5% stem extract of *Rosa indica* L. that is, 4mm (Table 1). Among the aqueous extracts highest inhibitory activity was shown by 15% stem extract of *Prunus armeniaca* L. (Table.1) and the lowest inhibitory activity was observed in 10% leaf extract of *Prunus amygdalus* L. That is, 4.2mm. Moreover hypha growth and sporulation have also been observed in most of the aqueous extracts and few ethanol extracts. Thonglem *et al.* [27] reported that *Penicillium digitatum* is the chief cause of green mold diseases in orange trees. Aggressive microorganisms were isolated from the various parts of healthy orange trees. A total of 121 isolates were collected. Among these 15 isolates showed inhibitory effect against *Penicillium digitatum* by agar disc diffusion method using culture filtrate from nutrient broth. The effect of culture filtrate on spore germination was also tested. Some of the

isolates showed significant antifungal activity. These isolates were of *Bacillus pumilus*. Results revealed plant rich in tannin and phenolic compounds have been shown to possess antimicrobial activities against a number of microorganisms. Roses are rich in these compounds so they greatly inhibit the growth of the fungus. The other two plants comparatively showed lesser inhibitory activity. It is also a common observation that *Rosa indica* L. flourish well in any type of climatic conditions and show resistance to certain types of fungi. The remaining two species *Prunus armeniaca* L. and *Prunus amygdalus* L. are easily susceptible to fungal attacks. Antifungal activity of *Aloe vera* was analyzed against *Aspergillus flavus* and *Aspergillus niger* and the method used was agar diffusion method. The maximum antifungal activity was observed in acetone extracts when compared with other extracts. They use three different solvents for the extraction such as water, ethanol and acetone [28].

Phytochemical constituents such as alkaloids, flavonoids, tannins carbohydrates, and oil and fatty compounds are secondary metabolites of plants that serve a defense mechanism against predation by many microorganisms, insects and other herbivores. The present study carried out on the plant samples revealed the presence of medicinally active constituents. The phytochemical constituents of the selected plants investigated are summarized in Table 3.

Table 3. Qualitative Phytochemical tests of the selected plants of family Rosaceae

S. No.	Botanical Name	Parts used	Alkaloids	Flavonoids	Tannin	Fixed oil and fats	Carbohydrates
1	<i>Rosa indica</i> L	Flower	+	+	+	-	+
2	<i>Prunus amygdalus</i> L	Fruit	-	+	+	+	-
3	<i>Prunus armeniaca</i> L	Fruit	-	+	+	+	-

Conclusion

The present study was undertaken and supports that traditionally use of raw plant extract either singly or in combinations, suggests that medicinal plants have plenty of curative properties that could be significantly used in improving human disorders which lead to infectious disease like cancer.

Authors' contributions

Conceived and designed the experiments: S Akhter, Performed the experiments: S Akhter, Analyzed the data: HA Begum, Contributed reagents/ materials/ analysis tools: M Hamayun & M Shakeel, Wrote the paper: HA Begum & T Yaseen.

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