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RESEARCH ARTICLE

PHYTOCHEMICAL ANALYSIS AND ANTIFUNGAL ACTIVITIES OF AZADIRACTA INDICA AGAINST DIFFERENT FUNGI ISOLATED FROM INFECTED SOLANACEOUS VEGETABLE FRUITS.

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Key words:-
Azadiracta indica, antifungal activity, Phytochemical analysis, fungal isolation from solanaceous vegetables.

Abstract

Aqueous extracts of Azadiracta indica was subjected to invitro antifungal assay against different fungi, Fusarium oxysporum, Penicillium digitatum, Aspergillus niger, and Alternaria alternata. Plant leaves were effective against all the tested organisms. Leaf extract of Azadiracta indica contains pharmacologically active constituents that may be responsible for its activity against different fungi.The chemical constituents present in the neem plant makes it a doctor tree due to its wide scope in biological activities associated with it and has become a global context today. A qualitative phytochemical analysis was performed for the detection of seccondry plant metabolites viz, Alkaloids, Glycosides, Terpinoids, Steroids, Flavinoids, Tannins and reduing sugars.

Introduction:-

Azadiracta indica commonly called neem is very common in india having great medicinal value and spread every were in the world. Neem has been extensively used in Ayurveda, Unani and Homoeopathic medicine and has become a centre of attraction of modern medicine. Chemical constituents of neem exhibits great medicinal properties. These chemical constituents were extracted from neem that includes Alkoloids, Terpinoids, Phenolic compounds, Carotenoids, steroids and ketones. Neem plant contains important Azadirachtin chemical constituent which is seven isomeric compounds labelled as Azadirachtin A-G and Azadirachtin E is more effective (Verkerk et al., 1993). Other compounds that were also isolated were Salannin, volatile oils, and Nimbin (Jacobson et al., 1990, Ahna et al., 2005).The main active constituents of the plant are nimbin, nimbinin, nimbidin, limocinol, limocinone, azadirol, naheedin, azadironolide, limbocinin (Zulfikar et al., 2010). Non wood products like flowers, fruits, seeds, oil, leaf, bark and gums also find various uses (Satesh, 1998). Well known megosa oil has its use in treating ulcers, leprosy and sprain (Ananymous, 1992).According to a report of WHO, more than 80% of world's populations depend on traditional medicine for their primary health care needs. Extraction is the separation of medicinally active portions of plant tissues using selective solvents through standard procedures.The products obtained from these plants are complex mixtures of metabolites, in liquid or semisolid state or after removing the solvent in dry powder form, and are intended for oral or external use (Handa et al., 2008). The use of phytochemicals in the treatment of various types of diseases may lie in their antioxidant properties (Akinmoladun et al., 2007). Secondary plant metabolites are largely unexplored in 'conventional' animal production systems. In the past, plant metabolites were generally considered as sources of antinutritional factors. Recent bans and restrictions on the use of animal antibiotic growth promoters stimulated interest in bioactive secondary metabolites of plan source as alternative performance

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enhancers (Greathed., 2003).Quercetin (a polyphenolic flavonoid) is known to have antifungal properties. The neem constituent have been divided into two major sections viz. I) isoprenoids, II) non-isoprinoids. The later category comprises glycerides, polysaccharides, sulphurones compounds, flavonoids and their glycosides, amino acids, aliphatic compounds etc. The action of leaf extracts of neem against two tomato pathogenic fungi Alternaria solani and Fusarium oxysporum were carried out. Evaluation of the activity of neem oil against Fusarium oxysporum and Alternaria tenius showed that the active antifungal fraction is a mixture of tetranortriterpenoids.Rhizopus sp., and Aspergillus sp., were inhibited with the crude aqueous and alcoholic extract of different aged leaves of Azadiracta indica (Mondali et al., 2009).Phytochemicals have increased attention of researchers to use them against microbes as these products have been found to be degradable and safe. (Ghosh et al., 2008; Kumar et al., 2008; Behbahani et al., 2013). An important member of Meliaceae family Azadirachta indica (Neem) is well known for its unique characters of fast growth and resistance to the drought conditions (Dalziel, 1955). Recently, neem has been of ecological importance, it has antiseptic, antifungal, antibacterial, antipyretic, anti-malaria, anti-diabetic and anti-fertility properties among several other uses (Nok et al., 1993, Natarajan et al., 2003; Fredros et al., 2007; Mbaya et al., 2010).

Materials and Methods:-

Collection of plant material:-

The experiment was conducted recently in the mycological research laboratory RDVV Jabalpur. Leaves were collected from the Azadiracta indica tree in the University campus. Leaves used were healthy and uninfected. The leaves were washed under running tap water and dried at room temperature for seven days. Dried leaves were powdered with mixer for further use.

Preparation of leaf extracts:-

100 grams of fresh leaves were poured with 250 mL of different solvents for 3-4 days and kept in shaking incubator for two days. The extracts were filtered by using Whatmann filter paper No.1 and then concentrated in vacuum at 40°-50°C using a rotary evaporator. Evaporation of solvent in the rotary evaporator results crude extract from different solvents and these extracts were subjected to the phytochemical analysis and antifungal activities.

Phytochemical analysis:-

The extracts were analyzed by the following procedures (Talukdar and Choudhary 2010).To test for the presence of the following metabolites like alkaloids, tannins, terpenoids, flavonoids, glycosides, and reducing sugars.

Tannins:-

To a portion of the neem extract diluted with water, 3-4 drops of 10% ferric chloride solution is added. A blue colour is obtained which indicates the presence of tannins.

Glycosides:-

2.5ml of dilute sulphuric acid was added to 5ml extract in a test tube and boiled for 15 minutes, cooled and neutralized with 10%NaOH, then 5ml of Fehling solution added. Glycosides are indicated by a brick red precipitate.

Alkaloids:-

2ml of extract was measured in a test tube to which picric acid solution was added. An orange coloration indicated the presence of alkaloids.

Flavonoids:-

4ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red color was observed for flavonoids.

Terpenoids:-

Four milligrams of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet colour was observed for determining the presence of terpenoids.

Chloroform extract:- Azadiracta indica leaves (100 g) were grind into fine powder (Himal Pauel Chhetri et al., 2008) using a stainless-steel grinder, and used in 100% chloroform (250 mL) for overnight. The chloroform fraction

was separated using sterile muslin cloth and filter through sterile Whatman filter paper no. 1. The filtered extract was eveoprated to dryness by a rotary film evaporator.

Ethanol extract:-
For preparation of Ethanol extract powder plant sample (100 g) was added in Ethanol respectively (250ml each case) and left for overnight at room temperature (Puri et al., 1995). The extracts were separated using sterile muslin cloth and filter through sterile Whatman filter paper no.1. Filtered extract was eveoprated to dryness by a rotary film evaporator so as to get dry sample.

Methanol Extract:-
100 grams of powderd plant material was extracted with 100 ml of methanol kept on a rotary shaker for 24 h. Thereafter, it was filtered with muslin cloth and filter through sterile Whatman filter paper no.1. Filtered extract was eveoprated to dryness by a rotary film evaporator.

Distilled water extract:-
100 grams of powdered plant material was extracted with 100 ml of distilled water kept on a rotary shaker for 24 h. Thereafter, it was filtered with muslin cloth and filter through sterile Whatman filter paper no.1. Filtered extract was concentrated by a rotary film evaporator so as to get dry sample.

Source of microorganisms:-
The organisms were isolated from infected Solanaceous vegetable fruit viz., Fusarium oxysporum, Penicillium digitatum, Aspergillus flavus,and Alternaria alternata. The organisms were isolated from the Mycological research lab RDVV Jabalpur Madhya Pradesh India.

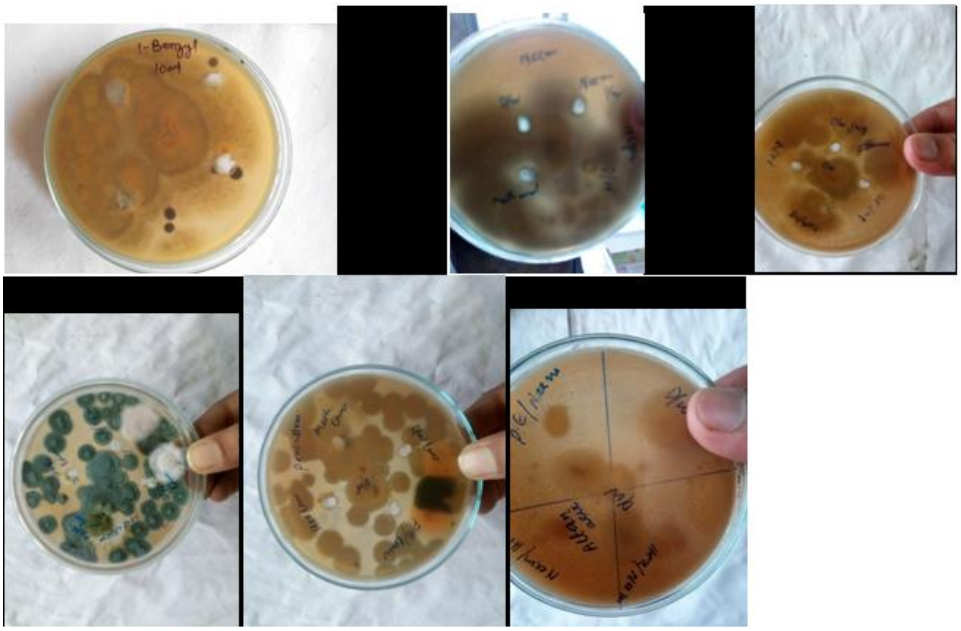
Determination of Antifungal Activity:-
The antifungal activity of the leaf extracts was determined using agar well diffusion method and Disc diffusion method. Potato dextrose agar was inoculated with the given microorganisms by spreading the fungal inoculums on the media. Wells were punched in the agar and filled with plant extracts. Control wells containing distilled water were also run parallel in the same plate. The plates were incubated at $28C^0$ for 3-4 days and the antifungal activity was assessed by measuring the diameter of the zone of inhibition. The antifungal potential of the different extracts was evaluated by comparing their zones of inhibition.

Results and Discussions:-

Plant	Extracts	Zone of Inhibition in mm															
		Aspergillus-flavus				Fusarium-oxysporum				Alternaria-alternate				Penicillium digitatum			
		24 H	48 H	72 H	96 H	24 H	48 H	72 H	96 H	24 H	48 H	72 H	96 H	24 H	48 H	72 H	96 H
Azadirachta indica	Chloroform	-	20	18	12	-	24	18	15	-	22	18	12	-	29	19	15
	Methanol	-	12	10	9	-	18	16	10	-	25	16	10	-	20	18	15
	Hexane	-	10	9	8	-	14	11	10	-	20	15	11	-	14	13	10
	D/W	-	12	9	9	-	12	10	9	-	10	9	9	-	12	11	9

Antifungal activity exhibited by all the extracts decreases with increasing the incubation period. There was significant difference among the activities of different solvents of neem extracts. The result indicated that all the solvent extracts of Azadiracta indica showed antifungal activity against all the tested fungi from 48-96 hours of incubation, there was no inhibition zone observed against any of the tested fungi till 24 hours.

Chloroform extract of neem revealed significantly higher inhibition (29mm) against penicillium species after 48 hours. But methanol extract of neem leaves showed highest inhibitory zone (25mm) against Alternaria alternate species than other extracts of plant parts. Highest inhibitory zone (24mm) was observed of chloroform extract against Fusarium oxysporum at 48 hours while no inhibition zone was observed at 24 hours. Methanol extract showed 12mm inhibition zone in Aspergillus flavus, 18mm in Fusarium species, 25mm in Alternaria alternate and 20mm in Penicillium species at 48 hours. In Fusarium species and Alternaria species inhibition zone remains same at 96 hours i.e., (10mm). Highest inhibition zone in distilled water solvent was measured 12mm.



Antifungal activities of neem extract against different fungus

Usha et al., 2009 observed antifungal activity against Fusarium species while (Sunita Bansood and Mahendra Rai, 2008) showed antifungal activity of Aspergillus niger using agar well diffusion method. Inhibition zone was measured 16mm at 100µg concentration of neem.

From phytochemical analysis of neem extract by the procedure of of (Talukdar and Choudhary, 2010) indicated the presence of Alkoloids, Tanins, Terpenoids, Flavinoids, Glycosides, and reducing sugars. Blue colour obtained showed presence of Glycosides. Orange colour indicates presence of Alkoloids while red was observed showing presence of Terpenoids.

Determination of phytochemicals from neem extract.

Phytochemical constiuents	Chloroform	Ethanol	Methanol	D/w
Alkoloids	+	+	+	+
Steroids	+	+	+	_
Glycosides	+	+	+	_
Flavinoids	+	+	+	+
Tanins	_	+	+	_

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