World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

EFFECT OF ANTIFUNGAL ACTIVITY OF SOME MEDICINAL PLANTS AGAINST PATHOGENIC FUNGI

V. S. Patil* and S. N. Dakhore

Department of Botany, Shri Shivaji College of Arts, Commerce and Science, Akola.

***Corresponding Author: V. S. Patil**
Department of Botany, Shri Shivaji College of Arts, Commerce and Science, Akola.

Article Received on 21/10/2019 **Article Revised on 11/11/2019** **Article Accepted on 01/12/2019**

ABSTRACT

Medicinal plants are used as a source of medicine for treatment of various diseases. These plants produce secondary metabolites that have antifungal properties. These fungal pathogens not only affect plants but also causes various diseases to human as well as other animals. These plants can provide another best alternative for producing chemical fungicidies that are relatively non-toxic and cost effective. Leaf extracts of *Azadirachtaindica, Ocimum sanctum, Adhatodavasica, Polyalthialongifolia, Tinosporacordifolia* were investigated for activity against selected pathogenic fungi such as *Mucor, Rhizopus, Aspergillus, Fusarium by agar well diffusion method.* Out of these four fungi the *Rhizopus, Mucor and Fusarium* had significant sensitivity to the plant extract than of *Aspergillus* and were capable for reducing growth of fungi responsible for various alternations. In particular extract it offers effective bioactive compound for growth inhibition of that fungi.

KEYWORDS: Medicinal plants, Pathogenic fungi, Antifungal activity.

INTRODUCTION

Pathogenic fungi are fungi that cause disease in humans, plants or other organism. These fungi use diverse strategies, some fungi kill their hosts, feed on dead materials, while others colonize the live tissue. Basically all fungi interfere with primary plant defence, secrete toxins to kill animal as well as plant tissue. These fungi are resistant to the newer or modern antibiotics. Also the problem posed by the high cost, adulteration & increasing toxic side effects of this synthetic drugs. Coincidently, the last decade has also witness increasing intensive studies on extracts & biologically active compounds, isolated from plant species used for natural therapies or herbal medicine.

Medicinal plants represent a rich source of antifungal agents. These plants synthesize hundreds of chemicals compounds for functions including defence against fungal diseases. A single plant contains widely diverse phytochemicals, the effect of using a whole plant as medicines are uncertain. These plants are widely used in non-industrialised societies, mainly because they are readily available & cheaper than modern medicines. The annual global export value of 50,000-70,000 types of plants with suspected medicinal properties. But these plants face both general threats such as climate change & habitat destruction and the specific threat of over collection to meet market demand. All plants produce chemical compound which give them as defending against herbivores & these phytochemicals have potential for use as drugs in modern medicine. Some plants contain alkaloids, glycosides, polyphenols, terpenesetc to help them threw predators or pathogen. A wide range of medicinal plant parts is used for extract as raw drugs & they possess varied medicinal properties. The different parts used include root, stem, fruit, twigs, exudates & modified plant organs. Although hundreds of plant species have been tested for antifungal properties, the vast majority of them have not been adequately evaluated.

*Species of Mucor, Rhizopus, Aspergillus, Fusarium*are commonly found in living as well as non-living substances such as soil, digestive systems, plant surfaces, vegetables, fruits & cause severe diseases. Mucormycosis, Aspergillosis, Septic Arthritis, Endopthalmitis, Osteomyelitis, Cystitis, brain abscess & some skin infection can cause by these fungi.

Azadirachtaindica, Ocimum sanctum, Adhatodavasica, Polyalthialongifolia, Tinosporacordifolia are commonly found in Akola region. These medicinal plants used to treat various diseases but they also have ability to cure fungal infection.

Considering the vast potentiality of these plants as source for antifungal agents, a systematic investigation was undertaken to screen the local flora for antifungal activity from these plants.

MATERIAL AND METHOD

I. Plant Collection

Plants were collected from the Botanical garden of ShriShivaji College, Akola campus during the month of January and February. Medicinal plants such as *Azadirachtaindica, Ocimum sanctum, Adhatodavasica, Polyalthialongifolia, Tinosporacordifolia***,** were taken for the antifungal study. These plants were identify with the help of Flora of Marathwada by V.N.Naik.

Morphological studies of medicinal plants-

1. Azadirachtaindica A. Juss

Family – Meliaceae

Common Name – Neem, Neem tree, Indian Lilac

It is a fast growing, evergreen tree. The leaves are opposite, pinnate, dark green, petioles short, flowers white, fragrant, drooping, axillary panicles; protrandrous, bisexual, fruit smooth, glabrous, olive like drupe, elongate, oval, roundish, bitter, yellowish white, fibrous ; seed white, one two or three elongate.(See Plate I, A)

Chemical Components:- Nimbin, Nimbinin, Nimbidin

Uses: -Leaves are used to prevent insect bite. The flowers are used in many Indian festivals. Leaves are used in bath. The tender shoots and flowers are eaten as a vegetable. The flowers are used in rasam. Neem gum is rich source of protein. Oil is used in soaps, shampoos, balms, cream, toothpaste and tongue cleaner. Oil is also used to grease cart wheels, acts as nitrification inhibitor, as plant protector in apiculture and as animal feed.

Medicinal uses: Use as anthelmintic, antifungal, antidiabetic, antibacterial, antiviral, contraceptive, sedative for skin diseases e.g. Eczema psoriasis, for healthy hair, to improve liver function, detoxify the blood , balance blood sugar level. It is used to treat sweet itch and mud fever.

2. *Ocimum sanctum* Linn

Family –Lamiaceae

Common Name –Holy basil, Tulsi

It is an erect, many branched, subshrub with hairy stems. Leaves green or purple, simple, petiolate, ovate, margin toothed, phyllotaxy opposite decussate, flowers purple, whorls on elongate racemes.(See Plate I,B)

Chemical Components: - Contain oleanolic acid, ursolic acid, rosmarinic acid, eugenol, carvacrol, linalool, β-caryophyllene, essential oils, luteolin, orientin, apigenin, terpenes, nerol, and pinene.

Uses: - Use in treatment of disease, in curries, insect repellent, relives fever. It is also planted in kitchen garden and as an indoor plant; tulsi is taken as herbal tea.

Medicinal Uses: -In respiratory, digestive and skin diseases, use for tumerous growth, immune-modulator, cytoprotective, anticancer, promotes healthy heart, anti ageing, in treatment of kidney stones, relieves headache, fight acne, relieves fever, eye health, oral health, rich source of vitamin K.

3. *Adhatodavasica*Nees

Family –Acanthaceae

Common Name –Adulsa, Malabar nut

It is a shrub, leaves are lance shaped, opposite, smooth edged, petiole short, green, hairy, flowers white, inflorescence axillary spike, fruits pubescent, capsule. (See Plate I, C)

Chemical Components: - Alkaloids, tannis, saponins, phenolics, flavonoids, vasicine, quinazoline.

Uses: - Medicinal applicants use the leaves, roots, flowers and stem bark of this plant. The leaves of these plants are widely used for home remedies.

Medicinal Uses: - Used in the treatment of chronic bronchitis, asthama, dysentery, diarrhea, a poultice of the leaves may be applied to the wounds for their antibacterial and anti-inflammatory properties. The herb is known for its antispasmodic expectorant and blood purifying qualities. It has been used to control both internal and external bleeding as peptic ulcers, haemorrhoids and bleeding gums.

4. *Polyalthialongifolia*Sonn

Family –Annonaceae

Common Name– Ashoka, Buddha tree, Indian mast tree, Indian fir tree

An evergreen tree; leaves simple, alternate, narrow lanceolate or linear lanceolate, slightly acute, margin wavy or undulate, apex long, acuminate, membranous, coriaceous, faintly aromatic, shining dark green, glabrous above, paler glaucous beneath, reticulate veinlets; flowers bisexual, axillary, solitary fascicled on very short umbels, yellowish green, pedicels slender, bracts submedian; sepals 3, ovate or triangular, base connate, apex acute, pubescent, petals 6, valvate, yellowish green, fleshy, glabrous, stamens numerous, anther broad, dorsal; carpel many, stigma sessile, ovary monocarpous, seed pale brown to yellow brown.(See Plate I,D).

Chemical Components: - It contains diterpenoids, alkaloids, tannins, mucilage.

Uses:-Leaves are used for ornamental decoration, in making of masts for sailing ships, for manufacturing small articles.

Medicinal Uses: - Used as an antipyretic agent, as an antimicrobial activity, cytotoxic function and hypotensive effects. Bark is useful in fever, in skin diseases, diabetes, hypertension, helminthiasis and vitiated conditions of vata and pitla in antitumor and anticancer.

5. *Tinosporacordifolia*(Thunb.) Miers
Family - Menispermaceae
Common Name – Giloy, guduchi, Heart leaved moonseed
It is large deciduous, climbing, shrubs, leaves simple, alternate, exstipulate, petiole long, roundish pulvinate, ovate or ovate cordate, flowers unisexual, small greenish yellow, axillary and terminal racemes, Male flowers clustered, female solitary, sepals 6, free in two series of three each, the outer ones are smaller than inner, petals 6, free, smaller than sepals, obovate, membranous; fruits aggregate, ovoid smooth drupelets, orange coloured. (See plate I, E)

Chemical Components: - Columbine, tinosporaside, jatrarhizine, palmatine, berberinetembeterine, tinocordifolioside, phenyl propene, diasaccharides, cholin, tinosporic acid, tinosporol, tinosporon.

Uses: - The decoction of guduchi and sunth is good combination for treating gout and rheumatic disorder. The root of guduchi is strong emetic and used for bowel obstruction. The starch of the plant serves as household remedies for chronic fevers relieves burning sensations.

Medicinal uses:- Decrease sneezing and nasal itching, discharge stuffy nose, used as anti-inflammatory, antiarthritic, antiallergic, antidiabetic, anti-impotency, antioxidant, cure fever, skin disease, gastrointestinal disorders, helps to enhance grasping power as well as memory, regulates blood circulation.

II. Sterilization of Plant Materials
The disease free & fresh plants were selected. About 2g of fresh & healthy leaves were taken for each solvent extraction. They were washed with distilled water for three times. Then surface sterilized with 0.1% mercuric chloride for 20 seconds. Again the leaves were washed thoroughly with distilled water (three times).

III. Preparation of plant extract
Two grams of sterilized plant leaves were kept in the 10 ml organic solvents such as n-butanol, methanol & aqueous. They were ground well with the help of Mortar & Pestle. The plant materials were subjected to centrifugation for 10-15 min (at 1000 rpm). It was filtered through Whatmann filter paper No.1. The supernatant was collected & made to known volume by adding sterile n butanol, methanol and aqueous for further antifungal screening purpose.

IV. Fungal cultures & growth conditions
The plant extract were assayed for antifungal activity against the fungal strain *Mucor*, *Rhizopus*, *Aspergillus*, *Fusarium* isolated from the *Annonasquamosa*, *Psidiumguajava*, *Mangiferaindica*. The fungus was grown on Asthana & Hawker Medium 'A' at 25°C (± 2°C) & maintain with periodic sub-culturing. These fungi were identify using the available literature in the laboratory.

Preparation of Asthana& Hawker Medium 'A'
5.0gm- Glucose
3.5gm- KNO_3
1.75 gm -KH_2PO_4
0.75gm -MgSO4.7H2O
15gm -Agar Agar
1000ml-Distilled Water

All ingredients were first weighed &then dissolved in 1000 ml distilled water. Finally the medium was sterilized at pressure 15 labs i.e. 121°C for 15 min.

Taxonomical Studies of Isolates
1) *Rhizopus* sp. Ehrenb
Family – Mucoraceae
The fungus was collected from the*Mangiferaindica.*

Morphology: - Hyphae broad, not or scarcely septate; rhizoids & stolon present; sporangiophores brown to yellow, solitary or in tufts on the stolon diverging from the points at which the rhizoids form; sporangia rather round; apophysis absent or scarcely apparent; sporangiophores ovoid. [See plate II A].

2) *Mucor* sp. Fresen
Family– Mucoraceae.
The fungus was collected from the *Mangiferaindica.*

Morphology:-Colonies are very fast growing, cottony to fluffy, white to yellow, becoming dark gray, with the development of sporangia. Sporangiophores are erect, simple or branch forming large, terminal globose, to spherical, multispored sporangia, without apophyses and with well developed subtending columella. Sporangiospores are hyaline, grey or brownish, globose to ellipsoidal, smooth walled or finely ornamented. [See plate II B].

3) *Aspergillus* sp. Michell ex link
Family– Trichocomaceae.
The fungus was collected from the *Annonasquamosa*
Morphology:- Colonies white initially but soon turn in black due to the production of conidia, hyphae hyaline, branched, septate, conidiophores unbranched, septate, hyaline terminating in globose vesicles; sterigmata flask shaped producing conidia inacropetal succession in chains, conidia globose, 1 celled,verrucose, dark brown to grayish black. [See plateII C]

4) *Fusarium* sp. Link ex. Fries
Family – Nectriaceae
The fungus was collected from *Psidiumguajava.*

Morphology:-Mycelium pale to dark brown, branched,septate, conidiophores simple, short, branched, bearing whorl philides; conidia hyaline, variable and of two kind in moist head; macro-conidia 5-6 celled, micro-conidia 1-celled, ovoid or oblong borne single on conidiophores intermediate conidia 2-3 celled, oblong or slightly curved.[See plate II D].

5) Screening for antifungal Assay

Antifungal activity test

Antifungal activity was screened by agar well diffusion method (Perez et.al.1990). Methanol, n-butanol, & aqueous extracts of five different medicinal plants were tested against *species of Mucor, Rhizopus, Aspergillus, and Fusarium.* The Asthana & Hawker's medium "A" was poured into the sterile petriplates & allowed to solidify. The test fungal culture was evenly spread over the media by sterile cotton swales. About 1cm wells were made in the medium using sterile cork borer. 200ml of each extracts were transformed into the separate wells. The plates were incubated at 27°C for 48-72 hrs. These plates were observed for formation of clear incubation zone around the well which indicates the presence of antifungal activity. The zones of inhibition were recorded.

PLATE I

A. *Azadirachtaindica* A. Juss
Family:-Meliaceae

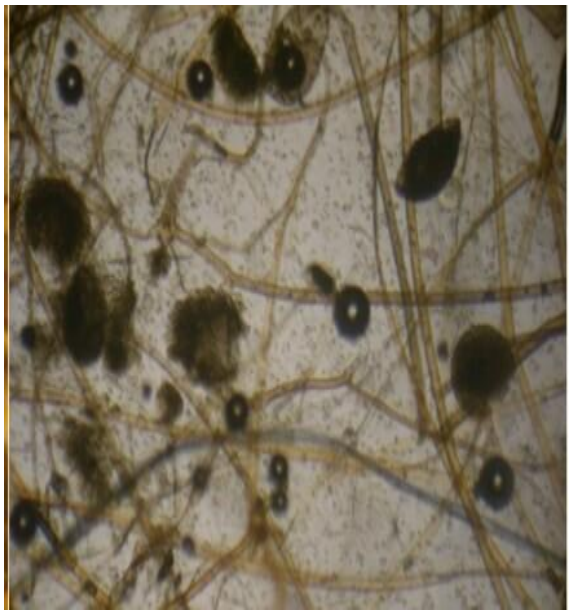
B. *Ocimum sanctum* Linn
Family:-Lamiaceae

C.*Adhatodavasica* (L.)Nees
Family: - Acanthaceae

D. *Polyalthialongifolia*Sonn
Family: - Annonaceae

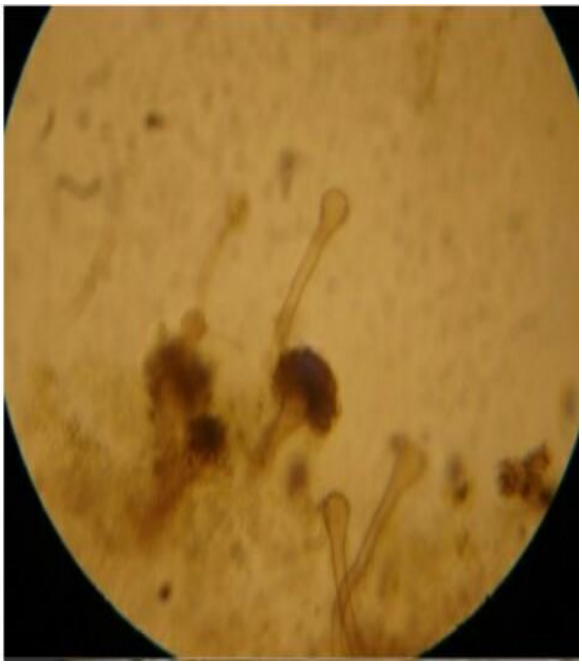
E. *Tinosporacordifolia* (Thunb.)Miers
Family: - Menispermaceae

PLATE II

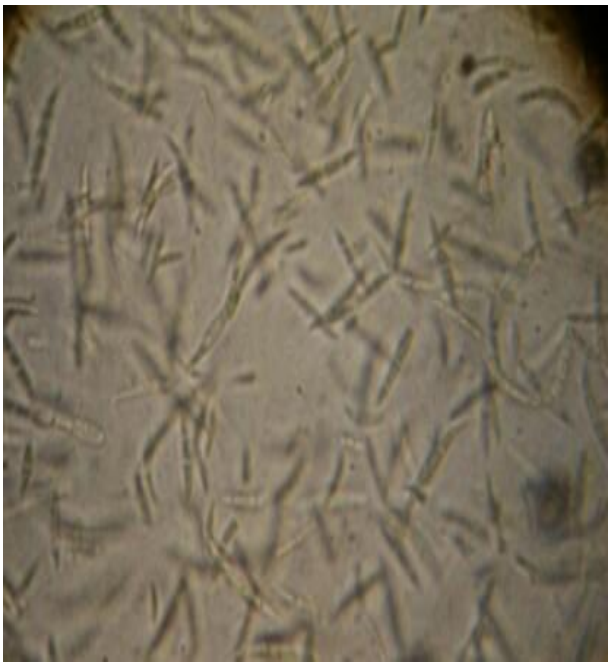


A. *hizopus* Ehrenb
Family – Mucoraceae

B. *ucor* Fresen
Family – Mucoraceae

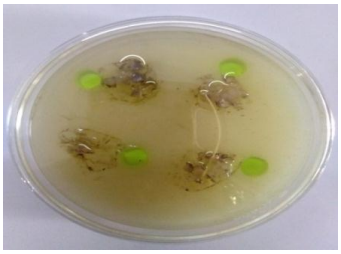


C.*Aspergillus*Michell ex link
Family – Trichocomaceae

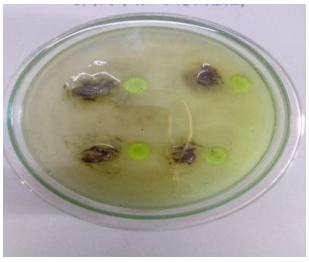


D. *Fusarium* Link ex. Fries
Family – Nectriaceae

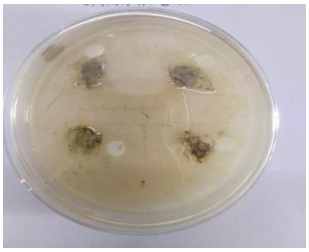
PLATE III
Growth inhibition of *Rhizopus sp.*



I.n-butanol extract of *Azadirachtaindica*

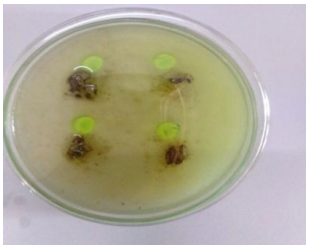


II. Methanol extract of *Azardirachtaindica*

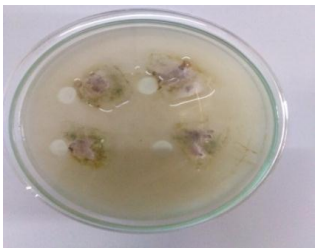


III. Distilled water extract of *Azardirachtaindica*

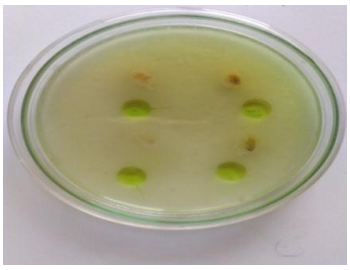
IV. n –butanol extract of *Ocimumsanctum*



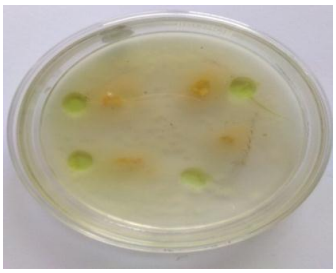
V .Methanol extract of *Ocimumsanctum*



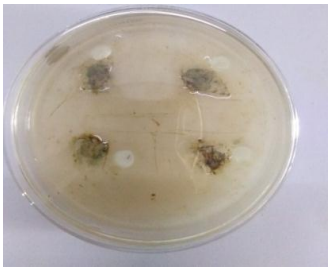
VI. Distilled water extract of *Ocimumsanctum*



VII. n-butanol extract of *Polyalthialongifolia*

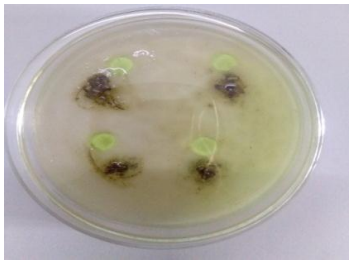


VIII. Methanol extract of *Polyalthialongifolia*

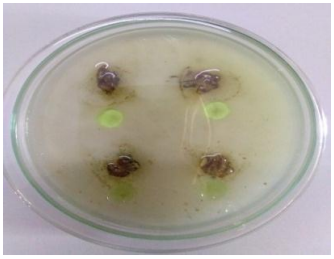


IX. Distilled water extract of *Polyalthialongifolia*

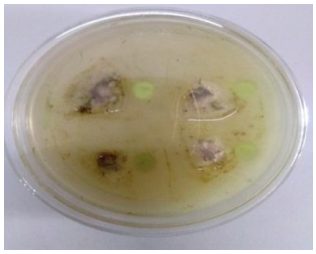
PLATE IV
Growth inhibition of *Rhizopus sp.*



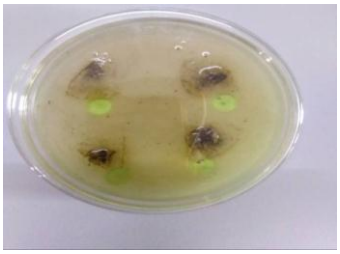
XI.Methanol extract of Tinosporacordifolia



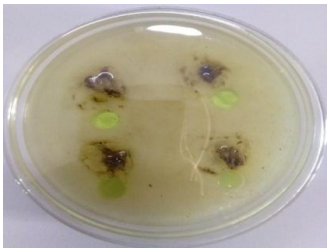
XII.Distilled water extract of Tinosporacordifolia



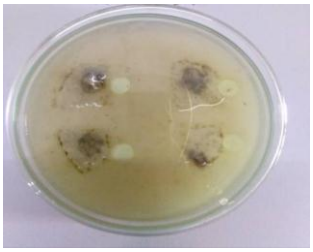
XII. Distilled water extract of *Tinosporacordifolia*



XIII. n-butanol extract of *Adhatodavasica*

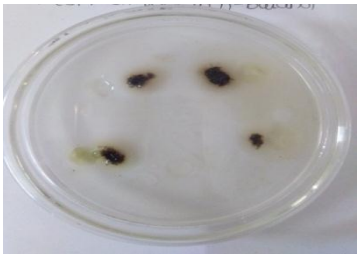


XIV. Methanol extract of *Adhatodavasica*

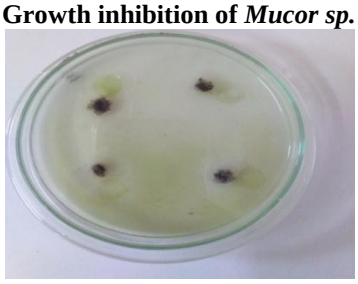


XV. Distilled water extract of *Adhatodavasica*

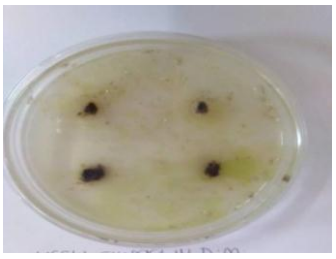
PLATE V
Growth inhibition of *Mucor sp.*



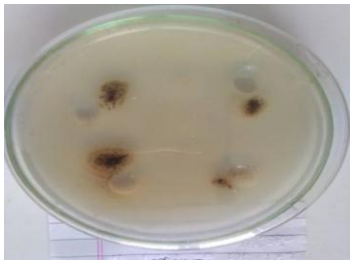
I.n-butanol extract of *Azadirachtaindica*



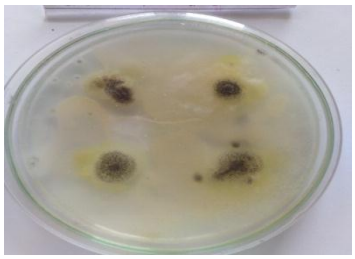
II.Methanol extract of *Azardirachtaindica*



III.Distilled water extract of *Azardirachtaindica*



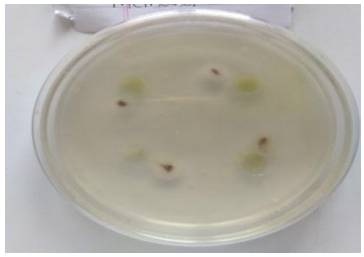
IV. n –butanol extract of *Ocimumsanctum*



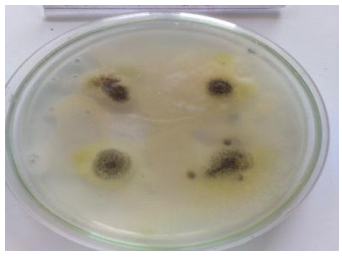
V .Methanol extract of *Ocimumsanctum*

VI. Distilled water extract of*Ocimumsanctum*

VII. n-butanol extract of *Polyalthialongifolia*

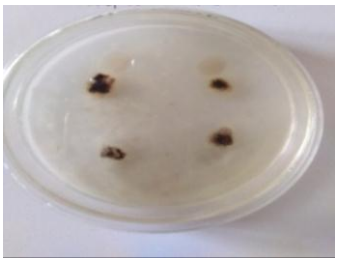


VIII. Methanol extract of *Polyalthialongifolia*



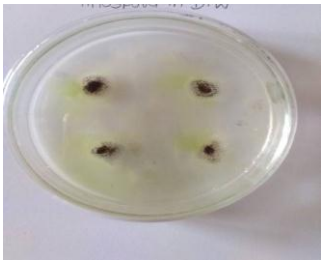
IX. Distilled water extract of *Polyalthialongifolia*

PLATE VI
Growth inhibition of *Mucor sp.*

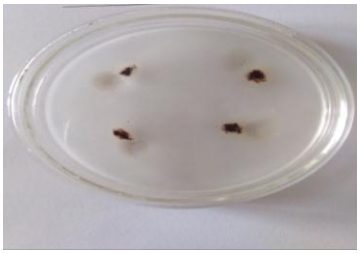


X. n-butanol extract of *Tinosporacordifolia*

XI. Methanol extract of *Tinosporacordifolia*

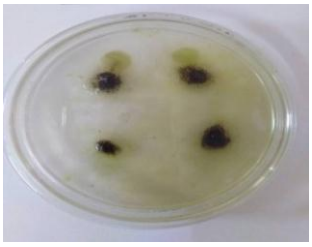


XII. Distilled water extract of *Tinosporacordifolia*



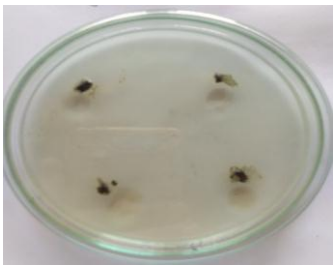
XIII. n-butanol extract of *Adhatodavasica*

XIV. Methanol extract of *Adhatodavasica*

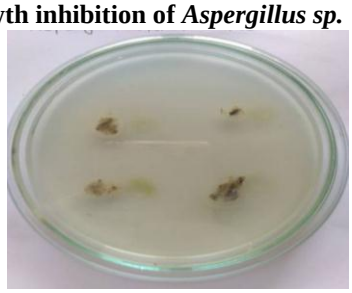


XV. Distilled water extract of *Adhatodavasica*

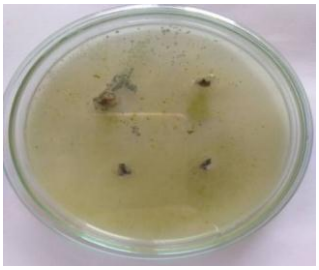
PLATE VII
Growth inhibition of *Aspergillus sp.*



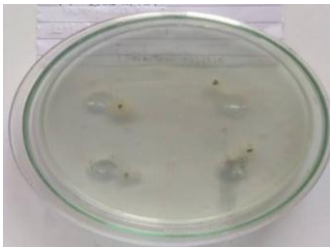
I.n-butanol extract of *Azadirachtaindica*



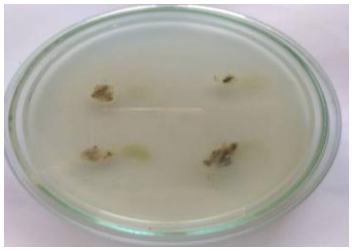
II.Methanol extract of *Azardirachtaindica*



III.Distilled water extract of *Azardirachtaindica*

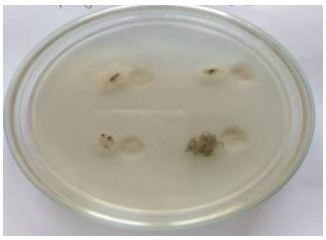


IV. n –butanol extract of *Ocimumsanctum*

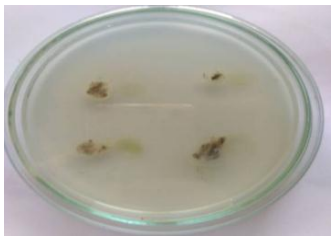


V .Methanol extract of *Ocimumsanctum*

VI. Distilled water extract of *Ocimumsanctum*



VII. n-butanol extract of *Polyalthialongifolia*

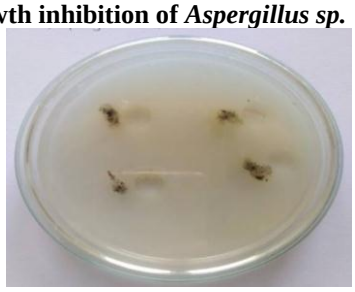


VIII. Methanol extract of *Polyalthialongifolia*

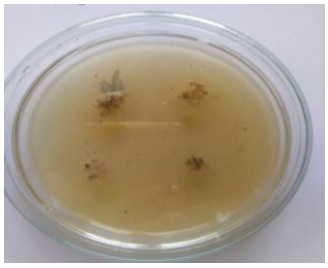
IX. Distilled water extract of *Polyalthialongifolia*

PLATE VIII
Growth inhibition of *Aspergillus sp.*

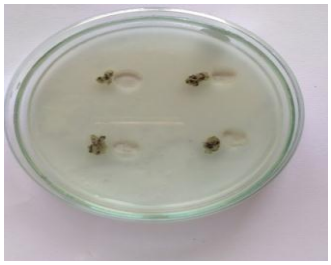
X. n-butanol extract of *Tinosporacordifolia*



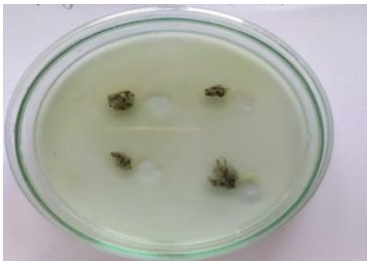
XI. Methanol extract of *Tinosporacordifolia*



XII. Distilled water extract of *Tinosporacordifolia*



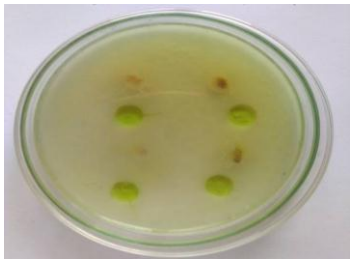
XIII. n-butanol extract of *Adhatodavasica*



XIV. Methanol extract of*Adhatodavasica*

XV. Distilled water extract of *Adhatodavasica*

PLATE IX
Growth inhibition of *Fusarium sp.*



I.n-butanol extract of *Azadirachtaindica*

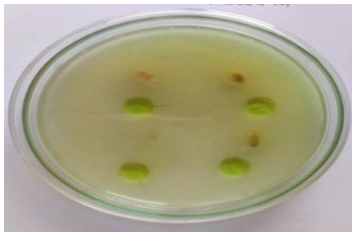
II. Methanol extract of *Azardirachtaindica*

III. Distilled water extract *Azardirachtaindica*

IV. n –butanol extract of *Ocimumsanctum*

V .Methanol extract of *Ocimumsanctum*

VI. Distilled water extract of*Ocimumsanctum*



VII. n-butanol extract of *Polyalthialongifolia*

VIII. Methanol extract of *Polyalthialongifolia*

IX. Distilled water extract of *Polyalthialongifolia*

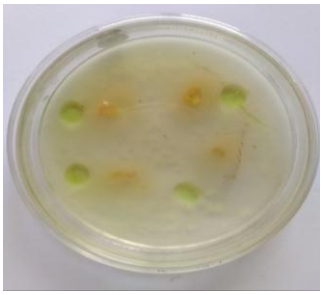
PLATE X
Growth inhibition of *Fusarium sp.*

X. n-butanol extract of *Tinosporacordifolia*

XI. Methanol extract of *Tinosporacordifolia*

XII. Distilled water extract of *Tinosporacordifolia*

XIII. n-butanol extract of *Adhatodavasica*



XIV. Methanol extract of *Adhatodavasica*

XV. Distilled water extract of *Adhatodavasica*

RESULT AND DISCUSSION

Methanol, n-butanol, and aqueous extract *of Azadirachtaindica, Ocimum sanctum, Polyalthialongifolia, Tinosporacordifolia, Adhatodavasica* was found growth inhibitory effect against species of *Rhizopus, Mucor, Aspergillus* and *Fusarium*. The zone of inhibition were observed and measured. The size of zone of inhibition has been incorporated in table.

Calculation was done by putting the value in the following formula.
Growth inhibition of species = dc-dt/dc
Where, dc- control reading of species,
dt- reading of target species

Table 1: Effect of antifungal activity of five medicinal plants against *Rhizopous sp.* Control reading 51.5mm.

Sr. No	Medicinal Plants	Zone of inhibition (mm)		
		n-butanol	Methanol	Aqueous
1.	*Azadirachtaindica*	32.6	35.6	15.7
2.	*Ocimum sanctum*	38.5	31.6	28.5
3.	*Polyalthialongifolia*	36.1	32	29.4
4.	*Tinosporacordifolia*	18.5	35.5	34.3
5.	*Adhatodavasica*	41.8	36.5	32.5

The n-butanol extract of Azadirachta *indica* shows the zone of inhibition upto 32.6 mm, methanolic extract shows the inhibition upto 35.6mm, while the aqueous extract of *Azadirachtaindica* shows the inhibition of mycelial growth upto 15.7 mm against *Rhizopus*. *Ocimum sanctum* shows the inhibitory activity against Rhizopusupto 38.5mm in n-butanol, 31.6 mm in methanolic extract while upto 28.5 mm in aqueous extract. Sequentially the n-butanol, methanol and aqueous exract of *Polyalthialongifolia* show the inhibitory activity against *Rhizopus* upto 31.6mm, 32mm and 29.4mm. *Tinosporacordifolia* shows the inhibitory activity against *Rhizopus* in n-butanol, methanol and aqueous extract upto 18.5mm, 35.5mm and 34.3mm. *Adhatodavasica* shows the inhibitory activity against *Rhizopus* in n-butanolupto 41.8mm, in methanol upto 36.5mm and in aqueous extract upto 32.5mm. See PLATE III (I, II, III, IV, V, VI, VII, VIII, IX) & PLATE IV (X,XI,XII,XIII,XIV,XV).

Table 2: Effect of antifungal activity of five medicinal plants against *Mucor*sp. Control reading 35mm.

Sr. No.	Medicinal Plants	Zone of inhibition(mm)		
		n-butanol	Methanol	Aqueous
1.	*Azadirachtaindica*	20.8	21.5	22.8
2.	*Ocimum sanctum*	11.6	1.5	4.5
3.	*Polyalthialongifolia*	16	13	8.5
4.	*Tinosporacordifolia*	14.8	17.5	8.8
5.	*Adhatodavasica*	22.6	14	18.3

Azadirachtaindica shows the inhibitory activity against *Mucor* in n-butanol, methanol and aqueous extract upto 20.8 mm, 21.5mm and 22.8mm. The n-butanol extract of *Ocimum sanctum* shows the zone of inhibition upto 11.6mm, methanol extract upto 1.5mm and aqueous extract upto 4.5mm against Rhizopus. Sequentially the n-butanol, methanol and aqueous extract of *Polyalthialongifolia* show the inhibitory action against Mucorupto 16mm, 13mm and 8.5mm. *Tinosporacordifolia* shows the inhibitory activity against *Mucor*in n-butanol, methanol and aqueous upto 14.8mm, 17.5mm and 8.8mm, *Adhatodavasica* shows the zone of inhibition upto 22.6mm in n-butanol, upto 14mm in methanol and upto 18.3mm in the aqueous extract against the *Mucor.* See PLATE V (I,II,III,IV,V,VI,VII,VIII,IX) & PLATEVI (X,XI,XII,XIII,XIV,XV).

Table 3: Effect of antifungal activity of five medicinal plants against *Aspergillus* sp. Control reading 48 mm.

Sr. No	Medicinal Plants	Zone of inhibition(mm)		
		n-butanol	Methanol	Aqueous
1.	*Azadirachtaindica*	36	31.3	29.5
2.	*Ocimum sanctum*	35.5	28.8	30
3.	*Polyalthialongifolia*	38.3	32.5	29
4.	*Tinosporacordifolia*	38.8	37.5	32.3
5.	*Adhatodavasica*	35.3	32	30.7

Sequentially the n-butanol, methanol and aqueous extract of *Azadirachtaindica* shows the antifungal effects against *Aspergillus*upto 36mm, 31.3mm and at 29.5mm. *Ocimum sanctum* shows the zone of inhibition in n-butanol, methanol and aqueous extract upto 35.5mm, 28.8mm and 30mm. *Polyalthialongifolia* shows the inhibition in growth upto 38.3mm in n-butanol, and upto 32.5mm and 29mm in methanol and aqueous extract against the *Aspergillus. Tinosporacordifolia* shows inhibitory activity against the *Aspergillus* in n-butanol, methanol and aqueous extract upto 38.8mm, 37.5mm and upto 32.4mm. *Adhatoda vasica* shows the zone of inhibition upto 35.3mm, 32mm and upto 30.7mm in n-butanol, methanol and aqueous extract against the *Aspergillus*. See PLATE VII (I,II,III,IV,V,VI,VII,VIII,IX) & PLATEVIII (X,XI,XII,XIII,XIV,XV).

Table 4: Effect of antifungal activity of five medicinal plants against *Fusarium* sp. Control reading 43.25mm.

Sr. No.	Medicinal Plants	Zone of inhibition(mm)		
		n-butanol	Methanol	Aqueous
1.	*Azadirachtaindica*	30.1	31.2	12.4
2.	*Ocimum sanctum*	24.4	31.4	19.9
3.	*Polyalthialongifolia*	29.4	26.2	22.6
4.	*Tinosporacordifolia*	29.7	26.9	16.9
5.	*Adhatodavasica*	29.2	29.4	23.4

Azadirachtaindica shows the zone of inhibition against the *Fusarium* in n-butanolupto 30.1mm, in methanol upto 31.2mm and in aqueous extract upto 12.4mm. The n-butanol, methanol and aqueous extract of *Ocimumsanctum* shows the inhibitory activity against Fusarium sequentially upto 24.4mm, 31.4mm and 19.9mm. *Polyalthialongifolia* in n-butanol, methanol and aqueous extract shows the antifungal effect against Fusariumupto 29.4mm, 26.2mm and 22.6mm. Sequentially n-butanol, methanol and aqueous extract of *Tinosporacordifolia* shows the inhibitory activity against the growth of *Fusarium*upto 29.7mm, 26.9mm and upto 16.9mm.*Adhatodavasica* shows the zone of inhibition upto 29.2mm, 29.4mm and 23.4mm in the extract of n-butanol, methanol and aqueous extract against the *Fusarium*. See PLATE IX (I,II,III,IV,V,VI,VII,VIII,IX) & PLATEX(X,XI,XII,XIII,XIV,XV).

Antifungal activity of five medicinal plant extract assayed by agar well diffusion method. The result revealed that the extract of medicinal plant shows significant reduction in growth of *Rhizopus, Mucor, Aspergillus and Fusarium.*

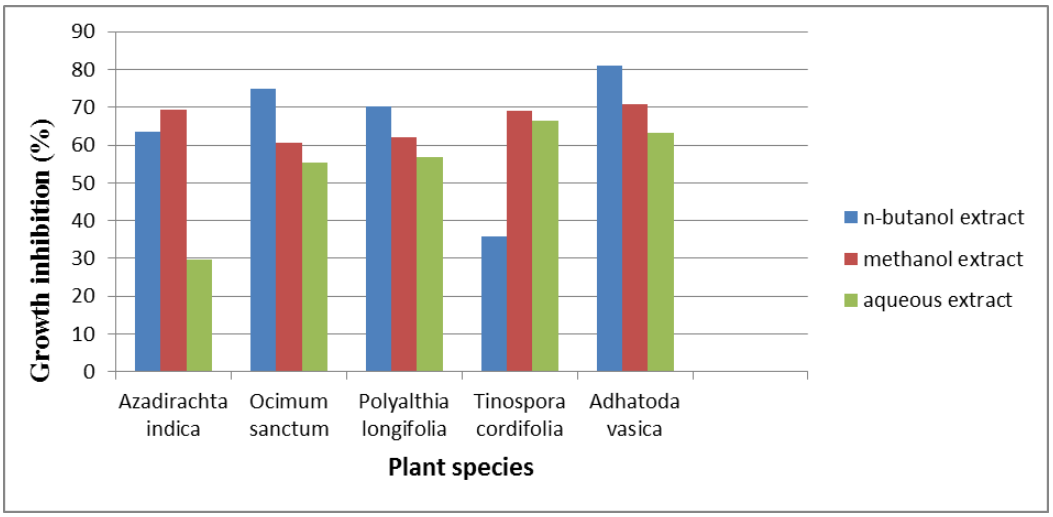


Figure 1: Growth inhibition of *Rhizopus* sp. by various extracts of medicinal plant.

In case of *Rhizopus*, among all the extract of five medicinal plants the aqueous extract of *Azadirachtaindica* and n-butanol extract of *Tinosporacordifolia* has been observed most effective. The n-butanol extract of *Ocimum sanctum* and *Adhatodavasica* found less effective on the *Rhizopus* as shown in Fig.1.

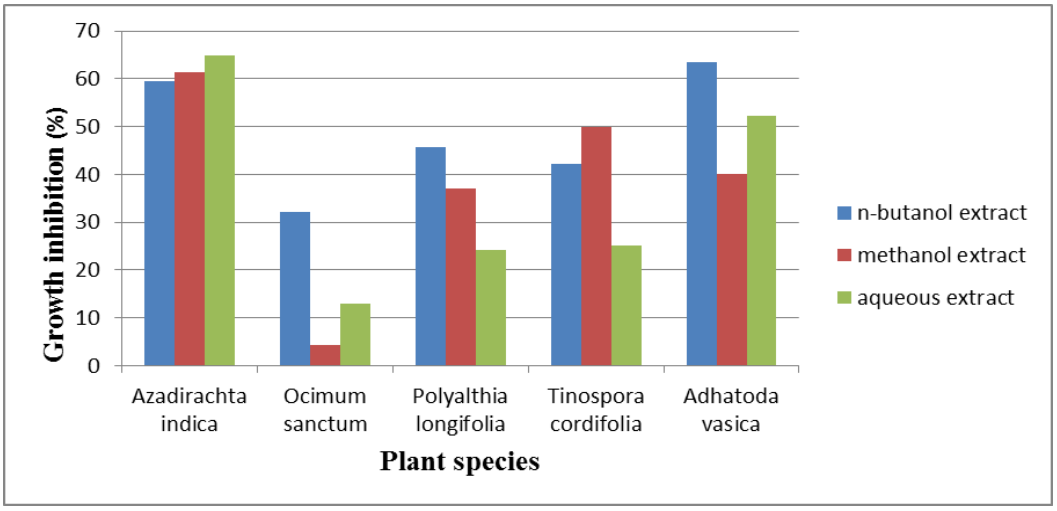


Figure 2: Growth inhibition of *Mucor* sp. by various extracts of medicinal plant.

In case of *Mucor* all the extracts shows the positive effects except the extract of *Azadirachtaindica*. Among all methanol and aqueous extract of *Ocimumsanctum* shows the most active antifungal effect against the *Mucor*. The n-butanol extract of *Adhatodavasica* found to be less effective against the *Mucor* as shown in fig 2.

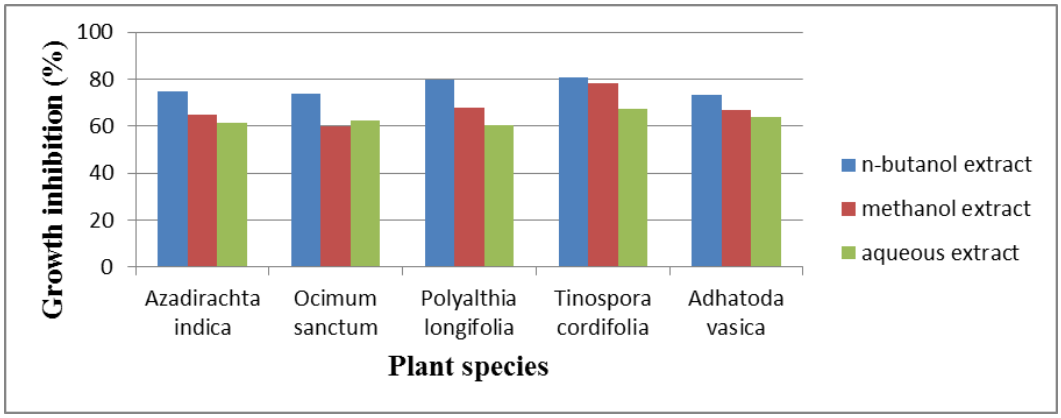


Figure 3: Growth inhibition of *Aspergillus*sp. by various extracts of medicinal plant.

The extracts of the five medicinal plants in various solvents are not more effective for the inhibition of growth of *Aspergillus*. But among all these extract, the methanol extract *Ocimum sanctum* and n-butanol extract of *Polyalthialongifolia* is quite active than that of other extract as shown in fig 3.

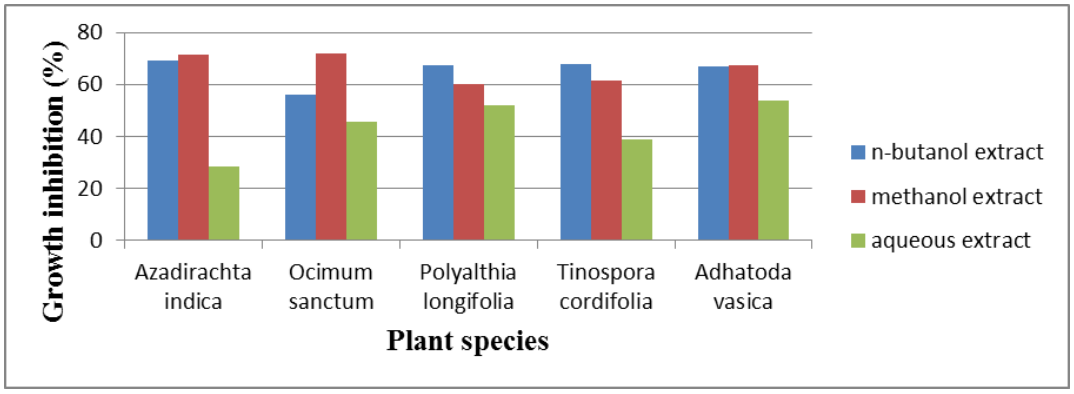


Figure 4: Growth inhibition of *Fusarium*sp. by various extracts of medicinal plant.

In case of *Fusarium*the aqueous extract of all five plants has been observed active in the inhibition of growth of *Fusarium.* The methanol extract of *Azadirachtaindica* and *Ocimum sanctum* are found to be less effective against *Fusarium* as shown in fig 4.

From above tables it shows that plant extract exhibit antifungal effects against *Rhizopus*, *Mucor, Aspergillus*, *Fusarium.* Out of these four fungi the *Rhizopus, Mucor and Fusarium* had significant sensitivity to the plant extract than of *Aspergillus* and were capable for reducing growth of fungi responsible for various alternations. In particular extract it offers effective bioactive compound for growth inhibition of that fungi.

The use of medicinal plant and herbs as medicines to treat various diseases could be traced as far back as the beginning of ancient human civilization. Medicinal plants are not only renewable in nature but also offer a wide variety of phytochemicals which are said to have significant antifungal active.

CONCLUSION

There is growing body of evidence indicating the benefits of medicinal plants for their use against pathogenic microorganisms. Plant based remedies used in human and animal medicine are an essential part of the primary health care system in many countries. Extensive screening programmes of plants mainly used in traditional medicine have resulted in the discovery of thousand of phytochemicals with inhibitory effects on different types of microorganism in vitro. There is need to exploit these bioactive compound in diseases caused by pathogenic fungi.

The extract of the plant part used showed prominent antifungal activity against the fungi which are severe pathogen. Thus the use of these plants in the treatment of pathogenic diseases associated with the infection of this pathogen is validated, scientifically supported by the results which are obtained in these studies. However, the further studies are to gain more clarity as to the specificity and biochemical mechanism responsible for the antifungal properties of these five plants in the different concentrations and on the other strains of these fungi. Natural plant derived fungicides may be a source of new alternative compounds, in particular with antifungal activity.

New milestone in the development of pharmaceutical products can be achieved by discovering bioactive natural products from these medicinal plants that address unfulfilled therapeutic needs against these fungi. Further investigations are warranted for safety, cost effectiveness have to be conducted. This study paves the way for the development of bioactive natural products with the added benefit of an environmentally safe and economically viable product. So these plants can be used to discover bioactive natural products that may serve as feed for the development of new pharmaceuticals compounds. Development of phytomedicine is relatively inexpensive and also suitable to our economic conditions.

REFERENCES

1. Abad MJ, Ansuategui M, Bermejo M (2007) Active antifungal substances from natural sources. ARKIVOC, 7: 116–145.
2. Achenbach H, Stocker M, Constenla MA (1988) Flavonoid and other constituents of Bauhinia Manca. Phytochemistry, 27: 1835–1841.
3. Adikaram NKB, Ewing DF, Karunaratne AM, Wijcratne EMK (1992) Antifungal compounds from immature avocado fruit peel. Phytochemistry, 31: 93–96.
4. Adou E, Williams RB, Schilling JK, Malone S, Meyer J, Wisse JH, Frederik D, Koere D, Werkhoven MC, Snipes CE, Werk TL, Kingston DG (2005). Cytotoxic diterpenoids from two lianas from the Suriname rainforest. Bioorg Med Chem, 13: 6009–6014.
5. Afolayan AJ, Meyer JJ (1997) The antimicrobial activity of 3, 5, 7-trihydroxyflavone isolated fromthe shoots of Helichrysumaureonitens. J Ethnopharmacol, 57: 177–181.
6. Ahmad I, Aqil F (2007) In vitro efficacy of bioactive extracts of 15 medicinal plants against ESbL-producing multidrug-resistant enteric bacteria. Microbiol Res, 162: 264–275.
7. Ahmad I, Beg AZ (2001) Antimicrobial and phytochemical studies on 45 Indian medicinal plantsagainst multi-drug resistant human pathogens. J Ethnopharmacol, 74: 113–123, 19 Antifungal Activities of Medicinal Plant Extracts and Phytocompounds 475.
8. Ahmad I, Mehmood Z, Mohammad F (1998) Screening of some Indian medicinal plants for theirantimicrobial properties. J Ethnopharmacol, 62: 183–193.
9. Aiyegoro OA, Okoh AI (2009). Phytochemical screening and polyphenolic antioxidant activity of aqueous crude leaf extract of Helichrysumpedunculatum. Int J MolSci, 10(11): 4990–5001.
10. Akinpelu DA, Obuotor EM (2000) Antibacterial activity of Nicotianatabacum leaves. Fitoterapia, 71: 199–200.
11. Alanı´s-Garza GM, Gonza´lez-Gonza´lez R, Salazar-Aranda N, Waksman de Torres VM, Rivas-Galindo (2007). Screening of antifungal activity of plants from the northeast of Mexico. J Ethnopharmacol, 114: 468–471.
12. Bahceevli AK, Kurucu S, Kolak U, Topc¸u G, Adou E (2005) Alkaloids and aromatics of Cyathobasisfruticulosa (Bunge) Aellen. J Nat Prod, 68: 956–958.
13. Banthorpe DV, Bilyard HJ, Brown OD (1989) Enol esters of caffeic acid in several genera of thelabiatae. Phytochemistry, 28: 2109–2113.
14. Barrero AF, Oltra JE, Alvarez M, Raslan DS, Saude DA, Akssira M (2000) New sources and antifungal activity of sesquiterpene lactones. Fitoterapia, 71: 60–64.

15. Bbos a GS, Kyegombe DB, Ogwal-Okeng J, Bukenya-Ziraba R, Odyek O, Waako P (2007) Antibacterialactivity ofMangiferaindica (L.).Afr J Ecol, 45: 13–16.
16. Bednarek P, Kerhoas L, Einhorn J, Franski R, Wojtaszek P, Rybus-Zaj M, Stobiecki M (2003). Profiling of flavonoid conjugates in Lupinusalbus and Lupinusangustifolius responding tobiotic and abiotic stimuli. J ChemEcol, 29: 1127–1142.
17. Bhakuni DS, Dhar ML, Dhar MM, Dhawan BN, Mehrotra BN (1969) Screening of Indian plantsfor biological activity. II. Indian J ExpBiol, 7: 250–262.
18. Bhakuni DS, Dhar ML, Dhar MM, Dhawan BN, Gupta B, Srimal RC (1971) Screening of Indianplants for biological activity. III. Indian J ExpBiol, 9: 91–102.
19. Bisoli E, Garcez WS, Hamerski L, Tieppo C, Garcez FR (2008) Bioactive pentacyclictriterpenes from the stems of Combretumlaxum. Molecules, 13: 2717–2728.
20. Dhawan BN, Patnaik GK, Rastogi RP, Singh KK, Tandon JS (1977) Screening of Indian plants forbiological activity. VI. Indian J ExpBiol, 15: 208–219.
21. Dhawan BN, Dubey MP, Mehrotra BN, Rastogi RP, Tandon JS (1980).Screening of Indian plantsfor biological activity. IX. Indian J ExpBiol, 18: 594–606.
22. Douros J, Suffness M (1881) New natural products under development at the National CancerInstitute. Recent Results Cancer Res, 76: 153–175.
23. Douros J, Suffness M (1980) The National Cancer Institute's Natural Products Antineoplastic, DevelopmentProgram. Recent Results Cancer Res, 70: 21–44.
24. Duke (2004) Biological activity summary for cocoa (Theobroma cacao L.). J Med Food, 3: 115–119.
25. Eloff JN (1998) Sensitive and quick microplate method to determine the minimum inhibitory concentration of plant extracts for bacteria. Planta Med, 64: 711–713.
26. Eloff JN, Mcgaw LJ (2006) Plant extracts used to manage bacterial, fungal and parasitic infectionsin South Africa. In: Ahmad I, Aqil F, OwaisM(eds) Modern phytomedicine: turning medicinalplants into drugs. Wiley, Germany, 97–119.
27. Eloff JN, Jager AK, van Staden JS (2001).The stability and relationship between anti-inflammatoryactivity and antibacterial activity of South African combretum species. S Afr J Sci, 97: 291–293.
28. Endo K, Kanno E, Oshima Y (1990) Structures of antifungal diarylheptenones, gingerenones A, B, C and isogingerenone B, isolated from the rhizomes of Zingiberofficinale. Phytochemistry, 29: 797.
29. Erdemoglu N, Ozkan S, Tosun F (2007) Alkaloid profile and antimicrobial activity of Lupinusangustifolius L. alkaloid extract. Phytochem Rev, 6:197–201.
30. Ezoubeiri A, Gadhi CA, Fdil N, Benharref A, Jana M, Vanhaele M (2005) Isolation and antimicrobialactivity of two phenolic compounds from Pulicariaodora L. J Ethnopharmacol, 99: 287–292.
31. Fabricant DS, Farnsworth NR (2001) The value of plants used in traditional medicine for drugdiscovery. Environ Health Perspect, 109: 69–75.
32. Fanos V, Cataldi L (2000) Amphotericin B-induced nephrotoxicity: a review. J Chemother, 12: 463–470.
33. Franich RA, Gadgil PD, Shain L (1983) Fungistatic effects of Pinusradiata needle epicuticularfatty and resin acids on Dothistromapini. Physiol Plant Pathol, 23: 183–195.
34. Freile M, Giannini M, Sortino M, Zamora M, Juarez A, Zacchino S, Enriz D (2006) Antifungalactivity of aqueous extracts and of Berberine Isolated from Berberisheterophylla. Acta FarmBonaerense, 25: 83–88.
35. Mandal P, Sinha SP, Mandal NC (2005) Antimicrobial activity of saponins from Acacia auriculiformis. Fitoterapia, 76: 462–465.
36. Manojlovic NT, Solujic S, Sukdolak S, Milosev M (2005) Antifungal activity of Rubiatinctorum,Rhamnusfrangula and Caloplacacerina. Fitoter, 76: 244–246.
37. Maregesi SM, Pieters L, Ngassapa OD, Apers S, Vingerhoets R, Cos P, VandenBerghe DA, Vlietinck AJ (2008) Screening of some Tanzanian medicinal plants from Bunda district forantibacterial, antifungal and antiviral activities. J Ethnopharmacol, 119: 58–66.
38. Marston A, Gafner F, Dossagi SF, Hostettmann K (1988) Fungicidal and molluscicidalsaponinsfrom Dolichoskilimandscharicus. Phytochemistry, 27: 1325–1326.
39. Marston A, Hamburger M, Sordat-Disercns I, Msonthi JD, Hostettmann K (1993) Xanthones from Polygala nyikensis. Phytochemistry, 33: 809–812.
40. Marthanda M, Subramanyan M, Hima M, Annapurna J (2005) Antimicrobial activity of clerodanediterpenoids from Polyalthialongifolia seeds. Fitoterapia, 76: 336.
41. Uma B, Prabhakar K, Rajendran S (2009) Anticandidal activity of Asparagus racemosus. IndianJ Pharm Sci, 71: 342–343.
42. Valsaraj R, Pushpangadan P, Smitt UW, Adsersen A, Nyman U (1997) Antimicrobial screening of selected medicinal plants from India. J Ethnopharmacol, 58: 75–83.
43. Vinaya K, Kavitha R, Ananda Kumar CS, Benaka Prasad SB, Chandrappa S, Deepak SA, NanjundaSwamy S, Umesha S, Rangappa KS (2009) Synthesis and antimicrobial activity of 1-benzhydryl-sulfonyl-4-(3-(piperidin-4-yl) propyl)piperidine derivatives against pathogens of Lycopersiconesculentum: a structure-activity evaluation study. Arch Pharm Res, 32: 33–41.

44. Viturro CF, Dela-Fuente JR, Maier MS (2004) 5-Methylcoumaranones from Mutisiafriesiana and their bioactivity. J Nat Prod, 67: 778.
45. Wachter GA, Hoffmann JJ, Furbacher T, Blake ME, Timmermann BN (1999) Antibacterial and antifungal flavanones from Eysenhardtiatexana. Phytochemistry, 52: 1469–1471.
46. Walsh TJ, Gonzalez C, Lyman CA, Chanock SJ, Pizzo PA (1996) Invasive fungal infections inchildren: recent advances in diagnosis and treatment. AdvPediatr Infect Dis, 11: 187–290.
47. Webster D, Taschereau P, Belland RJ, Sand S, Rennie RP (2008) Antifungal activity of medicinalplant extracts; preliminary screening studies. J Ethnopharmacol, 115: 140–146.
48. Wippich C, Wink M(1985) Biological properties of alkaloids. Influence of quinolizidine alkaloidsand gramine on the germination and development of powderly mildew, Erysiphegraminisf. sp. hordei. Experientia, 41: 1477–1479.
49. Wong JH, Ng TB (2005a) Vulgarinin, a broad-spectrum antifungal peptide from haricot beans(Phaseolus vulgaris). Int J Biochem Cell Biol, 37: 1626–1632.
50. Wong JH, Ng TB (2005b) Lunatusin, a trypsin-stable antimicrobial peptide from lima beans (Phaseoluslunatus L.). Peptides, 11: 2086–2092.
51. Woodward S, Pearce RN (1988) The role of stilbenes in resistance of Sitka spruce (Picea sitchensis (Bong) Carr) to entry of fungal pathogens. PhysiolMol Plant Pathol, 33: 127.
52. World Health Organization (1998). The World Health report. Life in the 21st century: a vision forall, 2 Measuring health. World Health Organization, Geneva, Switzerland, 39–60.
53. Wu TC (1994). On the development of antifungal agents: perspective of the US Food and Drug Administration. Clin Infect Dis, 19: S54–S58.
54. Xia L, Ng TB (2004). Actinchinin, a novel antifungal protein from the gold kiwi fruit. Peptides, 25: 1093–1098
55. Xiao D, Kuroyanagi M, Itani T, Matsuura H, Udayama M, Murakama M, Umehara K, Kawhara N(2001). Studies on constituents from Chamaecyparispisifera and antibacterial activity ofditerpenes. Chem Pharm Bull, 49: 1479–1481.
56. Yang MH, Wang JS, Luo JG, Wang XB, Kong LY (2009) Tetranortriterpenoids from Chisochetonpaniculatus. J Nat Prod, 72: 2014–2018.
57. Yemele-Bouberte M, Krohn K, Hussain H, Dongo E, Schulz B, Hu Q (2006) Tithoniamarin andtithoniamide: a structurally unique isocoumarin dimer and a new ceramide from Tithonia diversifolia. Nat Prod Res, 20: 842–849.
58. Yoganandam GP, Gowri R, Biswas D (2009) Evaluation of WedeliaBiflora (Linn) D.C foranthelmintic and antimicrobial activity. J Pharm Res, 2: 375–377.
59. Young MCM, Braga MR, Dietrich SMC, Gottlieb HE, Trevisan LMV, Bolzani VS (1992) Fungitoxic non-glycosidiciridoids from Alibertia macrophylla. Phytochemist, 31: 3433.
60. Zarnowski R, Suzuki Y, Yamaguchi I, Pietr SJ (2002) Alkylresorcinols in barley (Hordeumvulgare L. distichon) grains. Z Naturforsch, 57: 57–62.