ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

EVALUATION OF ANTIFUNGAL ACTIVITY OF PLANT EXTRACTS AGAINST THE FUNGAL DISEASES OF POMEGRANATE

M. LAKSHMI SRI, *SANDHYA DEEPIKA, D. and LAXMI SOWMYA, K.,
(Department of Botany, Andhra University, Visakhapatnam- 530003, Andhra Pradesh.)

Manuscript Info

Manuscript History:

Received: 15 May 2014
Final Accepted: 19 June 2014
Published Online: July 2014

Key words:
Pomegranate, anti fungal activity, plant extracts, fungal diseases

Corresponding Author

SANDHYA DEEPIKA, D

Abstract

In this present study Anti fungal activity of 10 plant extracts are used for examined against the fungal disease of pomegranate which is Cercospora, Sphaceolema, Certocystis fimbriata, Collitotrichum gleosporides, Fusarium solani, and Fusarium oxysporum, using agar well diffusion method. The Hexane, Choloform, Methanol, extracts of 10 plant extracts exhibited varying degrees of inhibition activity against the fungal pathogens of Pomegranate. Among the all plant extracts 9 plants shows low activity while Tribulus terristris methanol extract shows high activity inhibition zones

Introduction

Pomegranate (Punica granatum) is an important fruit crop it is also known as "fruit of paradise" belongs to the family Punicaceae. It is grown since ancient times for its fruit, ornamental and medicinal purposes. Pomegranate is a high value crop and has great therapeutic uses(Julie Jurenka, 2008) Though it's native is Iran cultivated extensively in Spain, Morocco, Egypt, Afghanistan, Arabia and other Mediterranean countries. In India, Pomegranate is commercially cultivated in Maharashtra, Karnataka, Andhra Pradesh and Himachal Pradesh (Chadha, K.L., 2001) Pomegranate is an important fruit crop of Maharashtra. Demand in the international market has widened the scope for earning higher dividends from this crop and there has been a steady increase in cultivation area for production of pomegranate in the country. Pomegranate fruits are good source of carbohydrates and mineral such as Ca, Fe and S and a moderate source of pectin (Waskar, 2006)

Pomegranate suffered from several fruit rot diseases (Kanwar and Thakur, 1973) caused by fungi, bacteria and nematodes. Among them some post-harvest and fungal diseases caused by these pathogens such as Cercospora, Sphaceolema, and wild complex by Certocystis fimbriata, Anthracnose by Collitotrichum gleosporides, fruit rot by Alternaria alternate and other fungal disease caused by Fusarium oxysporum and Fusarium solani. However, infected fruits generally are slightly off-color such as a pale red and may show some yellowish to brownish – red discoloration. In the storage period of the pomegranate faced with a lot of rot diseases. Many fungicides are used against these diseases and these fungicides leads to residue on the fruit. The use of herbal plant extracts gradually becoming a method of choice is management of plant diseases. This study is also important for human health, pomegranate storage problems, marketing and pomegranate production economy.

Materials and Methods:

Collection of Plant Material:

For the present study, ten plant species (Table-1) were selected based on the information collected from literature and through field observation. The plant materials were collected from Visakhapatnam District, Andhra Pradesh, India. Taxonomic identification of the plants was done by the reference to the flora of Visakhapatnam by Venkateswarlu et al. (1972) as well as comparison with the authenticated herbarium specimens available in the Botany department, Andhra University. The names adopted were the latest valid ones. Whole plants were screened for their antifungal activity. The collected materials were washed thoroughly under running tap water, finally with sterile distilled water and then the materials were air dried on a sterile blotter under shade. (M. K. Khokhar 2012)

Solvent Extraction of Plant Material:

The completely shade dried plant materials were coarsely powdered using electric blender. Then they were allowed to soxhlet extraction separately and successively with hexane, chloroform and methanol solvents. Later the soxhlet material was subjected to distillation to remove the solvent. After distillation different extracts obtained were concentrated with Rotary evaporator and brought to complete dryness over a water bath to yield the crude extract.

Collection of Microbial Cultures:

Infected pomegranate plant parts were collected and identified by the National Research Centre on Pomegranate, solapur, Maharashtra, India.

The Infected parts were cut, crushed in sterile saline and streaked on sterile potato dextrose agar (Hi-media) plate containing streptomycin (50µg/ml). The plates were incubated at 30^0c for 5 days. The isolated fungal cultures were identified based on morphology and maintained on sterile malt extract agar salt at 40^0c.

Preparation of Media and plates for Agar Diffusion Method

Agar well diffusion method is used (Perez et al., 1990) to screen for antifungal activity of the hexane, chloroform, and methanolic extract of 10 plant species. Dimethyl sulfoxide (DMSO) is used as solvent to dissolve the crude extract. The tested strain of fungal broth cultures was inoculated into the 20ml of potato dextrose agar medium. After the medium was solidified a well or cup was made in petriplates with the help of a cup borer of 6mm in diameter. The plates and each well was marked with marker. To determine the potential of the hexane, chloroform, and methanolic extracts, they were diluted up to 500mg/ml, 250mg/ml, 100mg/ml, 50mg/, 25mg/ml, 10 mg/ml, 5mg/ml, in DMSO solution. 40µl of each concentration was poured into the well according to the markings made on the plate and allowed to diffuse into the medium. Then plates were incubated at 25^0c for 36hrs (Seetha Lakshmi 2007). After proper incubation of cultures the fungal growth was determined by measuring the diameter of the zone of inhibition around the well/cup by using the Hianibiotic Zone Scale. The standard antifungal drugs mancozeb are used to determine the minimum inhibitory concentrations for comparison. In the present study mancozeb was used as positive control and streptomycin as antibiotic to inhibit the growth of bacteria.

Result and Discussion:

The effect of different extracts of selected plants was observed inhibition on fungal pathogens of pomegranate. All the preparations tested have shown antifungal activity against the selected fungal pathogens of pomegranate. Total 10 plant extract were screened for their antifungal properties at different concentrations. Out of 10 plant extracts nine plant shows very low activity at (500mg/ml) in different solvents such as hexane, chloroform, methanol. The methanol extract of Tribulus terristris have shown greater activity when compared to chloroform, hexane extracts at (5mg/ml).The largest inhibition zone has shown by Tribulus terristris with the whole plant methanolic extract against all the tested pathogens of pomegranate (Table-2).

The selected plants were well known for their antimicrobial properties, but most of the study was carried out with human pathogens (Almas k.2001), (kulkarni Dr. 1991).

Table 1: List of the plants collected and their parts used for different ailments.

S.No	Botanical Name	Family	Local/Vernacular Name	Parts Used	Uses
1.	Areva lenata(L.)	Amaranthaceae	Pindicura, Pindichettu	Whole plant	The plant is used for the treatment of snake bite, this plant is used for food for people. In the whole plant only the leaves are edible.
2.	Blumea mollis	Asteraceae	Kukka pogaku	Leaves, stem	This plant is for traditional & herbal medicine
3.	Eclipta alba(L.)Hassk	Asteraceae	Guntagalagaraku	Whole plant	It is used in ayurveda & sidda for the treatment of kapha and vata imbalance. A preparation obtained from leaf juice boiled with sesame or coconut oil is for anointing the head to render the hair black and luxuriant
4.	Jatropha carcus(L.)	Euphorbiaceae	Nepalam	Leaves & stem	The leaves, seeds and oil is used to treat ulcer, tumour, scabies, wound, skin diseases, seeds are used for bio-fuel.
5.	Leucas aspera	Lamiaceae	Thummichettu	Whole plant	Leucas aspera is reported to have antifungal, anti oxidant and cytotoxic activity. It is used in the traditional medicine. It is a herb that has the ability to reduce fevers. This plant is used commonly as an insecticide.
6.	Phyllanthus amarus	Phyllanthaceae	Nela Usiri	Whole plant	This plant is used in Indian Ayurvedic system of medicine which is used for problems of the stomach, liver ,kidney.
7.	Thespecia papulnia(L) sol.	Malvaceae	Gangaraavi	Bark & leaves	Bark is used to treat skin diseases. Leaves are applied to inflamed and swollen joints. Young fruit secrets a yellow stocky sap used to treat ringworm and other skin diseases, roots used tonic
8.	Triumfetta pantandra(L)	Malvaceae	Chirusitrika	leaves	The crushed leaf is applied in dressings for treatment of goiter
9.	Tribulus terristris L.	Zygophyllaceae	Palleru	Whole plant	It is best known for its clogging effects on. It is used in tonics in Indian Ayurvedic practice, where it is known by its Sanskrit name,' Gokshura Surrata' .Also used as an aphrodisiac, diuretic and nervier in Ayurveda, Unni or in another medical system in India.
10.	Tridax procumbens L.	Astaraceae	Gaddi chamanthi	Whole plant	It is used to treat Inflammation, hepatic disorder, wounds, microbial infections, reduce immunity, arthrosclerosis and neurological disorder

Table 2: Antifungal activity of Tribulus terristris

S. NO	Name of the pathogen	500			250			100			50			25			10			5			
		H	C	M	H	C	M	H	C	M	H	C	M	H	C	M	H	C	M	H	C	M	
1	Cercospora	nil	nil	21	nil	nil	19	nil	nil	18	nil	nil	17	nil	nil	15			14	nil	nil	13	High
2	Certocystis fimbriata	nil	nil	20	nil	nil	19	nil	nil	19	nil	nil	19	nil	nil	16	nil	nil	15	nil	nil	13	High

3	Sphaceolema	nil	nil	20	nil	nil	19	nil	nil	18	nil	nil	18	nil	nil	15	nil	nil	14	nil	nil	13	High
4	Alternaria alternata	nil	nil	19	nil	nil	18	nil	nil	18	nil	nil	17	nil	nil	16	nil	nil	14	nil	nil	13	High
5	Collitotrichum gleosporides	nil	nil	19	nil	nil	18	nil	nil	17	nil	nil	17	nil	nil	16	nil	nil	16	nil	nil	14	High
6	Fusarium oxysporum	nil	nil	19	nil	nil	18	nil	nil	18	nil	nil	15	nil	nil	14	nil	nil	14	nil	nil	13	High
7	Fusarium solani	nil	nil	19	nil	nil	18	nil	nil	18	nil	nil	17	nil	nil	17	nil	nil	15	nil	nil	13	High

H = Hexane
C = Chloroform
M = Methanol

Table 2: Zone of inhibition has shown by Tribulus terristris with the whole plant methanolic extract against all the tested pathogens of pomegranate

Conclusion

From the present study, it is concluded that the methanolic plant extract of Tribulus terristris have been proved to be more effectively inhibiting on tested pomegranate pathogens than the standard drug mancozeb taken for comparison. Although the nature and number of antifungal compounds involved in each extract of the present study is not fully discovered but the broad spectra of activity of these chosen plant extracts were promising. It requires further research to investigate and isolate pure compounds.

References

1. Almas K. The antimicrobial effects of seven different types of Asian chewing sticks, Odontostomatd Trop 2001;24:17-2
2. Chadha, K. L., Pomegranate. In: Handbook of Horticulture (Eds.) Shashi, A Verma and Somdutt, pp 297, Directorate of information and Publications of Agriculture, ICAR, New Delhi, 1031 (2001)
3. Julia Jurenka (MT 9ASCP) (2008): Therapeutic applications of pomegranate(punica granatum L.): A review , Alternative Medicine Review Vol.13 No.2
4. Kanwar ZS , Thakur DP (1973). Some new fruit rots of pomegranate in Haryana. Sci and Culture .36: 274-276.
5. Khokar MK, Gupta R, Sharma R, (2012) Biological Control of Plant Pathogens using Biotechnological aspects.
6. Perez C and Paul M and Bezique P(1990) An Antibiotic assay by the agar well diffusion method. Alta biomed. Group experiences. 15, 113.
7. Seetha Lakshmi B 2007. A search for antimicrobial agents in plant resources against the pathogens of Zea mays L. and Elevisine coracana (L.) Gaertn. Crops. (Unpublished thesis.
8. Udupa SL, UdupaAL, Kulkarni DR. Influence of (Tridax procumbence) on Lysyl oxydase activity and wound healing. Planta Med 1991;57:325-7.
9. Waskar DP (2006). Pomegranate (punica granatum) . Advances in arid Horti, Vol. II International Book Distributing Company, Lucknow. Pp. 375-394.

Venkateswarlu, J., Bhiravamurthy, P.V. and Narasimha Rao, P. 1972. The flora of Visakhapatnam. A.P. Akad. Sci., Hyderabad