



Antifungal activity of selected plant extracts against three pathogenic fungi of *Gossypium herbaceum*

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Abstract

The aim of the study was to evaluate the antifungal activity of seven different plant extracts against pathogenic fungi of cotton viz. *Alternaria alternata*, *Chaetomium globosum* and *Fusarium oxysporum*. Cotton (*Gossypium herbaceum* L.) is an important cash crop of Gujarat and Maharashtra in India. Methanolic fractions of selected plants exhibited more promising results than aqueous fractions in suppressing the fungal growth.

Key words – *Gossypium herbaceum* – Antifungal activity – Methanolic extract – *Alternaria alternata* – *Chaetomium globosum* – *Fusarium oxysporum*

Introduction

India is the largest consumer of pesticides in the world. Pesticides which include insecticides, fungicides, herbicides, rodenticides and fumigants are undoubtedly the largest group of toxic chemicals that are introduced profusely into the environment. They are defined as any substance or mixture of substances used for preventing, destroying, repelling or mitigating the pest. Most of the chemicals products fall within four main categories viz. organochloride insecticides, organophosphate insecticides, carbamate insecticides and pyrethroid. Pesticides have an innate capacity to cause damage to the biological system, which may involve human health or environment. The most dramatic of such effects on human are accidental acute poisoning (Sinha & Choudhary 2008).

Synthetic fungicides are currently used as the primary means for the control of plant diseases. However, the alternative control methods are needed because of the negative public perceptions about the use of synthetic chemicals, resistance to fungicides among fungal pathogens, and high development cost of new chemicals. Some fungicides are not readily biodegradable and tend to persist for years in the environment. This leads to third problem, the detrimental effects of chemicals on organisms other than target fungi. Because of these problems associated with the use of chemicals, researches are now trying to use environmentally safe alternative methods of fungal control.

The commonly used synthetic fungicides have been found to display side effects in form of carcinogenicity, teratogenicity and pollutive effects. Uses of less harmful and true eco – friendly products of plant origin are replacing the routine fungicides (Fawcett & Spencer 1970, Khanna & Chandra 1972, Dixit et al. 1983, Arya & Mathew 1990, Arya et al. 1995).

Plants produce diverse range of pre-infectious metabolites including alkaloids, chalcones, flavanones, organic acids, saponins, sesquiterpene lactones, steroids, sulphur containing amides, and terpenoids, many of which display a broad spectrum antifungal activity (Ebel 1986). These secondary metabolites with no direct effect on the growth and development of plants in which they have produced, have a potential bioactivity (Nychas 1995) and are attempted as natural fungicides (Benner 1993). Wilkins & Board (1989) reported approximately 1400 plants as potential sources of microbial agents with different classes of compounds and several other metabolites from new plant species being identified every year (Eksteen et al. 2001, Aqil & Ahmad 2003, Qasem & Abu-Blan 1996, Ushiki et al. 1996). Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine (Mann et al. 2008, Bobbarala et al. 2009). Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs. The secondary metabolites of the plants are a vast repository of biologically active compounds. Plant products still remain the principal source of pharmaceutical agents used in traditional medicine (Ibrahim 1997, Ogundipe et al. 1998). The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world (Reddy et al. 2001, Ateb & ErdoUrul 2003). Much work has been done on ethno medicinal plants in India (Maheshwari et al. 1986, Negi et al. 1993).

Materials & Methods

Isolates and morphology

The effect of aqueous and methanolic leaf extracts were obtained by Soxhlet Extraction method of seven plants was observed on test organisms. It was tested on three different pathogenic fungi. The healthy leaves of *Annona reticulata*, *Balanites roxburghii*, *Cochlospermum religiosum*, *Gliricidia sepium*, *Ferronia limonia*, *Sapindus emarginatus* and *Tephrosia jamnagarensis* were collected and washed well and dried in oven at 60 C for 48 hours. The dried leaves were powdered and stored in plastic bags. Twenty grams of leaf powder was extracted in a Soxhlet extractor with 200 ml methanol for 8 hours. The extract was concentrated then the residue was treated with 20 % of methanol. It was added to dry residue and water soluble compounds were filtered out. The leaf extracts were mixed with appropriate volume of medium (PDA) to obtain concentrations ranging from 5 to 25 % in the final volume of 100 ml of medium. This 100 ml medium was dispensed into 100 mm Petri plates with triplicates (Nene & Thapliyal 1979).

Fungal isolates were placed in the centre of each plate. Control sets were also prepared without plant extract. The plates were incubated at 25 ±2 C and growth of colony was measured after 7 days of inoculation. The radial growth of mycelium was measured at two points along the diameter of the plate and the mean of these two readings was taken as the diameter of the colony. The growth of the colony in control sets was compared with that of various treatments and the difference was converted into percent inhibition by following formula

$$\text{Percent inhibition} = \frac{\text{Diameter of control set} - \text{diameter of treated set} \times 100}{\text{Diameter of control set}}$$

Results and Discussion

In the present study seven types of leaf extracts were used against three pathogenic fungi viz. *Alternaria alternata*, *Chaetomium globosum* and *Fusarium oxysporum*. Table 1 shows the list of plants used for the antifungal study.

Table 1 List of plants used as biocontrol against pathogenic fungi.

Plants Used	Family	Chemical Constituents
<i>Annona reticulata</i> L.	Annonaceae	Alkaloids, Flavanoids, Glycosides, Triterpenoides
<i>Balanites roxburghii</i> Planchon.	Zygophyllaceae	Alkaloids, Glycosides, Saponins, Flavones and Phenolic compounds.
<i>Cochlospermum religiosa</i> (L.) Alst.	Cochlospermaceae	Flavanoids, Phytosterols, Saponins and Tanins
<i>Gliricidia sepium</i> (Jacq.) Kunth ex Walp.	Papilionaceae	Tannins, Isoflavanoids like Aformosin, Formentin, Glyricidin A and B and Medicarpin.
<i>Limonia acidissima</i> L.	Rutaceae	Essential oil containing estragol, Flavone, 3'OMe-quercetin nad Phenolic acids like p-hydroxy benzoic, vanillic, syringic, p-coumaric and ferulic acids.
<i>Sapindus emarginatus</i> Vahl.	Sapindaceae	Alkaloids, Phenols, Flavonoids, Saponins.

Methanolic fractions exhibited more promising results than aqueous fractions in suppressing the fungal growth (Fig. 2). The periodic data regarding fungal growth, exposed to various concentrations of plant extracts of *Annona reticulata*, *Balanites roxburghii*, *Cochlospermum religiosa*, *Gliricidia sepium*, *Limonia acidissima*, *Sapindus emarginatus* and *Tephrosia jamnagarensis* are present in Table 2.

Table 2 Percentage inhibition of *Fusarium oxysporum* at different concentration of leaf extracts.

Plant selected	Methanolic Extract			Aqueous Extract		
	5%	10%	25%	5%	10%	25%
<i>Annona reticulata</i>	12.7	23.6	72.3	9.4	13.2	15.6
<i>Balanites roxburghii</i>	31.2	79.2	100	12.0	19.0	35.3
<i>Cochlospermum religiosa</i>	43.3	68.0	100	19.3	36.6	49.8
<i>Gliricidia sepium</i>	28.01	61.05	87.9	1.3	5.8	7.6
<i>Limonia acidissima</i>	32.19	61.91	100	3.2	9.0	16.3
<i>Sapindus emarginatus</i>	24.9	42.8	66.7	12.0	27.0	39.0
<i>Tephrosia jamnagarensis</i>	27.67	51.36	90.4	16.06	38.97	69.96

- Each compound values are based on three replicates

Results were significant at $P \leq 0.05$ level by one way ANOVA

Earlier Jamuna Bai et al. (2011) showed the antimicrobial activity of *Cochlospermum religiosum*. Sivia et al. (2002) showed the antifungal activity of *Annona reticulata* leaf and stem extract against *Colletotrichum gloeosporioides*. The presence of antibacterial substances in the higher plants is well established (Srinivasan, 2001). Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health.

Phytomedicine can be used for the treatment of diseases as is done in case of Unani and Ayurvedic system of medicines or it can be the base for the development of a medicine, a natural blueprint for the development of a drug (Didry et al. 1998). Successive isolation of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure.

Table 3 Percentage inhibition of *Chaetomium globosum* at different concentration of leaf extracts.

Plant Selected	Methanolic Extract			Aqueous Extract		
	5%	10%	25%	5%	10%	25%
<i>Annona reticulata</i>	27.2	23.6	72.3	6.17	16.7	62.0
<i>Balanites roxburghii</i>	100	100	100	18.0	30.16	62.7
<i>Cochlospermum religiosa</i>	100	100	100	56.7	78.6	100
<i>Gliricidia sepium</i>	39.18	63.48	100	28.2	41.5	50.3
<i>Limonia accidissima</i>	82.27	100	100	9.1	52.0	65.9
<i>Sapindus emarginatus</i>	17.5	37.4	100	18.0	30.16	62.7
<i>Tephrosia jamnagarensis</i>	65.2	86.5	100	37.4	46.5	70.9

* Each compound values are based on three replicates

Results were significant at $P \leq 0.05$ level by one way ANOVA

The variation in antifungal activity of the extracts in different solvents may be attributed to the different chemical nature of the solvents. It is likely that different types of chemical were dissolved in different solvents that resulted in variable activity of the extracts from the same part of the plant in different solvents (Tables 3, 4). *Fusarium oxysporum* showed 100% inhibition to three plant extracts *Balanites roxburghii*, *Cochlospermum religiosa* and *Limonia accidissima* followed by *Tephrosia jamnagarensis* which showed 90.4 % inhibition at 25% extract, minimum inhibition of 66.7 % was recorded in plant extract of *Sapindus emarginatus*. *Chaetomium globosum* showed 100% inhibition to two plant extracts *Balanites roxburghii*, *Cochlospermum religiosa* in all concentrations viz 5%, 10% and 25%, *Gliricidia sepium* *Limonia accidissima*, *Sapindus emarginatus* and *Tephrosia jamnagarensis* showed 100 % inhibition at 25% extract, maximum inhibition (72.3%) was recorded in plant extract of *Annona reticulata* in 25% concentration of methanolic extract.

Table 4 Percentage inhibition of *Alternaria alternata* at different concentration of leaf extracts.

Plant Selected	Methanolic Extract			Aqueous Extract		
	5%	10%	25%	5%	10%	25%
<i>Annona reticulata</i>	20.4	48.5	75.2	5.9	14.5	32.0
<i>Balanites roxburghii</i>	8.3	38.8	100	27.3	26.0	32.8
<i>Cochlospermum religiosa</i>	1.3	25	100	18.7	33.2	60.2
<i>Gliricidia sepium</i>	29.77	40.81	100	5.2	9.7	13.5
<i>Ferronia accidissima</i>	2.0	37.5	100	8	12	43
<i>Sapindus emarginatus</i>	11.3	33.1	65.0	7.8	12.2	15.3
<i>Tephrosia jamnagarensis</i>	5.0	21.0	100	5.0	19.6	52.5

* Each compound values are based on three replicates

Results were significant at $P \leq 0.05$ level by one way ANOVA

Alternaria alternata depicted 100% inhibition to five plant extracts *Balanites roxburghii*, *Cochlospermum religiosa*, *Gliricidia sepium*, *Limonia accidissima* and *Tephrosia jamnagarensis*, whereas *Sapindus emarginatus* showed minimum inhibition of 65 %. Leaf extracts of seven different plants were tested against three pathogenic fungi *in vitro* (Table 4). In most of the cases 25% methanolic extract was more effective than 5% and 10% (Fig. 1). From the above results, it is depicted that the extracts of *Balanites roxburghii*, *Cochlospermum religiosa* and *Limonia accidissima* were most effective in the against all three pathogenic fungi as compared to the other plants.

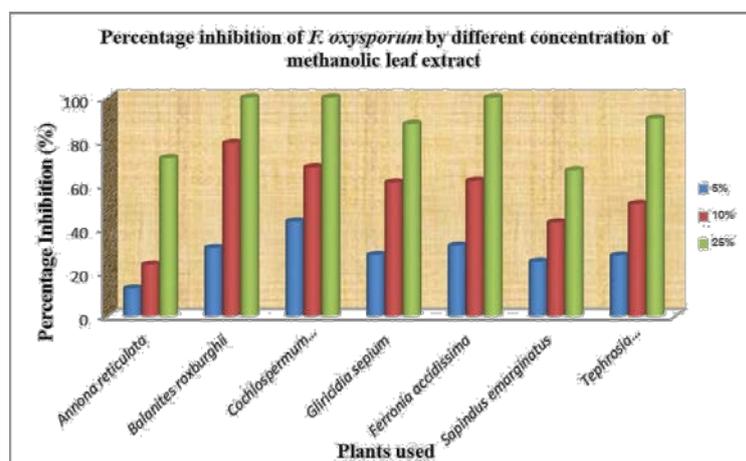


Fig. 1 – Percentage inhibition of *Fusarium oxysporum* by different concentrations of methanolic leaf extracts.

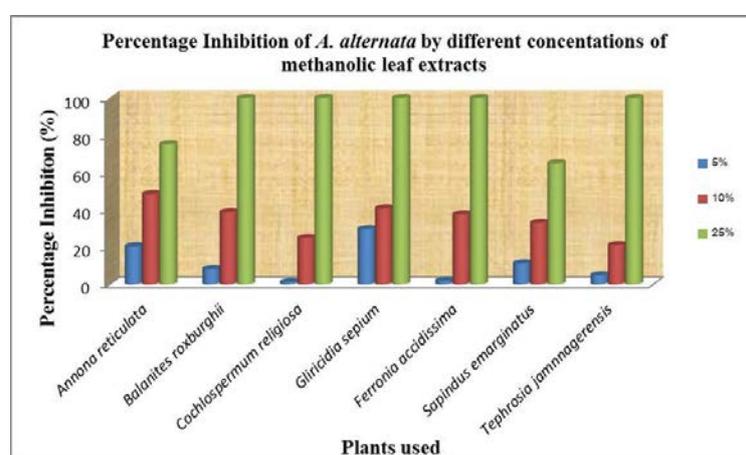


Fig. 2 – Percentage Inhibition of *Alternaria alternata* by different concentration of methanolic leaf extracts.

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