Chapter-2

REVIEW OF LITERATURE

2.1. *Fusarium oxysporum* f.sp. *lycopersici*

2.2. According to Snyder & Hansen (1940), classification of *Fusarium oxysporum* f.sp. *lycopersici* is as follows:

Kingdom- Fungi.

Phylum – Ascomycota.

Sub Phylum – Pezizomycotina.

Class- Sordariomycetes.

Order- Hypocreales.

Family- Nectriaceae.

Genus – Fusarium.

Species- *F. oxysporum* f.sp. *lycopersici*

Fungi are potent destroyers of foodstuffs, making them not only unfit for human consumption but also decreases their nutritive value as well by producing certain mycotoxins (Marin *et al.*, 1999; Janardhana *et al.*, 1998). Genus *Fusarium* and its species are examples of phytopathogenic and toxin producing fungi that have been reported to be widespread throughout the world, which can cause serious health problems like cell toxicity, cancer and adverse effects on growth and development of animals and humans (Harvey, 2001: Hussein, 2001). *Fusarium* is a soil-born and plant pathogenic fungus, and are responsible for destroying crops and dramatically reducing production yields (Matos and Ricardo, 2006: Agrios, 2005). *Fusarium* species can synthesize certain toxic mycotoxins, such as zearalenone, fusarins, fumonisins or trichothecenes which are detrimental to the consumer’s health (Koopmann *et al.*, 1994: Blat *et al.*, 1997: Desjardins, 2000). Among such toxins, enniatrin and fusaric acid are phytotoxins, whereas others, such as the mycotoxins, trichothecins and fumonisins, are toxic to animals (Agrios, 2005).

*Fusarium* species are usually found in cereals and often produces mycotoxins in the kernels of various cereal species. *F. oxysporum* causes vascular wilts on many crops,
whereas in other cases, Root and stem rot are caused especially by *F. solani*, and rots of seeds that are accompanied by the production of mycotoxins. Moreover, some *Fusarium* species can cause disease in immunocompromised human population (Agrios, 2005; Seifert, 2010). The species *F. avenaceum, F. culmorum, F. graminearum, F. poae, F. semitectum, F. tricinctum and F. sporotrichioides* are found in cereals; *F. nygamai, F. verticilloides* and *F. subglutinans* in corn; *F. thapsinum* and *F. chlamydosporum* in sorghum, while *F. nygamaiand and F. fujikuroi* are found in rice. In legumes *F. chlamydosporum* and *F. tumidum* are typically encountered. *F. solani* usually attack potatoes. The species *F. acuminatum, F. equiseti, F. oxysporum, F. proliferatum, F. solani* and *F. sambucinum* can attack a variety of fruits, vegetables and ornamental plants (Samuels, 2001).

The Fusarium wilt of tomato (*Lycopersici esculantum* Mill) caused by *Fusarium oxysporum* f. sp. *lycopersici* (Snyder and Hansen). It (Fol) is recognized as a devastating disease in tomato growing areas all over the world (Beckman, 1987; Bondad-Reantaso *et al.*, 2005) also in different regions of India from severe to moderate (50-60%) percentage (Sherf and Macnab, 1986; Jiskani *et al.*, 2007; Chakraborty and Chatterjee, 2009). Tomato (*Lycopersici esculentum*), is one of the most important vegetable in many countries has a worldwide economic and nutritive importance (Khoslo, 1994). *Fusarium oxysporum* f.sp. *lycopersici* is a soil isolated pathogen in the class Hyphomycetes that causes wilting in tomato as the only host of pathogen (Rai *et al.*, 2011). *Rhizoctonia solani* and *Fusarium oxysporum* are major soil isolated fungal pathogens known to cause great harm to the both greenhouse and field grown tomatoes in the warm vegetable growing areas of the world (Sneh *et al.*, 1986). *Fusarium oxysporum* infected the roots mainly through penetrating wounds and proceeds into and throughout the vascular system, leading to functional collapse, systemic wilting due to xylem clogging and often the death of the infected (wilted) plant (Bowers *et al.*, 2000). In India, Kanpur (U. P.), the vegetable cultivators and farmers suffer more than 25.14-47.94% crop damage due to *Fusarium* wilt of tomato. Singh and Kamal (2012), reported higher 10-90% loss in yield of tomato crop in temperate tomato growing regions due to this disease. Kapoor (2008) has evaluated that most of the common varieties of tomato and brinjal are susceptible and fungicides are frequently used to control the disease. However, it is difficult and uneconomical to control the soil-borne disease with chemicals alone. In
this subject, biological control is an alternative and environmental-friendly strategy for disease management (Bowers and Locke, 2000; Momin and Nair, 2001; Eziashi et al., 2007).

2.3. Plants with antimicrobial activity

“Eat leeks in March and wild garlic in May, and all the year after the physicians may play.” Traditional Welsh rhyme (Tyler, 1987).

All over the world, the use of medicinal plants has significantly helpful for primary health care (Maciel et al., 2002). Plants possess antimicrobial properties because of the presence a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, flavonoids etc. Microbiologists have strongly two reasons to be interested in the antimicrobial properties of plant extracts and their scopes in medical science. First, it is very likely discovered that these phytochemicals will find their way into the arsenal of antimicrobial drugs prescribed by physicians. It is reported that, approximately two or three antibiotics at an average rate derived from microorganisms are launched each year (Clark, 1996). Second reason is that, the public is becoming aware of problems with the over prescription and overdose with antibiotics and at the same time because of the adverse effects of allopathic medicinal system. It is estimated that there are 250,000 to 500,000 species of plants on Earth (Borris, 1996). A relatively very small percentage (1 to 10%) of these are used as foods by both humans and other animal species. It is possible to use them more for medicinal purposes (Moerman, 1996).

Plant secondary metabolites are of low-molecular weight compounds that are not important for sustaining life, but are essential for the survival of the producing organism (Hadacek, 2002). Hydrophilic compounds (such as alkaloids, flavonoids, tannins, and saponins) are stored in the vacuole while the lipophilic SMs (such as terpenoids) are sequestered in resin ducts, laticifers, oil cells, trichomes, or in the cuticle. Bioactive compounds affect the tissues and cells of fungi via interference. The major targets portions includes biomembrane, proteins and nucleic acids. These bioactive compounds are still regarded as a valuable pool for discovering novel mode of action (Engelmeier and Hadacek, 2006).
Nowadays, public pressure to reduce the use of synthetic fungicides in agriculture has increased. Concerns have been raised for both the environmental impact and the potential health risks. Hence, there is a great demand for safer antifungals belonging to a wide range of structural classes, which are selective on new targets with less side effects (Abad et al., 2007). Every year huge crop damage is caused by various diseases and among them fungal diseases is very serious. According to an evaluation, about 10 to 20% of staple foods and cash crops are destroyed by plant pathogens (Hewitt, 2000).

The antimicrobial bioactive compounds are divided into 5 main classes consisting: Terpenoids and essential oils; phenolics and polyphenols; alkaloids; polypeptides and mixtures (crude extract) (Cowan 1999).

2.4. Essential oils

Essential oils, volatile oils or simply the "oil" are hydrophobic liquids containing volatile aroma of the plant from which they were extracted, such as “oil of lemongrass”. Most of these volatile natural compounds belong to monoterpenoids compounds (Hanson, 2003). These essential oils have been traditionally used for treatment of numerous infections and diseases all over the world for centuries (Rios and Recio, 2005). The essential oils are important because of their antibacterial, antifungal, antioxidant and anti-carcinogenic point of view (Tzortzakis, 2007). Although numerous researches have been reported the antifungal activity of essential oils when applied directly to fungus (Alizadeh et al., 2010; Bansod and Rai, 2008; Tullio et al., 2007; Cavaleiro et al., 2006; Pinto et al., 2006; Pawar and Thaker, 2006), the studies concerning about the antifungal activity of volatile vapors of the essential oils are relatively limited. (Jain and Agrawal, 2002) and few studies concerning about the inhibitory effect of essential oils have been reported, antifungal activity of volatile components extracted from leaves, stems and flowers of Lantana camara, Malvaviscus arboreus and Hibiscus rosa-sinensis were tested against Alternaria solani, Botrytis cinerea, Fusarium solani f. sp. cucurbitae, F. oxysporum f. sp. niveum, Pythium ultimum, Rhizoctonia solani and Verticillium dahlia (Boughalleb et al., 2005). Volatile components extracted from the flowers of L. camara at concentration of 100 mg/ml, showed the strongest antifungal effect (38%).
against tested fungi. However, *P. ultimum* was not inhibited by the extracts of any of the four plants tested in the experiment (Boughalleb et al., 2005).

Tzortzakis and Economakis (2007) investigated the antifungal activity of lemongrass (*Cymbopogon citratus*) oil against *Colletotrichum coccodes*, *Botrytis cinerea*, *Cladosporium herbarum*, *Rhizopus stolonifer* and *Aspergillus niger*. The results showed that fungal spore production was inhibited up to 70 to 100% at 25 to 500 ppm by lemongrass oil concentration. However, lemongrass oil up to 100 ppm inhibited spore germination for *A. niger*.

Ranasinghe et al., (2002) reported that essential oils of *Cinnamomum zeylanicum* and *Syzygium aromaticum* at concentration of 0.03 to 0.11% (v/v) exhibited strong antifungal activity for *Fusarium proliferatum*, *Lasiodiplodia theobromae* and *Colletotrichum musae*, the causal agents responsible for the crown rot and anthracnose of banana.

Chang et al., (2008) investigated the antifungal activity of essential oil and its constituents from *Calocedrus macrolepis* var. *formosana* on the growth of plant pathogenic fungi. Their experiments showed that sesquiterpenoid components were more effective than monoterpenoid components of the leaf oil.

Razzaghi-Abyaneh et al., (2008) investigated the inhibitory effect of essential oil from *Satureja hortens* against *Aspergillus parasiticus* well known to produce aflatoxins. In their study they found that both carvacrol and thymol compounds were able to significantly inhibit fungal growth.

Wilson et al., (1997) evaluated 49 essential oils for their antifungal activity against *B. cinerea*. According to The Wealth of India, *Cinnamomum zeylanicum* and *Eugenia caryophyllata* demonstrated the most effective antifungal activity against *B. cinerea*. Cinnamon oil is isolated from the bark of the plant *Cinnamomum zeylanicum* Blume (Lauraceae). The bark yields approximately 0.35% oil containing cinnamaldehyde and eugenol as major constituents. Presence of cinnamyl acetate, linalool, 1,8-cineol, p-cymene, cuminaldehyde is also reported. The oil is an active fungicide, germicide, carminative, stimulant and highly aromatic.
According to Satyavati et al., (1976) and Nadkarni, (2010) eucalyptus oil is isolated from the leaves of *Eucalyptus globulus* Labill (Myrtaceae) which possess over 80% cineol, with other constituents as p-cymene, alpha-pinene, limonene, geraniol and camphene. The oil is widely used to treat headache, body pains, fever, chronic bowel complaints and dysentery. Its oil has antiseptic and disinfectant property and is used especially in the treatment of infection of upper respiratory tract and in certain skin problems.

Singh et al., (2011) and Paschapur et al., (2009) find out that, Kapur tulsi oil is obtained from the leaves of *Ocimum kiliandsharicum* (Lamiaceae). The major components isolated from the oil are camphor (56.07%), limonene, camphene, 4-terpineol, beta-ocimene, linalool, alpha-terpineol and L-phellandrene. The oil is used as multifunctional medicine for variety of ailments like cough, bronchitis, viral infection, anorexia and healing of wounds. It also possesses insect repellent and antimicrobial property.

Rabadi et al., (2011) evaluated essential oils of black pepper, cardamom, cumin, boswellia and patcholi. The results indicated that all the oils inhibited fungal strains in varying degrees of dilutions. Essential oil of boswellia was found to be the most effective in antifungal activity against *Candida tropicalis* and essential oil of cardamom against *Trichophyton mentagrophytes*.

Prabuseenivasan et al., (2006) investigated essential oils against four gram-negative bacteria, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and two gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* at four different concentrations (1:1, 1:5, 1:10 and 1:20) using disc diffusion method. The MIC of the active essential oils were tested by using two fold agar dilution method at concentrations ranging from 0.2 to 25.6 mg/ml. Out of 21 essential oils tested, 19 oils showed strong antibacterial activity against one or more strains. Cinnamon, clove, geranium, lemon, lime, orange and rosemary oils exhibited significant inhibitory effect. Cinnamon oil showed effective inhibitory activity even at low concentration, whereas eucalyptus and camphor oils were least active against the tested bacteria. On the other hand *B. subtilis* was the most susceptible. On the other hand, *K. pneumoniae* exhibited low degree of sensitivity.
Batish et al., (2008) found out that antifungal activity of *Eucalyptus* essential oil as a natural pesticide and antifungal agent.

Farzaneh et al., (2006) have studied the chemical composition and antifungal activity of the essential oils of three species of *Artemisia* on some soil born phytopathogens.

Iscan et al., (2002) have studied the antifungal activity of *Mentha piperita* essential oil against 21 plant and human pathogens.

Mishra and Dubey (1990) have studied the antifungal activity of *Prunus persica* oil against various phytopathogens.

Mishra and Dwivedi (1989) have isolated essential oils from leaves of *Chenopodium ambrosioides, Cinnamomum zeylanicum, Citrus medica, Melaleuca lucadendron, Ocimum canum* and *O. gratissium*. These oils have demonstrated strong fungitoxicity against *Aspergillus flavus* at 200, 300, 400 and 500 ppm and most of them have shown to be very effective.

Verma et al., (2011) have studied the seasonal variation in essential oil content and composition of thyme, *Thymus serpyllum* working at Central Institute Of Medicinal and Aromatic Plants (CIMAP), CSIR Research center Pantnagar., cultivated in western Uttarakhand hills. This oil is used against various microorganisms.

Siripornvisal (2010) evaluated the essential oil extracted from mature seeds of ajowan (*Trachyspermum ammi* Lin.) against three strains of *Fusarium oxysporum, F. oxysporum* f.sp. *lycopersici, F. oxysporum* f.sp. *cubense* and *F. oxysporum* f.sp. *capsici* the wilt pathogen of tomato, banana and chili respectively. The *in vitro* assay by poison food method indicates that ajowan oil, in solution phase, possesses strong antifungal activity against the test fungi.

Djordjevic et al., (2013) examined that essential oils of *Mentha piperita, Eucaliptus globulus, Pinus sylvestris, Rosmarinus officinalis, Pimpinella anisum* and *Origanum vulgare* for antifungal effect towards tomato pathogen (*Fusarium oxysporum* f.sp.
lycopersici) In-vitro. Antifungal effect of oils was expressed through calculating percentage of inhibition of radial growth of mycelia of pathogen, and by determining MIC (Minimum Inhibitory Concentration) and MFC (Minimum Fungicidal Concentration). Results indicated that all of examined oils expressed antifungal activity in different concentrations. Results showed that essential oil of oregano (Origanum vulgare) was the most efficient in inhibition of mycelial growth with total inhibition applied at lowest concentration of 0.04 µl/ml of air. Oils of anise (Pimpinella anisum) and menthe (Mentha piperita) were also very effective.

Monteiro et al., (2013) examined the antifungal activity of essential oils of Thymus vulgaris, Cinnamomum zeylanicum and Syzigium aromaticum on mycelial growth and conidial germination of Fusarium oxysporum f. sp. cubense in vitro. The obtained results indicated that plant extracts and essential oils of Cinnamomum zeylanicum and Syzigium aromaticum were effective at 500 ppm with direct effect against mycelial growth. The volatile essential oils of Cinnamomum zeylanicum and Syzigium aromaticum showed good inhibition effects in controlling the disease in both 1000 and 2000 ppm. At the same time extracts and essential oils of Cinnamomum zeylanicum and Syzigium aromaticum showed efficacy to inhibit conidial germination.

According to Krifa et al., (2011) the species Citrus carvi, Foeniculum vulgare, Pimpinella anisum and Piruranthos tortuous of family Apiaceae, exibite a strong inhibitory effect against the growth of Fusarium species. The essential oil P. tortuous is one of the oils showing the lowest MIC with a value of 3.6 mg/L. This results, because of the presence of high monoterpenoids contents in its oil. These substances have the potential of altering the morphology of hyphae by reducing the diameter each time they interact with cell membranes of pathogen agents.

Prieto et al., (2011) evaluated the species Zanthoxylum monophyllum, Zanthoxylum fagara and Zanthoxylum rhoifolium against Fusarium oxysporum, and it was found that essential oils obtained from the fruits of these species show similar or higher results compared to the positive controls used. Z. monophyllum was the most active, followed by Z. fagara and Z. rhoifolium. These results are because of the presence of some compounds that are in these fruits essential oils in low concentrations such as...
(E)-caryophyllene, T-muurolol, and α-cadinol, compounds reported as antifungal substances by other researchers.


Suncica et al., (2012) have studied the effect of the oregano extract (*Origanum vulgare* L.) on the growth of *Fusarium* and *Penicillium* species isolated from cakes and fresh salads. The oregano extract showed the efficiency to reduce mould growth at all applied concentrations. Stronger inhibitory effect on the growth of *Penicillium* species, contrary to *Fusarium*, was determined. At extract concentration of 2.50 mL/100 mL, growth of *P. aurantiogriseum*, *P. glabrum* and *P. brevicompactum* was completely inhibited during 14 days of incubation. At the same concentration, growth of *Fusarium proliferatum* was inhibited by 81.71%, *F. oxysporum* by 85.84%, *F. verticillioides* by 86.50%, *P. chrysogenum* by 86.2% and *F. subglutinans* by 88.85%.

Candan et al., (2003) investigated the common yarrow (*Achillea millefolium*), and its essential oil isolated from its stem and leaves presents higher antimicrobial activity than its respective extracts (methanol extract separated by chloroform into parts that were not all soluble). The oils prevented the growth of *Streptococcus pneumoniae*, *Clostridium perfringes* and *Candida albicans* and slightly inhibited *Mycobacterium smegmatis*, *Acinetobacter lwoffii* and *Candida krussei*.

Betoni et al., (2006) have studied the antimicrobial action of 70% methanol extracts from leaves of *Mikania glomerata* (guaco), *Psidium guajava* (guava), *Baccharis trimera* (“carqueja”), *Mentha piperita* (peppermint) and *Cymbopogon citratus* (lemongrass), and *Allium sativum* (garlic), *Syzygium aromaticum* (clove) and *Zingiber officinale* (ginger) plants, all performed good antimicrobial activity.
against *Staphylococcus aureus*, and the most effective extracts were from clove at the concentration of 0.36 mg/mL and guava at 0.56 mg/mL.

Rosato *et al.*, (2007) have studied the essential oils from *Pelargonium graveolens* (geranium) present low values of minimum inhibitory concentration against *Bacillus cereus* (0.36 mg/mL), *Bacillus subtilis* (0.72 mg/mL) and *Staphylococcus aureus* (0.72 mg/mL), on the other hand *Origanum vulgare* (oregano) oils also exhibit antimicrobial activity against the same bacteria, in addition to *E. coli*; however, in the latter, a concentration of 0.35 mg/mL is required to inhibit *B. subtilis* whereas 0.70 mg/mL is necessary to inhibit the other bacteria.

Costa *et al.*, (2008) tested the inhibitory effect of essential oils from *Croton zehntneri* (wild cinnamon) leaves against *Shigella flexneri*, *Salmonella typhimurium*, *E. coli*, *Staphylococcus aureus* and *Streptococcus β-hemolyticus* strains and found antimicrobial activity against all bacteria, except *Salmonella*. Similarly the inhibitory action against *S. flexneri* was highly significant, with a minimal inhibitory concentration of 25 µg/mL.

In another study, Silva *et al.*, (2009) investigated essential oils from rosemary (*Rosmarinus officinalis*), clove (*Caryophyllus aromaticus* L.), ginger (*Zingiber officinalis*), lemongrass (*Cymbopogon citratus*), peppermint (*Mentha piperita*) and cinnamon (*Cinnamomum zeilanicum* Blume) that were tested against *Staphylococcus aureus* and *E. coli* strains. The oils showed some antimicrobial action, ginger essential oil was the most efficient against *S. aureus* while cinnamon and clove were the most effective against *E. coli* at 0.09%v/v.

Barbosa (2010) have studied the antimicrobial activity of essential oils from oregano (*Origanum vulgare*), thyme (*Thymus vulgaris*), marjoram (*Origanum majorana*) and basil (*Ocimum basilicum*) against strains of *Listeria monocytogenes* and *Salmonella Enteritidis*. It was reported that all oils showed antibacterial activity and also gram-positive bacteria were more susceptible.
Bergkvist et al., (2007) have studied the volatile oils of *Syzygium aromaticum*, *Thymus serpyllum*, *Lavandula angustifolia* and *Lavandula x intermedia* for antimicrobial activity using the microatmosphere method. The oils were tested against five organisms; methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococci* (VRE), *Pseudomonas aeruginosa*, *Streptococcus pyogenes* and *Candida albicans*. The results showed considerable variability in the size of zone of inhibition depending which oil was used and no essential oil was observed to be the “best” against all organisms. *Pseudomonas aeruginosa* was the only bacterium not susceptible for any of the tested oils and *C. albicans* was the only microorganism that was susceptible to essential oils after an exposure time of 6 h. This study revealed that the volatile compounds from the tested essential oils have good antimicrobial activity.

Amini et al., (2012) examined the Minimum inhibitory concentration (MIC) and Minimum fungicide concentration (MFC) of three medicinal plant essential oils of *Zataria multiflora*, *Thymus vulgaris* and *Thymus kotschyanus* on the mycelial growth of four pathogenic fungi including *Pythium aphanidermatum*, *Rhizoctonia solani* (AG4), *Fusarium graminearum* and *Sclerotinia sclerotiorum*. The rates of growth inhibition were measured after placing active mycelial plugs of each fungus on Petri dishes containing PDA amended with specific concentrations of essential oils and incubated at 28 ± 1 ºC. The results showed that these essential oils were very effective on the four studied plant pathogenic fungi.

Al-Rahmah et al., (2013) studied the antifungal activity of five methanolic plant extracts from *Lantana camara*, *Salvadora persica*, *Thymus vulgaris*, *Zingiber officinale* and *Ziziphus spina-christi* on tomato phytopathogenic fungi, *Fusarium oxysporum*, *Pythium aphanidermatum* and *Rhizoctonia solani*, the causative agents of tomato damping-off diseases. Three of five plant extracts were effective against these phytopathogenic fungi. *T. vulgaris* and *Z. officinale* extracts were strongly active and showed fungistatic and fungicidal activities against the phytopathogenic fungi with minimal inhibitory concentration (MIC of 4 mg ml-1 and minimal fungicidal concentrations (MFC) of 8 mg ml-1 except *F. oxysporum* which was less sensitive and its MFC reached to 16 mg ml-1 of *Z. officinale* extract. On the other hand, *S. persica* extract showed a moderate antifungal activity while *L. camara* and *Z. spina-christi* were not effective against tomato phytopathogenic fungi except *P.*
aphanidermatum which was completely inhibited at 10 mg ml-1 of L. camara extract. Carbendazim fungicide was more effective than all methanolic plant extracts inhibiting mycelial growth of all phytopathogenic fungi at 8 ppm and a huge concentration reached to 8 mg ml-1 of the effective plant extract was required to attain the same effect. Analysis of the effective plant extracts by GC/MS revealed that T. vulgaris extract was mainly composed by thymol (38.73%), carvacrol (19.31%), β-cimene (10.13%) and α-terpinolene (5.94%) while Z. officinale was mainly composed by Gingerol (46.85%), cedrene (8.39%), zingiberene (7.41%) and α-curcumene (7.32%) respectively. These effective plant extracts may help to develop effective and environmentally safer alternative fungicide to control tomato damping-off phytopathogenic fungi.

Jovanka et al., (2011) have studied the antimicrobial activities of three essential oils on five bacterial species (Escherichia coli, Salmonella choleraesuis, Proteus mirabilis, Staphylococcus aureus, Enterococcus faecalis) obtained from the American Type Culture Collection. The plants used for the study were oregano (Origanum vulgare L.), thyme (Thymus vulgaris L.) and wild thyme (Thymus serpyllum L.), all members of Lamiaceae family. The strongest antibacterial effect was shown by oregano essential oil, while the oil extracted from wild thyme was least potent.

Sokolic-Mihalak et al., (2012) have studied the effects of the essential oil of Thymus serpyllum L. and of its components thymol and total phenols (total phenolic content, TPC) extracted from the plant on the growth and mycotoxin production of Aspergillus ochraceus, A. carbonarius, and A. niger.

Eweis et al., (2012) examined the Antifungal activities of the Thymus serpyllum L. oil with special reference to inhibition zones diameter, Minimal inhibitory (MIC) and Minimal fungicidal (MFC) concentrations of the oil. The antifungal efficacy of this essential oil was evaluated on Aspergillus parasiticus growth and mycotoxin production. The essential oil from T. serpyllum obtained by hydro distillation was observed by GC/MS. The major components of T. serpyllum oil recorded were thymol (64.2 %), β–phellandrene (13.5 %), cis-sabinene hydroxide (8.09 %) 1,8 – cineole (1.9%), and β – pinene (1.3%). Static growth effects of the above oil
against *A. parasiticus* were at 250 ppm and lethal effects of *T. serpyllum* was 500 ppm of the oil. Aflatoxin production was inhibited at 250 ppm.

Sevil Toroglu (2007) have studied *Thymus eigii* (M. Zohary and P.H. Davis) Jalas, *Pinus nigra* Arn. sub sp *pallasiana* and *Cupressus sempervirens* *L*. The study was formulated to examine the antimicrobial activities of essential oils of these plants by the disc diffusion and minimum inhibitory concentration (MIC) methods. In addition, antimicrobial activity of *Thymus eigii* was researched by effects when it was used together with antibiotics and even when it was combined with other essential oils. When the results were compared with vancomycin (30 mcg) and erytromycin (15 mcg) standards, it was found that *Thymus eigii* essential oil was particularly found to possess stronger antimicrobial activity, whereas other essential oils showed susceptible or moderate activity.

Bayoub et al., (2009) verified the antibacterial effect of ethanol extracts of 13 plants (*Artemisia Herba Alba, Lavandula officinalis* *L.*, *Matricaria Chamomilla, Eugenia caryophylata, Cistus salvifolius, Mentha suaveolens* subsp. *Timija, Thymus serpyllum* *L., Lippia citriodora, Cinnamomum Zeylanicum, Rosa centifolia, Thymus vulgaris* *L., Rosmarinus officinalis* and *Pelargonium graveolens*) against *Listeria monocytogenes* and other pathogenic strains by agar well diffusion method. The major components of extracts tested were identified by gas chromatography coupled with mass spectrometry (GC/MS) analysis. The obtained results showed that *in vitro* anti-*Listeria monocytogenes* activities of all the extracts. Also, the extracts of clove, mint timija, cinnamon, cistus, rose, thyme, wild thyme, artemisia, rosemary, geranium and camomile presented in this order promises inhibitory capacity with MIC value between 0.25 mg/ml for clove extract and 6.75 mg/ml for chamomile extract.

Wani et al., (2013) examined the antibacterial activity of methanolic extract of aerial parts of *Thymus serphyllum* *L.* growing wild in Kashmir Himalaya by agar well diffusion method and broth dilution assay against nine human pathogenic bacterial strains, known to cause serious infections. The extract was also screened for the presence of various bioactive compounds present in the plant. The extract possess appreciable potential of inhibiting the growth of all the bacterial strains at all tested concentrations. The highest sensitivity was exhibited with mean zones of inhibition
20.66 and 20 mm against *Staphylococcus epidermidis* MTCC-435 and *Staphylococcus*.

Amrouche *et al.*, (2011) have studied the antifungal activity of the oils extracts of *Citrullus colocynthis* L., *Linum usitatissimum* L., *Nigella sativa* L. collected from Bechar Department in the region of (Algeria). Two methods radial growth on solid medium and biomass on liquid medium were used in this investigation. The oils extracts were obtained by Soxhlet extraction of the seeds part. The values of physicochemical indices of our oils such as acid, acidity and peroxide were also determined. The results of the antifungal potency proved that the seeds oils exhibited different degrees of inhibition against *Aspergillus flavus* MTTC 2799. However, evaluation of radial growth on *Potatoes Dextrose Agar* (PDA) solid medium showed slight mycelial growth proportional to oil concentration added to the medium. Antifungal study allowed as to put our oils in the order of effectiveness: *L. usitatissimum* (29%) > *C. colocynthis* (26.5 %) > *N. sativa* (18.75 %).

### 2.5. Crude extracts

Initial screenings of plants for determining their antimicrobial activities usually begin with crude aqueous or alcohol extractions, followed by various organic fractionation methods. The way of extraction procedure usually depends on the nature of the source material and specially to the compounds to be isolated (Hanson, 2003). From researches it proves that most of the identified components from plants, active against microorganisms are aromatic or saturated organic compounds, basically obtained through extraction with ethanol or methanol (Cowan, 1999).

Thilza *et al.*, (2010) evaluated the *in vitro* antibacterial activity of the water extract of *Moringa oleifera* leaf stalk extract on *Pseudomonas aerogenosa*, *Staphylococcus albus*, *Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus pyogenus* and *Enterobacter aerogenes*. The results were obtained by paper disc diffusion method at dilution of 1000mg/ml, 700mg/ml, 400mg/ml and 200mg/ml only mild activity against *Escherichia coli* and *Enterobacter aerogenes* was noticed. while *Pseudomonas aerogenosa*, *Staphylococcus albus*, *Staphylococcus aureus* and, *Staphylococcus pyogenus* was resistant at these concentrations. The highest activity was recorded by *Escherichia coli* at 1000 mg/ml which comparably is less than that
of the standard drug tetracycline (250mg/ml). This study has shown that the water extract of *Moringa oleifera* posses some degree of antimicrobial activity especially at high dose.

Hassan (2011) isolated *Candida albicans* at 35.7% from 70 samples of infected women badly suffered from vaginal thrush compared with other infected agent. In vitro antifungal activities of ethanolic and aqueous extracts of *Quercus infectoria* galls with hot and cold water treatments were tested against growth of *C. albicans* and *C. glabrata* in different concentration. Results showed that ethanolic extracts of *Quercus infectoria* was more effective against *C. albicans* at its 700mg/ml concentration while the aqueous ( hot and cold distilled water) extracts were more effective against *C. glabrata* compared with growth of *C. albicans* at same extracts.

Olayemi *et al.*, (2011) have subjected the extracts of leaves, stem bark, roots and fruits of *Solanum macranthum* Dunal to preliminary phytochemical screening for the presence of plant secondary metabolites and in vitro antibacterial and antifungal studies respectively. The results of the investigation confirms the presence of alkaloids, the steroidal nucleus, saponins, tannins, cardiac glycosides, flavonoids, reducing sugars and anthraquinones. The in vitro antimicrobial activity was done using agar well diffusion technique against six clinical strains of human pathogenic microorganisms, comprising two gram positive, two gram negative bacteria and two fungi. The various plant extracts varied in their high inhibitory activity to *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* at 1000 mg/ml comparable to the reference antibacterial drug, gentamicin at 2.5 mg/ml. High activity was exhibited against *Bacillus subtilis* while *Candida albicans* and *Aspergillus niger* were moderately inhibited at 2000 mg/ml concentration.

Odunbaku *et al.*, (2008) evaluated the ethanolic leaf extract of *Ficus exasperate* for antibacterial activity against *Escherichia coli* and *Staphylococcus albus*. The satisfactory MIC of the plant extract against *E. coli* is 300 mg/ml while that of *S. albus* is 700 mg/ml. The study also revealed that the combination of the crude plant extract and the protein synthesis inhibitors had the highest inhibitory activity.
Jassim and Nushia (2014) Crude extracts from dried garlic (*Allium sativum*) and cloves (*Eugenia caryophyllus*) were studied for antimicrobial activity against *Staphylococcus aureus, Staphylococcus epidermidis* and *Pseudomonas aeruginosa*. A total 60 clinical specimens were collected from patients clinically diagnosed as cases of bacterial infections in Marghan Hospital in Babylon. Gram positive bacteria were isolated from 43 specimens and 17 specimens were gram negative bacteria. Gram positive bacteria identified by cultural and biochemical tests, among those isolates, 24 isolates were *Staphylococcus epidermidis* and 19 isolates were *Staphylococcus aureus*. Gram negative bacteria identified by cultural and biochemical tests and all isolates were *Pseudomonas aeruginosa*. The effect of ethanolic and water extract were tested against isolating bacteria by using agar well diffusion method. Forty microliters of the dilution of crude extracts was transferred to each well and sterile water served as a control. Extract from cloves showed the highest activity with minimum inhibitory concentration (MIC) ranging from 100 to 400 mg/ml. Garlic extract showed no inhibitory effect against these bacteria.

Tahany *et al.*, (2010) investigated antimicrobial activity among combined action of six active constituents isolated from *Moringa pregrina, Achillea fragrantissima* and *Coleome droserifolia*. In this experiment synergistic combination mixtures were detected against different pathogens, indicating the high efficacy of combination mixtures over monotherapy treatments.

Ethanolic extract of 40 higher plants representing 23 families were studied by Begum *et al.*, (2007) for testing their antifungal activity against 6 phytopathogenic fungi (*Alternaria alternata; Curvularia lunata; Fusarium equiseti; Macrophomina phaseolina; Botryodiplodia theobromae* and *Colletotrichum corchori*). The results showed that the two most active plants extract with antifungal potential were *Acorus calamus* and *Piper betel*.

Yegan *et al.*, (1992) have studied the fungitoxic effects of six selected plants from Turkey. Results indicate that aqueous and essential oils of *Thymbra spicata, Satureja thymbra, Laura nobilis, Mentha spicata, Salvia faticasa* and *Inula viscosa* are fungitoxic to *Fusarium moniliforme, Rhizoctonia solani, Sclerotinia sclerotiorum*. 
Pandey and Dubey (1997) have studied the antifungal activity of some higher plants against *Pythium* species causing damping-off in tomatoes.

Satish *et al.*, (2009) represented the antifungal activity of Maize, Paddy and Sorghum against *Fusarium* species, collected from Mysore district, Mysore, India.

Sekine *et al.*, (2007) have studied the antifungal effects of volatile compounds from black zira (*Bunium persicum*), other spices and herbs against *Fusarium* species.

Shrivastava (2008) have seen the antifungal activity of *Pseudomonas Fluorescens* against phytopathogenic fungi.

Upadhyaya and Gupta (1990) have demonstrated the inhibitory effects of some medicinal plants on growth of *Curvularia lunata* (Cochliobolus lunatus). Ethanol extract of garlic followed by those of *Ocimum sanctum, Datura alba* and *hemp* were found to be most inhibitory to growth of the fungus. While aqueous extracts were less effective.

Kumar and Tripathi (1991) have screened the leaf extracts of 18 plant species belonging to 11 families for their control on *Pythium debaryanum, Fusarium oxysporum, Rhizoctonia solani,* and *Sclerotium rolfsii.*

Manoharachary and Gourinath (1988) have determined the efficacy of some tropical plant extracts against four phytopathogenic fungi. Plant extracts from roots, stems, leaves, flowers and fruits of one hundred plants belonging to 37 families were screened for fungitoxicity against, *Fusarium solani* and other microorganisms.

Adeleye and Ikotun (1989) in the department of Agricultural Botany at the University of Ibadan have found that the extracts from a wild variety of *Doiscorea bulbifera* (L.) has some antifungal activity against plant pathogenic fungi namely *Sclerotium rolfsii, Curvularia lunata, Fusarium moniliforme.*

Bandra *et al.*, (1988 & 1989) at the university of Peradeniya in Sri Lanka evaluated three rhizomatous plants perennial herbs used in native medicine for their antifungal
and antibacterial properties. The herbs tested were: *Acorus calamus* (Araceae), *Zingiber zerumet* and *Curcuma longa* (Zingiberaceae). These were effective against *Fusarium solani*, *Phytophthora infestans* and *Pythium* spp.

The antifungal activity of *Aloe vera* (syn: *Aloe barbadensis*) leaf pulp (gel) and its liquid fraction were evaluated upon mycellium growth of *Rhizoctonia solani*, *Fusarium oxysporum*, and *Colletotrichum coccodes* (Rodriguez et al., 2005). The results showed an inhibitory effect of both pulp and liquid fraction of *A. vera* on *F. oxysporum* at 104 μg/l. Further the liquid fraction reduced the rate of colony growth at a concentration of 10^5 μg/l in *R. solani*, *F. oxysporum*, and *C. coccodes*.

Saks and Barkai-Golan (1995) assayed the antifungal activity of *Aloe vera* leaf pulp at 1 to 10^5 μl/l on four postharvest fruit pathogens: *Penicillium digitatum*, *Penicillium expansum*, *Botrytis cinerea*, and *Alternaria alternate*. The inhibitory effect was evaluated by the suppression of spore germination and mycelial growth.

Lee et al., (2007) reported the effect of aqueous, methanol and acetone fractions of some Chinese medicinal plants against some food borne pathogens. The results showed that acetone extracts of *Cinnamomum cassia* against *Borytis cinerea* and *Glomerella cingulata*.

Harish et al., (2007) investigated 50 plant species, two leaf extracts, from *Nerium oleander* and *Pithecellobium dulce*. Results showed the higher inhibition against mycelial growth (77.4 and 75.1%) and spore germination (80.3 and 80.0%) of *Bipolaris oryzae* (*Cochliobolus miyabeanus*) causal agent of rice brown spot.

Pinto et al., (2010) screened methanol extract from 200 plant species against *Colletotrichum lindemuthianum*, the causal agent of bean anthracnose in Brazil. The experiments showed that the extract of *Miconia argyrophylla* was most promising for preventing both mycelia growth and conidial germination of *C. lindemuthianum*. Under greenhouse conditions, the extract of *M. argyrophylla* and *Origanum vulgare* had the biggest effect in reducing the disease severity in the local effect assay by 41.82 and 37.65%, respectively. In the systemic effect assay, the extract of *Inga marginata*, *M. argyrophylla*, *Myrica fallax*, *Malva sylvestris*, *Ocimum gratissimum*,
*O. vulgare* and *Siparuna arianeae* showed the best reduction in disease severity to value below 35%.

Muthukumar *et al.*, (2010) found out the antifungal effects of 66 medicinal plants belonging to 41 families against *Pythium aphanidermatum*, the causal agent of chilli damping-off. The Zimmu (*Allium sativum* L. × *Allium cepa* L.) leaf extract at 10% concentration had the highest inhibitory effect (13.7 mm) against mycelial growth of *P. aphanidermatum*.

Curtis *et al.*, (2004) investigated the fresh garlic extract (*Allium sativum*) prepared by juicer against a range of plant pathogenic fungi: *Alternaria brassicicola*, *Botrytis cinerea*, *Fusarium tabacinum*, *Magnaporthe grisea*, and *Phytophthora infestans*. The results showed that growth of *A. brassicicola*, *B. cinerea*, *M. grisea* and *P. cucumerina* were inhibited by garlic extract. In the experiment performed on plant diseases a prominent reduction was observed for rice blast caused by *M. grisea*, downy mildew of *Arabidopsis* caused by *Hyaloperonospora parasitica* and tuber blight caused by *P. infestans*. In all the cases, the reduction in disease confirmed by *in vitro* experiment. In the *in vitro* experiments, the highest reduction in disease occurred with *M. grisea* and *H. parasitica*, when plants were treated 24 h before infection.

Castillo *et al.*, (2010) observed antifungal activity of plant extracts from *Larrea tridentata*, *Flourensia cernua*, *Agave lechuguilla*, *Opuntia* sp. and *Yucca* sp. with alternative organic solvents (lanolin and cocoa butter) and water against the *Rhizoctonia solani*. Their results showed that extracts of *F. cernua* and *L. tridentate* using lanolin and cocoa butter inhibited 100% the *R. solani* growth at 2000 and 1000 ppm. The authors concluded high recovery of polyphenolic molecules with strong antifungal activity against *R. solani* with that of that lanolin and cocoa butter solvents.

Abdel-Monaim *et al.*, (2011) studied the effect of water extract and organic solvents from some plant species against *Fusarium oxysporum* f. sp. *Lupine* casual agent of damping-off and wilt diseases of lupine plants. Their experiments revealed that solvent extracts of *Eugenia jambalaya*, *Nerium oleander* and *Citrullus colocynthis* are most effective against *F. oxysporum* f. sp. *lupini*. Amongst the tested organic solvents, the butanolic and ethereal extracts were highly effective in reducing diseases than the
other tested extracts. Under field conditions, ethereal and butanolic extracts of *N. oleander* and *E. jambolana* leaves and *C. colocynthis* not only reduced the percentage of wilt severity in fruits but also improved plant growth parameters and increased total seed yield/hectare compared with control treatment, while protein content in seeds was not affected.

Choi *et al.*, (2004) investigated methanol extracts of 57 plants species for their antifungal activity against rice blast (caused by *Magnaporthe grisea*); rice sheath blight (caused by *Corticium sasaki*); tomato gray mold (caused by *Botrytis cinerea*); tomato late blight (caused by *Phytophthora infestans*); wheat leaf rust (caused by *Puccinia recondita*) and barley powdery mildew (caused by *Blumeria graminis* f. sp. *hordei*). The results indicated that none of the plant extracts was effective against tomato gray mold. The methanol extracts of *Chloranthus japonicas* and *Paulownia coreana* displayed the highest antifungal activity. The *C. japonicus* extract controlled the development of rice blast, rice sheath blight, and wheat leaf rust more than 90%, and tomato gray mold and tomato late blight more than 80%. The *P. coreana* extract displayed control values of more than 90% against rice blast, wheat leaf rust, and barley powdery mildew and more than 80% against tomato gray mold. The extract of *Rumex acetosella* roots significantly reduced barley powdery mildew.

Joseph *et al.*, (2008) reported the efficacy of different plant extracts to control brinjal (*Solanum melongena*) wilt pathogen (*Fusarium solani* f. sp. *melongenae*). The results showed *Azadirachta indica* water extract at its 20% concentration was most effective, followed by *Rheum emodi*, *Eucalyptus globulus*, *Artemesia annua* and *Ocimum sanctum* respectively against *Fusarium solani* f. sp. *melongenae*.

Wong and Ng (2005) isolated an antifungal peptide named vulgarinin from the seeds of *Phaseolus vulgaris*. This peptide displayed considerable antifungal activity against fungal species such as *Fusarium oxysporum*, *Mycosphaerella arachidicola*, *Physalospora piricola* and *Botrytis cinerea*.

In another investigation Sidhu *et al.*, (2009) examined the individual and combined methanolic plant extracts for their efficacy against growth and aflatoxin produced by *Aspergillus flavus*. The experiments revealed that combined extracts of various plant
species have satisfactory antifungal and antitoxin activity as compared to their individual one. Combined methanolic extract of *Azadirachta indica* and *Pongamia pinnata* oils inhibited 57.32% of fungal growth. However, the combined effect of *Cymbopogon nardus* (Citronella) essential oil and methanolic extract of *Citrullus colocynthis* roots inhibited 85.67% of fungal growth and more than 90% of aflatoxin produced as compared to that of control.

Extracts from 345 fresh plants were evaluated for their antifungal activity against *Botrytis cinerea* (Wilson *et al.*, 1997). Of the plants tested, *Allium* and *Capsicum* species at their 10% dilution species showed the greatest antifungal activity and completely inhibited spore germination of *B. cinerea* after 24 and 48 h.

Methanolic extracts of *Pimpinella anisum* and *Illicium verum* were studied for their potential antifungal activities against some filamentous fungi (Yazdani *et al.*, 2009). The results indicated that methanolic extract of *P. anisum* seeds did not have any inhibitory effect on *Aspergillus flavus* mycelial growth, while extracts of *I. verum* fruits at 16 mg/ml concentration was found to be effective against growth of *A. flavus*.

Wang *et al.* (2005a) reported a chitinase with antifungal activity isolated from *Phaseolus mungo* seeds. This protein exerted antifungal action towards *Fusarium solani*, *Fusarium oxysporum*, *Mycosphaerella arachidicola*, *Pythium aphanidermatum* and *Sclerotium rolfsii*. *Phaseolus mungo* also yielded a novel lysozyme exhibiting antifungal activity toward *Botrytis cinerea*.

Small cysteine rich peptides Defensins produced by *Trigonella foenum-graecum*, exhibited high antifungal activity at 100 μg concentration against the *Rhizoctonia solani* and *Phaeoisariopsis personata* (Olli and kirti, 2006).

Methanol and aqueous extract of *Ocimum gratissimum* and *Aframomum melegueta* on spore germination and mycelial growth of *Aspergillus niger* and *Fusarium oxysporum* was studied by Okigbo and Ogbonnaya (2006). The results showed that ethanol extraction was more effective than water extraction and antifungal activity of *O. gratissimum* leaf extracts was more effective than *A. melegueta* against spore germination and mycellial growth of *A. niger* and *F. oxysporum*.
Fawzi et al., (2009) investigated the antifungal activity of 5 plant extracts; *Cinnamomum zeylanicum* (Cinnamon), *Cymbopogon proximus* (Halfa barr), *Laurus nobilis* (Laurel), *Persea Americana* (Avocado) and *Zingiber officinale* (Ginger) performed with either cold distilled water (CDW) or boiling (BDW) on two pathogenic fungi, *Alternaria alternata* and *Fusarium oxysporum*. The results revealed that plants extracts with CDW had a strong antifungal activity with significant inhibition on the growth of the 2 tested fungi and their hydrolytic enzymes, β-glucosidase, pectin lyase and protease. Extracts of halfa barr and ginger were the most effective to inhibit the growth of the tested fungi followed by avocado, cinnamon and laurel. This study confirmed that plant extracts can be used as natural fungicides to control pathogenic fungi, thus reducing the dependence on the synthetic fungicides. Halfa barr, which was found to be the most efficient extract (75% inhibition), might be an essential material for controlling these fungi for future aspects.

Baka et al., (2014) examined that the aqueous extracts from five wild traditional medicinal plants (*Achillea fragrantissima, Balanites aegyptiaca, Peganum harmala, Rumex vesicarius*, and *Urtica urens*) which were collected from different locations in Egypt against the predominant fungal pathogens (*Alternaria alternata* f. sp. lycopersici, *A. solani, Fusarium oxysporum* f. sp. lycopersici and *Rhizoctonia solani*) infested tomato seeds. All the aqueous plant extracts significantly inhibited the mycelial growth and spore germination of these fungi, but the extract of *A. fragrantissima* exhibited the strongest antifungal activity. The maximum seed germination, plant emergence and seedling vigor was analyzed after the treatment of tomato seeds with 10% *A. fragrantissima* extract. In greenhouse experiment, the aqueous extract of *A. fragrantissima* decreases disease severity but increased total pigments followed by total phenolics and fruit yield.

Dwivedi and Enespa (2012) examined that the antifungal activity of extracts of *Tinospora cordifolia* (leaves), *Moringa oleifera* (bark) and *Trachyspermum ammi* (seeds) at three concentrations 25, 50, 75% (v/v) *in vitro* by poisoned food technique against *Fusarium oxysporum* f. sp. *lycopersici* and *Fusarium solani* causing wilt disease on tomato and brinjal plants. The antifungal activity was assessed in terms of percentage of inhibition of mycelial growth of the test fungi. All the plant extracts showed significant inhibition in the mycelial growth of the test pathogens. Among the
extracts evaluated, *M. oleifera* against *Fusarium oxysporum* f. sp. *lycopersici* and *T. cordifolia* against *Fusarium solani* completely inhibited mycelial growth at their 75% concentration followed by *T. ammi* seed extract.

Boulenouar *et al.*, (2012) investigated that *Fusarium oxysporum* f. sp. *albedinis* (Foa) is a soil borne fungus causing the most serious disease of date palm (*Phoenix dactylifera* L.) called “Bayoud”. Five medicinal plants from the Algerian Sahara (Southwest of Algeria): *Limoniastrum feei* (aerial part, roots), *Launeae arborescens* (Batt.) Murb. (aerial part, roots), *Fredolia aretioides* Moq. et Coss. (aerial part, roots), *Asteriscus graveolens* (Forsk) (leaves, stems) and *Acacia raddiana* (leaves, bark), were used to evaluate their extracts for antifungal activity against Foa. Two parts from each plant were used for extraction by four solvents: methanol, ethyl acetate, dichloromethane and hexane. The antifungal test was conducted using disc diffusion technique and relative virulence (RV) test (on potato tuber tissue). For both tests, four extract quantities were used (200, 400, 800 and 1,600μg). Among all solvents, methanol had the best extraction yield (mean: 6.35%, minimum: 2.27%, maximum: 9.80%). The highest frequency of antifungal effect on Foa was presented by ethyl acetate extracts (32.50% of detectable effect). The best effect was observed for ethyl acetate extract of *Limoniastrum feei* (aerial part). The virulence test showed a decrease in RV up to 30% for ethyl acetate extract of *Launea arborescens* aerial part. The increase in RV was observed mostly for hexanic extract from *Fredolia aretioides* reflecting its high toxicity compared to the other extracts.

Moghimipour *et al.*, (2014) examined the antifungal activity of saponin extracted from the *Glycyrrhiza glabra* against *Candida* species (*Candida albicans*, *Candida tropicalic* and *Candida glabrata*). Antifungal activity *Quillaja saponaria* total saponin (QST) was also evaluated. The roots of the plant were dried, powdered and prepared with petroleum ether in a soxhlet apparatus. The air dried powder was successively extracted with methanol, n-butanol and diethyl ether. The antifungal activity of the saponins was carried out using well diffusion method and also the value of Minimum Inhibitory Concentrations (MIC) was calculated. Clotrimazole was used as positive controls to determine the sensitivity of the species. According to the results, *C. albicans*, and *C. tropicalic* were sensitive to the saponins of *G. glabra*, and *Q. saponaria*, while saponin isolated from *G. glabra* just could inhibited the growth
of *C. glabrata*. *In vitro* studies have demonstrated that saponins extracted from *G. glabra*, and *Q. saponaria* can serve as potential candidates for the development of new antifungal agents.

Rajesh *et al.*, (2014) examined the antimicrobial activity of various extracts and fractions of *Muntingia calabura* (Elaeocarpaceae) root against a selected panel of microorganisms. Antifungal activity of different solvent extracts of *M. calabura* L. root, tested against *Alternaria solani*, *Fusarium oxysporum* f.sp. *lycopersici*, *Pythium* species, *Phytophthora* species, *Aspergillus niger*, *Colletotrichum* species and *Rhizoctonia solani*, were evaluated by agar well diffusion assay. The chromatographic fractionation of the extract resulted in the isolation of antifungal metabolite stigmasterol. The structure of the stigmasterol was confirmed using GC-MS, IR and NMR spectroscopic characterization. The stigmasterol had a potent antifungal activity with a minimum inhibitory concentration of 1 mg/ml against *A. solani*. Stigmasterol was subjected to docking studies carried out against fungal elicitor cryptogein. A better docking score of 12.59 with glide energy -42.56 was obtained for the complex fungal elicitor cryptogein. The interaction was done in chain A Tyr 47 [O–H…O] residue with a distance of 2.7 Å.

Mishra *et al.*, (2014) investigated the *in-vitro* antifungal efficiency of extracts of 20 plant species by poisoned food technique against the test pathogen. The extract of *Ageratum conyzoides* exhibited maximum toxicity (95.57%) against the *Fusarium oxysporum* f.sp. *lycopersici*. Significant results were also observed in extracts of *Ageratum haustoridanum*, *Clerodendrum inerme* and *Terminalia bellirica* showing inhibition of 90.33%, 84.97% and 79.19% respectively. Result obtained with plant extracts are easily available for controlling plant diseases, non pollutive, cost effective non hazardous and eco-friendly.

Subban *et al.*, (2011) have studied that antifungal activity of eleven different medicinal plants namely *Aloe vera*, *Alpinia calcarata*, *Acalypha indica*, *Carum copticum*, *Leucas aspera*, *Ocimum sanctum*, *Piper betle*, *Phyllanthus niruri*, *Solanum trilobatum*, *Mennycolon umbellatum* and *Tridax procumbens* against plant pathogenic fungus *Fusarium oxysporum* by agar well-diffusion method. The plants leaves were extracted with various solvents like ethyl acetate, diethyl ether and water (aqueous).
Among the different plants tested, all the 3 solvent extracts of the *Memycelon umbellatum* showed maximum (21 mm) antifungal activity against the plant pathogen. Whereas the other plant extracts were also effective from moderate to minimum antifungal activity.

Shukla and Dwivedi (2012) examined that *in-vitro* efficacy of different plant extracts *viz.* Bitter guard, Turmeric, Garlic and Black pepper has been tested to control both fusarial species *viz.* *Fusarium udum* (causing wilt in pigeonpea) and *Fusarium oxysporum* f.sp.ciceri (causing wilt in chickpea). Both pathogenic fungi have been isolated from infected plant parts and identified on the basis of their morphological and cultural characteristics. Different concentration i.e. 5%, 10% and 15% of plant extracts are taken in the swot. All the plant extracts showed considerable diminution in the growth of pathogens. Growth of *Fusarium udum* has been reduced by 15% concentration of turmeric (89.2%) followed by garlic (88.26%) and black pepper (82.22%). In case of *Fusarium oxysporum* f.sp.ciceri, 15% concentration of garlic, turmeric and black pepper reduced the growth upto 94.63%, 87.96% and 77.74% (at \( p< 0.01 \)) respectively. From the above observations it can be postulated that growth of both the pathogens has been significantly reduced (at \( p<0.01 \)) by garlic and turmeric extracts followed by extract of black pepper. The bitter guard extract is found least effective against both the pathogens at all concentrations. In addition to this 10% concentration of garlic and turmeric is also found effective against both the pathogens.

Ahumuza and Kirimuhuzya (2011) tested the ethanolic leaf extract of *Pentas decora*, a plant known in the local community as “Kabyakyasha” (in Runyankole Language), for its antifungal activity against *Candida albicans*, *Epidermophyton floccosum*, *Microsporum canis* and *Trichophyton rubrum*, and qualitatively analyzed for its phytochemical composition. The disc diffusion method was used to determine the antifungal activity. Minimum inhibitory concentrations (MICs) and Minimum Fungicidal Concentrations (MFCs) were also determined using the tube dilution method. The *Pentas decora* ethanolic extract exhibited antifungal activity against *C. albicans* and *M. canis*, with MICs of 1000 and 1500 mg/ml respectively, while the corresponding values for the standard drug (clotrimazole) were 50 and 100 mg/ml respectively. The MFC values of the extract for *C. albicans* and *M. canis* were 2000
and 2500 mg/ml respectively while the corresponding MFC values for clotrimazole were 100 and 150 mg/ml respectively. However, both the extract and the standard drug had no activity against *E. fluccosum* and *T. rubrum*. The extract was also found to contain alkaloid and terpenoids. It can be concluded that the plant has some antifungal activity but there is need to do more tests and first ascertain its toxicity profile before it is declared sufficiently efficacious and safe for use by the community.

Enespa and Dwivedi (2014) have viewed the pathogenic fusaria *viz.*, *Fusarium solani* f. sp. *melongena* and *F. oxysporum* f. sp. *lycopersici* causing brinjal and tomato wilt were isolated from soil as well as from the infected plant parts. *In vitro* efficacy of three medicinal plants *viz.*, *Azadirachta indica* (leaf extract), *Psidium guajava* (leaf extract), *Eucalyptus camaldulensis* (bark extract) and three fungal antagonists *viz.*, *Trichoderma harzianum*, *T. atroviride* and *T. longibrachiatum* were tested at 25, 50 and 75% (v/v) by poisoned food technique against both the pathogens. The assessment of fungitoxicity was carried out in terms of percent mycelial growth inhibition against the test fungi. Among different medicinal plant extracts, *Azadirachta indica* (leaf) was found significantly superior to the rest in suppressing the growth of *Fusarium oxysporum* f. sp. *lycopersici* as 100% inhibition was recorded at 50 and 75% concentration followed by *Psidium guajava* and *Eucalyptus camaldulensis* on 7th day of inoculation. On the other hand, among different microbial antagonists, *T. longibrachiatum* against both the test fungi was highly effective and there was 100% inhibition of mycelial growth at 50 and 75% concentration, while *T. harzianum* was effective against *F. oxysporum* f. sp. *lycopersici* followed by *T. atroviride* as it completely inhibited the mycelial growth at 75% concentration.

Verastegui *et al.*, (1996) investigated the antifungal activity of several widely distributed plants in the vegetation of northern Mexico and the southern U.S.A. The plants were evaluated against yeasts and moulds: *Candida albicans*, *Candida krusei*, *Candida rugosa*, *Cryptococcus neoformans*, *Cryptococcus laurentis*, *Cryptococcus albidus*, *Microsporum canis*, *Microsporum gypseum*, *Trichophyton tonsurans*, *Epidermophyton floccosum* and *Sporotrix schenckii*. The extracts analysed showed good antifungal activity against more than one organism.
Schmourlo et al., (2005) evaluated that antifungal agents was done on medicinal and fruit bearing plants used against skin diseases by the Brazilian population. The results, evaluated by the diameter of the inhibition zone of fungal growth, indicate that out of sixteen only six plant species, showed significant activity against three fungi: Candida albicans, Trichophyton rubrum and Cryptococcus neoformans.

Ethanol extracts from the leaves and/or roots of thirty five medicinal plants commonly used in Brazil were also screened for anti-Candida albicans activity (Duarte et al., 2005). Extracts from thirteen plants showed activity.

Muschietti et al., (2005) evaluated methanol extracts from eleven traditionally-used Argentine medicinal plants in vitro for antifungal activity against yeasts, hialohypomycetes as well as dermatophytes by microbroth dilution method. Of these, the most pronounced effect was observed by Eupatorium bunifolium H.B.K. (Asteraceae) and Terminalia triflora (Griseb.) Lillo(Combretaceae).

As part of a European screening leaf extracts of Camelia sinensis L. (Theaceae), Cupressus sempervivens L. (Cupressaceae) and Pistacia lentiscus L. (Anacardiaceae), and the seed extract of Glycine soja Sieb. et Zucc. (Papilonaceae) were tested against yeast and yeast like species implicated in human mycoses. Only extracts of C. sinensis exhibited widespread activity ( Turchetti, et al., 2005).

Tadeg et al., (2005) investigated the antifungal activities of some selected traditional Ethiopian medicinal plants used in the treatment of skin disorders. Hydroalcohol extracts of Acokanthera schimperi (D.C.) Benth. et Hook. (Apocynaceae), Calpurna aurea L. (Leguminoseae), Kalanchoe petิตitiana (Engl.) Cufod. (Crassulaceae), Lippia adonensis Hochst. (Verbenaceae), Malva parviflora L. (Malvaceae), Olinia rochetiana L. (Oliniaceae), Phytolacca dodecandra L’Herit (Phytolaccaceae) and Verbascum sinaicum Bentham (Scrophulariaceae) were screened for antifungal activity against different strains of fungi which are known to cause different types of skin infections. Of all the plants tested, L. adoensis and O. rochetiana were found to be the most effective.
Zaidi and Crow (2005) reported the antifungal activity of the following four important medicinal plants from Balochistan, Pakistan: *Grewia erythraea* Schwein f. (Tiliaceae), *Hymenocrater sessilifolius* Fisch. (Lamiaceae), *Vincetoxicum stocksii* Ali & Khatoon (Asclepidaceae) and *Zygophyllum fabago* L. (Zygophyllaceae). The extracts of *Z. fabago* and *V. stocksii* showed good activity against *Candida albicans*.

Thirty six extracts derived from ten plant species used by traditional Thai healers were assayed for their antifungal activity against clinical isolates of *Candida albicans*, *Cryptococcus neoformans* and *Microsporum gypseum* (Phongpaichit, 2005).

The chloroform extract of *Alpinia galanga* (L.) Willd. (Zingiberaceae) and *Boesenbergia pandurata* (Robx.) Schltr. (Zingiberaceae) had efficient antifungal activity against *Cryptococcus neoformans* and *Microsporum gypseum*, but exhibited weak activity against *Candida albicans*. Both plants are excellent candidates for the development of a remedy for opportunistic fungal infections in acquired immunodeficiency syndrome patients. Examples of other antifungal crude extracts of traditional medicinal plants also included those of plants used against venereal diseases in South Africa, (Buwa and Staden, 2006) and some medicinal plants from the Soqotra island in Yemen (Mothana and Lindequist 2005).

Loizzo et al., (2004) investigated the antifungal activity of methanol, ethyl acetate, dichloromethane, *n*-hexane, *n*-butanol and chloroform extracts of *Senecio inaequidens* D.C. and *Senecio vulgaris* L. (Asteraceae). The hexane extract of *S. vulgaris* showed strong activity against *Trichophyton tonsurans* (IC$_{50}$ of 0.031 mg/ml). Examples of other antifungal crude extracts from the Asteraceae family also included *Cynara scolymus* L. extracts (Zhu, et al., 2005).

The dichloromethane extract of the aerial part of *Blumea gariepina* D.C. was shown to be active against the phytopathogenic fungus *Cladosporium cucumerinum*, (Queiroz et al., 2005), and aqueous and petroleum ether extracts of *Spilanthes calva* D.C. was observe to be active towards *Fusarium oxysporum* and *Trichophyton mentagrophytes* (Rai et al., 2004).
Shams *et al.*, (2006) evaluated that in Liliaceae family, antifungal activity concern mainly the *Allium* genus. By using an agar dilution assay, the antifungal activity of aqueous extracts from *Allium cepa* L. (onion) and *Allium sativum* L. (garlic) were evaluated against *Malassezia furfur*, *Candida albicans* as well as several strains of various dermatophyte species. The results indicate that onion and garlic might be promising sources of drugs for the treatment of fungal associated diseases from the important pathogenic genera *Candida*, *Malassezia* and the dermatophytes. Onion and garlic were also investigated for their antifungal activity on two important dermatophytes, *Trichophyton rubrum* and *Trichophyton mentagrophytes* (Ghahfarakhi, *et al.*, 2004) (Iwalokun *et al.*, 2004).

Amin and Kapadnis (2005) investigated the antifungal activity from another *Allium* species, *Allium ascalonicum* O. Fedtsch, against twenty three strains of fungi. Among them, *Aureobasidium pullulans* and *Microsporum gypseum* were the most sensitive (IC$_{50}$ of 0.15 mg/ml).

Taguchi *et al.*, (2005) investigated the antifungal activity of medicinal species belonging to the Myrtaceae family include the herbal food clove *Syzygium aromaticum* L. and extracts of *Eucalyptus globulus* Labill., *Eucalyptus maculata* Hook. and *Eucalyptus viminalis* Labill. which significantly inhibited the growth of the fungus *Trichophyton mentagrophytes*.

A plant known as alfavaca, has been popular as having *in vitro* activity against the dermatophytes *Microsporum canis*, *Microsporum gypseum*, *Trichophyton rubrum* and *Trichophyton mentagrophytes* (Silva *et al.*, 2005) *Trichophyton rubrum*, the most common etiological dermatophytosis in Goiania, state of Goias, Brazil, was the most susceptible dermatophyte. The plant alfavaca was also active towards twenty five isolates of *Cryptococcus neoformans*, the etiological fungus responsible for cryptococcal infections (Lemos, *et al.*, 2005). Another Lamiaceae species, *Satureja khuzistanica* Jamzad was also effective against *Candida albicans* (Amanlov *et al.*, 2004).

Masoko *et al.*, (2005) investigated the antifungal activities of six South African *Terminalia* species (Combretaceae), *Terminalia prunioides* M.A. Lawson,
Terminalia brachystemma Welw. ex Hiern, Terminalia sericea Burch ex D.C., Terminalia gazensis Bak. f., Terminalia mollis Laws and Terminalia sambesiaca Engl. & Diels. against five fungal animal pathogens (Candida albicans, Cryptococcus neoformans, Aspergillus fumigatus, Microsporus canis and Sporothrix schenckii). T. sericea extracts were the most effective against almost all the microorganisms tested.

Agbenin and Marley (2006) have studied the effect of crude extracts of neem (Azadirachta indica) leaf, neem seed and garlic (Allium sativum) at concentrations ranging from 5% to 30% of the material in 100 ml of Potato Dextrose Agar on mycelial growth of Fusarium oxysporum f. sp. lycopersici. All the extracts inhibited mycellial growth specifically. Dry neem seed extract gave 100% inhibition of mycelial growth. Fresh neem leaf extract reduced mycelial growth with increasing concentration while in case of garlic there were no differences in growth inhibition among the various concentrations used. However garlic extracts decreased sporulation with increasing concentration.

Phongpaichit et al., (2005) examined the partially-purified fraction obtained from column chromatographic preparation of the crude methanol extract of Acorus calamus Linn. rhizomes for its antimicrobial activities on various microorganisms including bacteria, yeasts and filamentous fungi. It exhibited high activity against filamentous fungi: Trichophyton rubrum, Microsporum gypseum, and Penicillium marneffei with IC<sub>50</sub> values of 0.2, 0.2 and 0.4 mg/ml, respectively. However, it showed moderate activity against yeasts: Candida albicans, Cryptococcus neoformans and Saccharomyces cerevisiae (MIC 0.1-1 mg/ml) and low activity against bacteria (MIC 5->10 mg/ml). Scanning with electron microscopic observation resolved that hyphae and conidia treated with this fraction were shrunken and collapsed, due to cell fluid leakage.

Devi and Chhetry (2013) have studied the antifungal activity of aqueous extracts of locally available plants known for their medicinal value in vitro against Drechslera oryzae, the causal organism of brown leaf spot of rice. The plants extracts at 5%, 10%, 15% and 20% were tested against the mycelial growth of D. oryzae by poisoned food technique. Among the plants extracts, Acorus calamus extract at its 20%
concentration alone showed 80.0% inhibition of mycelial growth whereas the other tested plants showed less inhibitory effect. In field trial, aqueous extract of *Acorus calamus* showed maximum percentage of disease control and reduced the disease incidence by 45.29% as compared with control plot.

Mungkornasawakul *et al.*, (1996) examined the dry and powdered rhizomes of *Acorus calamus* L. which was extracted with ultrasonic bath using dichloromethane as solvent. Various concentrations (0.01-0.15 %) of the extract were determined for antifungal activity on PDA agar against *Alternaria* spp. isolated from leaf spot and *Fusarium* spp. isolated from wilt diseases of cruciferous vegetable, as well as *Botrytis* spp. isolated from gray mold rot of roses and *Septoria* spp. isolated from leaf spot of chrysanthemum. The results indicated that all of the molds tested were sensitive to *Acorus calamus* extract. The growth of all tested fungi was completely inhibited at the concentration of 0.10 % upward.

Kumar *et al.*, (2010) have studied the *in vitro* antimicrobial activity of ethanolic extracts of the medicinal plant *Acorus calamus* against three bacterial (*Pseudomonas* sp., *Bacillus* sp., *Staphylococcus aureus*) and three fungal (*Aspergillus niger*, *Aspergillus flavus*, *Trichoderma* sp.) species. The ethanolic extract of *Acorus calamus* exhibited antimicrobial activity moderately on *Pseudomonas* sp., *Staphylococcus aureus*, and *Aspergillus flavus*. The phytocompounds that are present in the rhizomes of *Acorus calamus* was screened by Gas Chromatography and Mass Spectroscopy (GCMS) method. The GC-MS study revealed about 10 active phytocompounds present in the rhizomes including alkaloids, aromatic, palmitic and linoleic acid.

Barik *et al.*, (2010) evaluated the endophytic fungus isolated from *Acorus calamus* rhizomes, which is an important medicinal plant. Colonial morphological trait and microscopic observation revealed *Fusarium* sp. in taxonomy. Based on 18S rRNA gene sequence the fungus was identified as *Fusarium oxysporum*. The crude metabolite of the fungus displayed considerable antimicrobial activity against some clinically significant microorganisms. Its metabolite was highly effective against both Gram positive and Gram negative microorganisms and moderately effective against fungal pathogens. The fungus was placed phylogenetically which revealed that it has evolved from saprophytic ancestor and co-exists with a pathogenic strain.
Phylogenetic tree was generated using maximum parsimony method to establish relationship of the fungus with other *F. oxysporum* isolates those existing in different forms.

Asha and Ganjewala (2009) have studied the antimicrobial activity of *Acorus calamus* rhizome and leaf extracts obtained with different solvents viz., petroleum ether, chloroform, hexane and ethyl acetate. Ethyl acetate extracts were found to be highly effective. Rhizomes and leaf ethyl acetate extracts exhibited pronounced antifungal activity with diameter zone of 20-28 and 18-25 mm as well as anti yeast activity with diameter zone of 22-25 and 20-23 mm, respectively. The minimum inhibitory concentration (MIC) of the rhizome and leaf extracts for antifungal activity measured was 2-4mg/ml, except *Penicillium chrysogenum* whereas against yeasts was relatively higher, 4-5 and 6-8 mg/ml. MIC value for antibacterial activity was comparatively very high ~16-42 mg/ml.

Kumar et al., (2014) evaluated the antimicrobial activity of *Acorus calamus* rhizome extracts with different solvents viz., water, methanol, ethanol, butanol, hexane, petroleum ether and ethyl acetate. From the phytochemical analysis flavonoid, glycoside, saponin, resin and steroid were found in the rhizome extract and which are responsible for their antimicrobial activities. Extract obtained with ethyl acetate and ethanol among other were found to be highly effective. Rhizomes ethyl acetate and ethanol extracts exhibited pronounced antibacterial activity against MRSA with diameter zone of inhibition (22-26 mm) and antifungal activity against *Aspergillus niger* with diameter zone of inhibition (20mm). The minimum inhibitory concentration (MIC) of the rhizome extracts for antimicrobial activity was done by serial dilution viz., 100mg/500μl, 50mg/500μl, 25mg/500μl, 12.5mg/500μl and 6.25mg/500μl.

Pereira et al., (2005) examined the antimicrobial activity of rough extracts from leaves of *Arctium lappa* against microorganisms, commonly found in the oral cavity, specifically in endodontic infections, such as *Enterococcus faecalis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis* and *Candida albicans*. The agar-diffusion method revealed hexanic phase as an inhibitor of microbial growth. Bioautographic assays identified antimicrobial substances in the extract. The results
showed the existence, in the rough hexanic phase and in its fractions, of constituents that have retention factors (Rf) in three distinct zones, thereby suggesting the presence of active constituents with chemical structures of different polarities that exhibited specificity against the target microorganisms.

Ionescu et al., (2013) have studied the antimicrobial activity of some hydroalcoholic extracts obtained from three vegetable species with choleretic-cholagogue action: artichoke (Cynara scolymus), dandelion (Taraxacum officinale) and burdock (Arctium lappa). The antimicrobial activity of these products was tested by serial dilution method against bacterial strains (Staphylococcus aureus ATCC 6538, Escherichia coli ATCC 8739 and Salmonella abony NCTC 6017). The hydroalcoholic extracts obtained from the studied vegetable species, have shown an antimicrobial activity against the bacterial strains of Escherichia coli and Salmonella abony, but they have not shown any antimicrobial activity against Staphylococcus aureus.

Moskalenko (1986) evaluated the antibacterial activity for Arctium lappa against Gram negative (E. coli, Shigella flexneri, and Shigella sonnei), Gram positive (Staphylococcus aureus, Bacillus subtilis) and Mycobacterium.

Perin et al., (2002) have studied that lyophilized extract of Arctium lappa was effective against Bacillus subtilis and Candida albicans. Ethyl acetate fraction was used as intracanal medication for 5 days in teeth infected with C. albicans, E. coli, Lactobacillus acidophilus, Pseudomonas aeruginosa and Streptococcus mutans that inhibited microbial growth after 14 days treatment.

Gentil et al., (2006) evaluated the antimicrobial activity of rough extracts from leaves of Arctium lappa and tested in vitro against microorganisms commonly found in the oral cavity, specifically in endodontic infections, Enterococcus faecalis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis and Candida albicans. The Arctium lappa constituents exhibited a great microbial inhibition potential against the tested endodontic pathogens.

Rongai et al., (2012) examined the antifungal activity of botanic extracts on the development of Fusarium oxysporum f.sp. lycopersici. The tests in vitro were carried
out in a multi-well plate assay. The tested plants were classified based on the optical density reached by germinating conidia 24, 48 and 72 hours after inoculation. Among 500 plant species tested, about 84% did not exert significant inhibition, 7.6% showed low inhibition, 5.2% had an intermediate level of antifungal activity, and only 3% inhibited fungal germination completely. These findings suggest that some botanic extracts tested possess antifungal activities against *Fusarium oxysporum* and could be used as potential antifungal agents for the control of fungal plant diseases.

Pereira *et al.*, (2005) have studied the antimicrobial activity of rough extracts from leaves of *Arctium lappa* and their phases. The following microorganisms, commonly found in the oral cavity, specifically in endodontic infections, were used: *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Candida albicans*. The agar-diffusion method allowed detection of the hexanic phase as an inhibitor of microbial growth. Bioautographic assays identified antimicrobial substances in the extract. The results showed the existence, in the rough hexanic phase and in its fractions, of constituents that have retention factors (Rf) in three distinct zones, thereby suggesting the presence of active constituents with chemical structures of different polarities that exhibited specificity against the target microorganisms. It may be concluded that the *Arctium lappa* constituents exhibited a great microbial inhibition potential against the tested endodontic pathogens.

Dabiri and Karbasizade (2013) evaluated ethanol extract of edible burdock (*Arctium lappa*) root on *Bacillus cereus* spores. In this investigation, the suspensions of tested microorganisms were cultured in sporulating agar. Sporulation process was assessed by optical microscopy following the staining of spores. Then the produced spores were exposed to various concentrations (100, 150, 200, 250, 300 mg/mL) of ethanol extract of edible burdock (*Arctium lappa*) root and finally the remaining spores were counted. With increasing concentrations of ethanol extract, the number of spores declined. The most effective extract concentration was found to be 300 mg/mL.

Uddin *et al.*, (2012) evaluated the Asparagus racemosus against eight bacterial strains including *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas alkaligenes*, *Proteus specie*, *Shegella*, *Salmonella typhi*, *Vibrio cholera* and *Staphylococcus aureus*. The zone of inhibition was studied by agar cup diffusion method at
concentration of 1μg/μl, 0.1μg/μl, 0.01μg/μl, 0.001μg/μl and 0.0001μg/μl in DMSO (Dimethyl Sulfoxide). Methanolic extract particularly at concentration of 1μg/μl was found to be effective against all bacterial strains. All the antimicrobial activities were compared with Amoxicillin, a standard antibiotic.

Zape et al., (2009) examined the extract of different parts of ten medicinal plants which were evaluated against chickpea wilt pathogen (Fusarium oxysporum f.sp. ciceri) with three concentrations (1000, 500 and 250 μg/ml) at five different time internals. The fungitoxicity of alcohol extract of medicinal plants against wilt causing pathogen significantly varied with concentration and time intervals. All plant extracts inhibited the mycelial growth of the fungus in vitro. As concentration of extracts decreased, the effectiveness of extracts were also decreased against wilt pathogen. The maximum growth inhibition was recorded at 1000 μg/ml concentration and the per cent inhibition was observed maximum in bawchi at 8th day (45.55%) and at 7th day (38.17%) followed by ashwagandha at 8 th day (37.84%) and at 7th day (36.25%). At 4th, 5th and 6th day, the per cent inhibition in alcohol extract at 500μg/ml concentration increased with increased in time upto 8 th day. Maximum per cent inhibition was observed in bawchi at 8 th day (44.44%) and at 7th day (36.36%) followed by ashwagandha at 8th day (33.33%) and at 7th day (32.38%). Bawchi treatment showed significantly highest per cent inhibition at 8 th day over rest of the treatments. The extracts at 250 μg/ml concentration were failed to inhibit the mycelial growth of these pathogens. At 250 μg/ml concentration, only ashwagandha, bawchi and kali Haldi were found to decrease the mycelial growth of the pathogen to some extent.

Bashar and Chakma (2014) evaluated seven soil fungi viz. Aspergillus flavus, A. fumigatus, A. niger, A. terreus, Penicillium sp., Trichoderma harzianum and T. viride associated with the rhizosphere, non rhizosphere and rhizoplane of brinjal plants to observe their antagonistic potential against the test fungi Fusarium oxysporum and F. solani. Out of seven soil fungi T. harzianum was found to be most effective to control the growth of both the test fungi. Plant parts extract of Allium sativum, Asparagus racemosus, Azadirachta indica, Cassia alata, Ocimum sanctum, Zingiber officinale and Datura metel were evaluated for their in vitro efficacy at 5, 10 and 20% concentration against the test fungi. Datura metel, and C. alata and A. indica was
found most efficient inhibitor of *F. solani* and *F. oxysporum*, respectively. Five fungicides *viz.*, agridazim 50 wp, cozeb 80 wp, newban 50 wp, sunvit 50 wp and vitavax 200 B were evaluated for their *in vitro* efficacy at 500 ppm concentration against *F. solani* and *F. oxysporum*. Vitavax 200B was found most efficient inhibitor of both the test fungi.

Patel and Patel (2013) examined the crude extracts from the leaf of *Asparagus racemosus Willd* using different solvents like petroleum ether, methanol, Chloroform, acetone, ethyl acetate and water. The effect of different extracts were tested on Gram positive bacteria like *Bacillus subtilis, Staphylococcus aureus* and Gram negative bacteria *E. coli, Pseudomonas* and the yeast *Candida utilis* by *in-vitro* agar well diffusion method. This study scientifically supports the usage of whole plant as a remedy for various superficial bacterial and fungal infections in traditional medicine.

Mathur *et al.*, (2011) examined the antifungal activity and minimum inhibitory concentration (MIC) of various plant extracts in different solvents such as hydro-alcohol (50 % *v*/v) and hexane of plants traditionally used as medicines as *Valeriana jatamansi* (Sugandhbala), *Coleus barbatus* (Pathar choor), *Berberis aristata* (Kingore), *Asparagus racemosus* (Satrawal), *Andrographis paniculata* (Kalmegha), *Achyranthes aspera* (Latjiri), *Tinospora cordifolia* (Giloei), *Plantago depressa* (Isabgol) were evaluated against *Aspergillus niger* and *Candida albicans*. Hydro-alcoholic extracts of all the plants were found to have maximum antifungal activity in comparison to hexane extracts. Hydroalcoholic extracts of *Andrographis paniculata* and *Achyranthes aspera* showed maximum effectiveness against *Aspergillus niger* and *Candida albicans* at highest MIC value of 0.5 and 0.3 mg/ml respectively. Hexane extracts of *Andrographis paniculata* showed highest MIC value of 0.7 mg/ml against *Aspergillus niger*.

Sinha and Biswas (2011) have studied the bactericidal activity of crude extracts from *Asparagus racemosus* against eight pathogenic strains which belongs to *Bacillus subtilis, Staphylococcus aureus, Micrococcus luteus, Escherichia coli, Vibrio cholerae, Shigella dysenteriae, Shigella flexneri* and *Pseudomonas aeruginosa*. The chloroform and ethanolic extract exhibited predominant antibacterial activity against all the bacteria tested. The chloroform extract showed higher zone of inhibition than
ethanolic extract. Gram positive bacteria were found to be more sensitive than those of Gram negative bacteria in both extracts. The inhibition of both Gram positive and Gram negative bacteria by the solvent extracts indicated the presence of broad spectrum antibacterial substances in the plant root. The result was promising and supported the use of *Asparagus racemosus* traditionally in several ailments.

Panghal *et al.*, (2011) examined the antimicrobial activity for *Asphodelus tenuifolius* Cav., *Asparagus racemosus*, *Balanites aegyptiaca* L., *Cestrum diurnum* L., *Cordia dichotoma* G. Forst, *Eclipta alba* L., *Murraya koenigii* (L.) *Spreng.* , *Pedalium murex* L., *Ricinus communis* L. and *Trigonella foenum graecum* L. against bacterial pathogens *Staphylococcus aureus* (23.2%), *Escherichia coli* (15.62%), *Staphylococcus epidermidis* (12.5%), *Pseudomonas aeruginosa* (9.37%), *Klebsiella pneumonia* (7.81%), *Proteus mirabilis* (3.6%), *Proteus vulgaris* (4.2%) and the fungal pathogens were *Candida albicans* (14.6%), *Aspergillus fumigatus* (9.37%) by modified Kirby-Bauer disc diffusion method. MIC and MFC were investigated by serial two fold micro broth dilution method. Out of 40 cases, 35 (87.5%) were observed as neutropenic. Eight medicinal plants (*A. tenuifolius*, *A. racemosus*, *B. aegyptiaca*, *E. alba*, *M. koenigii*, *P. murex*, *R. communis* and *T. foenum graecum*) showed significant antimicrobial activity (P < .05) against most of the isolates. The MIC and MFC values were ranged from 31 to 500 μg/ml. *P. aeruginosa* was observed highest susceptible bacteria (46.6%) on the basis of susceptible criteria.

Kaushik *et al.*, (2014) evaluated the antimicrobial and antifungal potential of medicinally important four plants used in Indian folklore medicine viz. *Solanum torvum*, *Adhatoda vasica*, *Terminalia chebula* and *Asparagus racemosus* with their extracts prepared in organic solvents namely methanol, ethanol and methanol-ethanol (1:1) and were tested against fungal pathogens *Fusarium oxysporum* and *Aspergillus parasiticus* by agar well diffusion assay. Irrespective of the extraction solvent used all the plants extracts showed certain degree of antimicrobial activity against *Aspergillus parasiticus*. Zone of inhibition were obtained against *Fusarium oxysporum* in case of methanolic and ethanolic extracts only. The plants with highest antifungal activity recorded were *Solanum torvum* and *Adhatoda vasica* against *Fusarium oxysporum*. Similarly *Asparagus racemosus* and *Adhatoda vasica* were also effective against
Aspergillus parasiticus. Rest of the plant extracts exhibited moderate to minimal antifungal activity.

Ngeny et al., (2013) evaluated the antimicrobial from Hagenia abyssinica, Fuerstia africana and Asparagus racemosus against pathogenic bacteria. Results exhibited antibacterial activity with minimum inhibitory concentration of ≤ 6.25mg/ml against Staphylococcus aureus, MRSA and Pseudomonas aeruginosa. However, the plants studied had weak antifungal activity. H. abyssinica and F. africana extracts were found to be cytotoxic with CC50 of < 90 μg/ml. These extracts were tested for acute toxicity and found to be safe at 5000 mg/kg body weight per day. The results of the study support the medicinal use of these plants and indicate that useful compounds from Hagenia abyssinica and Fuerstia africana can be isolated for further exploitation.

Shamim et al., (2004) evaluated the activity of Allium sativum Linn., Aloe barbadensis Mill., and Solanum nigrum Linn. against some common fungal species associated with superficial mycoses. The ethanol and aqueous extracts of these plants were tested to establish the antimycological effects against dermatophytes, saprophytes, and Candida species isolated from infected hospitalized patients. The in-vitro antifungal activity was established by observing and measuring the zones of inhibition formed on selective nutrient media. Zones of inhibition were as very high (41–50 mm), high (31–40 mm), medium (21–30 mm), and low (11–20 mm). High zones of inhibition were noted with ethanol extracts of Allium sativum, Aloe barbadensis, and Solanum nigrum.

Rauf et al., (2013) determined antifungal potential of different parts of Chenopodium album L. against Fusarium oxysporum Schlechtend. f. sp. cepae (Hans.)

Snyder and Hansen, the cause of basal rot disease of onion (Allium cepa L.). In screening bioassays, the effect of different concentrations (0.5, 1.0, ...3.0%) of methanolic leaf, stem, root and inflorescence extracts of Candida album was investigated. Extracts of different parts of the test plant species showed variable antifungal activity. The highest antifungal activity was exhibited by inflorescence extract. Different concentrations of this extract suppressed fungal growth by 24-80%.
Methanolic inflorescence extract was successively extracted with n-hexane, chloroform, ethyl acetate and n-butanol. The highest antifungal activity was shown by ethyl acetate fraction resulting in 68-100% reduction in fungal biomass. From ethyl acetate fraction, three unknown compounds viz. A, B and C were isolated through thin layer chromatography (TLC). TLC fraction A exhibited the highest antifungal activity with minimum inhibitory concentration (MIC) of 250 µg/mL. Thus, antifungal constituents of ethyl acetate fraction of methanolic inflorescence extract of C. album can be used as natural fungicides for the management of basal plate rot of onion.

Moghimipour et al., (2014) determined the antifungal activity of saponin extracted from the Glycyrrhiza glabra against Candida species (Candida albicans, Candida tropicalis and Candida glabrata). Antifungal activity Quillaja saponaria total saponin (QST) was also evaluated. The roots of the plant were dried, powdered and defatted with petroleum ether in a soxhlet apparatus. The air dried powder was successively extracted with methanol, n-butanol and diethyl ether. The antifungal activity of the saponins was carried out using well diffusion method and also the value of minimum inhibitory concentrations (MIC) was calculated. Clotrimazole was used as positive controls to determine the sensitivity of the species. According to the results, C. albicans, and C. tropicalis were sensitive to the saponins of G. glabra, and Q. saponaria, while saponin isolated from G. glabra just could inhibited the growth of C. glabrata.

Ethanolic and petroleum ether extracts of Centella asiatica plant shows significantly higher rate of antifungal activity against various fungal strains like Aspergillus niger, Aspergillus flavus and Candida albicans when compare to water extracts (Jagtap et al., 2009). While its hexane, carbon tetrachloride, chloroform and aqueous soluble fractions of methanolic extract showed antimicrobial activity against various yeast and mold strains like Aspergillus niger, Saccharomyces cerevisiae and Candida albicans (Ullah et al., 2009).

Methanolic extract of Centella asiatica showed significant inhibitory effect on efficiency of spore germination against various fungal strains like Alternaria, Cercospora, Curvularia, Drechslera and Fusarium. The inhibitory effect on spore
germination of the above fungus strains was increased proportionately with the increase in the concentration of methanolic extracts of the leaves (Areekul., 2009: Singh., 2000).

Duangkamol et al., (2008) evaluated the crude extracts of Asiatic Pennywort (Centella asiatica (Linn.) Urban) and Water Pennywort (Hydrocotyle umbellata Linn.) extracted with three kinds of solvent; hexane, ethanol and water, at their 1,000 mg/mL, 800 mg/mL, 400 mg/mL, 200 mg/mL, 100 mg/mL and 50 mg/mL concentrations against S. aureus ATCC 25923. From TLC results, asiatic acid was detected in crude extract of asiatic pennywort extracted with hexane and ethanol. Asiaticoside was detected in crude extract of asiatic pennywort extracted with ethanol and water. Neither asiatic acid nor asiaticoside was detected in crude extract of water pennywort. From disc diffusion results, water crude extract of Centella asiatica demonstrated that average inhibition zones ranged from 6.54-17.72 mm in diameter. Determination of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were performed using agar dilution method.

Bobbarala et al., (2009) examined the antifungal activity of forty nine plants including Centella against Aspergillus niger fungi using agar well diffusion method. Among the 49 plants studied the methanolic extracts of 43 plants including Centella exhibited varying degrees of inhibition activity against the above fungi.

Methanol, chloroform and acetone extracts of Centella asiatica showed significant inhibitory effect on growth and sporulation tendency of Colletotrichum gloeosporioides (Johnny, 2010).

Alcoholic extracts of Centella asiatica did not showed antimicrobial activity against yeasts like Pichia anomala and Saccharomyces cerevisiae and molds like Aspergillus niger and Penicillium pinophilum (Areekul., 2009).

Dash et al., (2011) have examined the antimicrobial activity of petroleum ether, ethanol, chloroform, n-hexane and water extracts of Centella asiatica herb by agar well diffusion assay against bacterial strain Proteus vulgaris, Staphylococcus aureus, Bacillus subtilis and Escherichia coli and fungal strains Aspergillus niger and
Candida albicans. Zone of inhibition produced by different extracts against the selected strains was measured and compared with standard antibiotic ciprofloxacin (10μg) and ketocanozole (10μg). The present study demonstrated that the petroleum ether, ethanol and chloroform extracts of Centella asiatica have higher antimicrobial activities (average 12-19 mm zone of inhibition) than n-hexane and water extracts (average 8-14 mm zone of inhibition) whereas n-hexane extract showed no activity against E. coli. All the extracts showed better results against the tested fungal strains comparing with ketocanozole (10μg). The results obtained in the present study suggest that the different extracts of Centella asiatica revealed a significant scope to develop a novel broad spectrum of antibacterial and antifungal herbal formulations.

Kumar et al., (2013) have studied the screening of anticomulsive effect by marble-burying model in mice. Study of marble-burying behaviour with locomotor activity in albino mice was carried out by specially designed model for anticomulsive activity and actophotometer for locomotor activity. For marble burying study, 7 groups (n=6) for control, test (50 and 100 mg/kg of aqueous and ethanolic extract) and standard drug (5 and 10 mg/kg of fluoxetine) while another 7 groups for control, test (50 and 100 mg/kg of aqueous and ethanolic extract) and standard drug (5 and 10 mg/kg of fluoxetine), of mice were used for locomotor activity. The data was analyzed by one way analysis of variance followed by Dunnett’s test (P < 0.01). The ethanolic extract of Centella asiatica (50 and 100 mg/kg) was found significant in dose dependently (p<0.01), reduced the number of marbles buried without affecting the motor activity. The aqueous extract (100 mg/kg) also reduced (P<0.01) the number of marbles buried with slight reduction (13%) in locomotor activity when compared with fluoxetine. The finding of this study was suggested that ethanolic extract of Centella asiatica may significantly reduce anticomulsive behaviour comparable as fluoxetine.

Kannabiran et al., (2009) have studied the antimicrobial potential of aqueous and solvent (ethanol, methanol, acetone and ethyl acetate) extracts and saponin fraction isolated from the leaves of Solanum xanthocarpum and Centella asiatica against selected bacterial and fungal species. The antimicrobial activity was tested by agar disc diffusion and agar well diffusion method. The aqueous, ethanol, acetone and ethyl acetate extracts of S. xanthocarpum and C. asiatica were inhibited the growth of bacterial pathogens. The most susceptible Gram -negative bacterial pathogens were
*Klebsiella pneumoniae* (20 mm) and *Escherichia coli* (17 mm). The saponin fraction of *S. xanthocarpum* and *C. asiatica* inhibited the growth of Gram positive bacterium *S. aureus* (21 mm and 22 mm). The antimicrobial activity exerted by the saponin fraction was higher than the aqueous and organic solvent extracts against tested pathogenic bacteria and fungi and standard antibiotics. *Aspergillus fumigatus* was more susceptible fungal pathogen than *Aspergillus niger*. Preliminary phytochemical screening revealed the presence of saponins; phytosterols and carbohydrates in both the plants. Based on the results of this study, it can be concluded that the saponin fraction might be responsible for the antimicrobial capacity of *S. xanthocarpum* and *C. asiatica*.

Udoh et al., (2012) determined the inhibitory concentration of *Centella asiatica* (Gotu kola) on some microorganisms of clinical importance using standard microbiological methods. The phytochemical screening revealed the presence of alkaloids, saponins, tannins, flavonoids, anthraquinones, cardiac glycosides and phlobatannins. The antimicrobial tests were carried out with ethanolic and aqueous extracts using agar disc diffusion method against bacterial species used *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Proteus* species, *Shigella* species, *Salmonella typhi* and *Vibrio cholerae*. The results obtained for ethanolic extracts revealed that *Staphylococcus aureus* exhibited the largest zones of inhibition 23.0 mm (35mg/ml) while *Shigella* and *Escherichia coli* had the smallest of 15.0 mm (15mg/ml). For the aqueous extracts *Proteus* species had the largest zone of inhibition of 18.0mm (35mg/ml) while *Vibrio cholerae* had the least zones of inhibition of 12.1mm (15mg/ml). The higher inhibitory effects of these ethanolic extracts may be due to some bioactive substances present in the extracts. The minimum inhibitory concentrations ranged from 100mg/ml to 500mg/ml for both aqueous and ethanolic extracts. The results obtained qualify *Centella asiatica* to be a medicinal plant that is recommended for use in the treatment of some diseases and infections.

Kalita and Saikia (2012) determined the antifungal properties of the three plants based on highly uses by the Tiwa tribes in gastrointestinal troubles and skin diseases. against enteropathogenic bacteria *E.coli* (MTCC723), *Bacillus subtilis* (MTCC10619) and *Staphylococcus aureus* (MTCC96). Antifungal activity were tested against *Aspergillus niger* and *Candida albicans*. *Centella asiatica* and *Nerium indicum*...
showed good results against *E. coli* and *Bacillus subtilis* and *Cuscuta reflexa* showed higher activity against *S. aureus*. In case of antifungal activity, *Centella asiatica* results were found effective in comparison to the others.

Lalitha *et al.*, (2013) evaluated the antifungal activity of aqueous extract of leaf of *Centella asiatica* L. against seven test fungi and four bacterial species isolated by following standard procedure. The extract were tested at 10 to 100% concentration. All the test fungi recorded a significant activity in all the concentration and recorded Minimum Inhibitory concentration (MIC) in the range of 80% to 90%. All the results were compared with synthetic fungicides. Among the four bacterial species tested, all the test bacteria recorded significant activity and recorded an inhibition from 29mm to 31mm.

Jagtap *et al.*, (2009) determined the antimicrobial activity of petroleum ether, ethanol and water extract of *Centella asiatica* plant by agar diffusion method. Zone of inhibition produced by petroleum ether, ethanol and water extract in dose of 62.5, 125, 250, 500 and 1000 μg/ml against some selected strains was measured and compared with standard antibiotics ciprofloxacin (10μg/ml). The present study demonstrated that the ethanolic extract of *Centella asiatica* has higher antimicrobial activity than its petroleum ether and water extracts.

Arunugam *et al.*, (2011) have studied the antibacterial activity of leaf and callus of *Centella asiatica*. Leaf explants of *C. asiatica* were cultured on MS medium supplemented with different concentration of plant growth regulators for callus initiation. The maximum percentage of callusing was achieved in medium supplemented with 6-benzylaminopurine 4.0 mg/L and 2,4-dichlorophenoxyacetic acid 2.0 mg/L. In the preliminary phytochemical screening, alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins, saponins and reducing sugars were present in most of the tested extracts of leaf and *in vitro* grown callus of *C. asiatica*. Methanol, acetone, chloroform and water extracts of leaf and callus were evaluated for *in vitro* antibacterial activity against *Bacillus cereus, Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* by agar plate well diffusion method. All the extracts from leaf and callus of *C. asiatica* showed significant antibacterial activity against the
tested organisms. However, methanol extracts of leaf and callus showed maximum antifungal potential.

Devi et al., (2013) examined Twenty plant leaf extracts viz., Acalypha indica, Azadiracta indica, Alternanthera sessilis, Aloe vera, Vitex negundo, Wedelia calendulaceae, Centella asiatica, Ocimum tenuiflorum, Gilericidia maculate, Nila nirgundi, Leucas aspera, Lantana camera, Solanum trilobatum, Tephrosia purpurea, Hibiscus canabinus, Cissus quadrangularis, Mentha arvensis Polyanthes tuberoso, Polygala elata and Solanum xanthocarpurum against the growth of a sunflower leaf blight causing pathogen Alternaria helianthi by poisoned food technique under in-vitro conditions. Among them, leaf extracts of Acalypha indica at 10 per cent concentration inhibited the mycelial growth, sporulation and spore germination to about 78.38 per cent, 85.90 per cent and 52.48 per cent respectively. The A. indica leaf extract was very effective against A. helianthi.

Rathnavijaya et al., (2011) evaluated the antifungal activity of Centella asiatica against Aspergillus tumifaciens. The maximum zone of inhibition of antifungal activity was observed on medium containing with 100 μl within 5 days culture. Three different extract used only acid methanol extract was show the maximum zone frequency of antifungal activity. The high length of zone observed in 100 μl of acid methanol. The low length of zone observed in 100 μl of methanol extract.

Jacob and Shenbagaraman (2011) have studied the antimicrobial activity of the Amaranthus tristis, Centella asiatica, Hibiscus sabdariffa, Moringa oleifera, Sesbania grandiflora and Solanum trilobatum against E.coli, Staphylococcus aureus and Klebsiella pneumonia. Study showed strong antioxidant activity of ethanolic extracts of Hibiscus sabdariffa, Centella asiatica and the aqueous extract of M.olifera. Antimicrobial activity was observed from the ethanolic extracts of only three samples.

Gauniyal and Teotia (2014) examined the antimicrobial activity of ethanolic extract of medicinal plants using well diffusion method against Streptococcus mutans, Enterococcus faecalis, Lactobacillus acidophilus, Candida albicans and Candida tropicalis. Ethanolic extracts of Aloe barbadensis, Cinnamum zeylanicum and Tinospora coridfolia, were not effective against Streptococcus mutans and
Enterococcus faecalis respectively. However, Azadirachta indica, Centella asiatica, Zingiber officinale were showing week and the extract of Allium sativum, Curcuma longa, Glycyrrhiza glabra, Ocimum sanctum, Piper nigrum, displaying strong antimicrobial activity against most of the test species. The ethanol extract of Syzygium aromaticum showing strong antimicrobial activity against all test species. The results provide justification for the use of the medicinal plants to treat various oral infections.

Vadlapudi et al., (2012) have studied the antimicrobial activity of aerial parts of Calotropis procera and Centella asiatica Linn., that have been popularly used as folk medicines. The organic solvent plant extracts are tested on the various microorganisms including bacteria and fungi using agar well diffusion technique. The length of the inhibition zone was measured in millimeters from the edge of the well to the edge of the inhibition zone. C. procera showed significant to moderate activity against (14 mm) Pseudomonas marginalis and (21 mm) Streptococcus mutans with 100 mg/ml DMSO plant drug concentration. The results of (MICs) values are lowest at 66 and highest at 152 mg/ml for C. procera whereas 0 to 155 mg/ml for C. asiatica. The extracts were assessed to validate the potential activity of the medicinal plants against microbes.

Samy and Chow (2011) have studied the antibacterial studies using hexane, dichloromethane and methanol extract of leaves of Centella asiatica by disc-diffusion method against gram-positive and gram-negative bacteria. The methanol and dichloromethane extracts of leaf showed a broad spectrum antibacterial activity. Thus the results support the traditional usage of this plant as a medicine.

Johnny et al., (2011) evaluated the antifungal activities of the leaves extract of 15 selected medicinal plants; Alpinia galanga (L.) Willd., Alstonia spatulata Blume., Annona muricata L., Blechnum orientale L., Blumea balsamifera L., Centella asiatica L., Dicranopteris linearis (Burm. f.) Underw., Dillenia suffruticos (Griff ex Hook.f. and Thomson) Martelli, Litsea garciae Vidal., Melastoma malabaricum L., Momordica charantia L., Nephrolepis bisserrata (Sw.), Pangium edule Reinw., Piper betle L. and Polygonum minus Huds. against plant pathogenic fungus, Colletotrichum capsici which was isolated from chilli. The antifungal assay was carried out in potato dextrose media in five different treatments, which were distilled water as the negative
control, crude extract of leaves in methanol, chloroform, acetone and Kocide 101 as the positive control. They were carried out in three replicates. The two-way analysis of variance (ANOVA) was carried out on all the data to justify the difference between critical difference (CD) of mean (\( P = 0.05 \)) and coefficient of variation (CV %) in terms of mean percent reduction in colony diameter, sporulation and minimum inhibitory concentration (MICs) of \( C. \ capsici \) to take statistical decisions. Crude extract of \( P. \ betle \) in all the solvents was found to be the most effective and exhibited the highest antifungal activities. Crude extract of \( P. \ betle \) in methanol inhibited 85.25% of radial growth of \( C. \ capsici \) followed by 78.53% leaves crude extract in chloroform and 73.58% leaves crude extract in acetone at the concentration of 10 \( \mu \)g/ml (\( p < 0.05 \)). The exact concentrations that had definite potential to fully restrict the growth (100% inhibition) of \( C. \ capsici \) (MIC) by \( P. \ betle \) was 12.50 \( \mu \)g/ml in methanol, 17.50 \( \mu \)g/ml in chloroform and 15.00 mg/ml in acetone. The sporulation assay also revealed that, \( P. \ betle \) leaves crude extracts showed the highest inhibition of spore germination rate of \( C. \ capsici \) overall at the concentration of 10 \( \mu \)g/ml; with 80.93% inhibition by leaves crude extracts in methanol, 74.09% by leaves crude extracts in chloroform and 72.91% by leaves crude extracts in acetone. Concentration of plant leaves crude extracts that inhibited 50% or more of the radial growth and sporulation was considered as effective (LC \( \geq 50 \)). As a conclusion, the leaf crude extracts that exhibited effectiveness by showing more than 50% inhibition against \( C. \ capsici \) should be considered for further evaluation. \( P. \ betle \) leaf crude extracts was the most effective in inhibiting the fungus respectively.

Taemchuay et al., (2009) evaluated the crude extracts of asiatic pennywort (\( Centella \ asiatica \) (Linn.) (Urban) extracted with either ethanol or water for antibacterial activity against 30 isolates of \( Staphylococcus \ aureus \) from milk samples of dairy cows. The antibacterial sensitivity of crude extracts were tested by the disc diffusion test, and results showed that ethanol extracts and water extracts had average inhibition zones ranged from 6.44-6.49 and 6.54-17.72 mm in diameter, respectively. The modified resazurin microtiter-plate was used to determine the minimum inhibitory concentration (MIC), and the minimum bactericidal concentration (MBC) was determined by touching the loop from each well of MIC plate and streaking it on a mannitol salt agar. Results showed that the ethanol extracts had an MIC50 value of 8 mg/mL, the water extracts of leaf powder had an MIC50 value of 32 mg/mL, and the
water extracts of fresh leaves had an MIC value of 32-256 mg/mL. The ethanol extracts had an MBC value of 16 mg/mL. The water extracts could not kill *S. aureus*. In conclusion, the ethanol extracts had more potential antibacterial activity than the water extracts.

Songsri and Lertcanawanichakul (2012) have examined the antifungal activity of water or ethanolic extracts of medicinal plants by assaying 10 extracts of 5 medicinal plants, i.e., galangal minor (*Alpinia officinarum*), galingale (*Boesenbergia rotunda* L. Mansf.), guava (*Psidium guajava* L.), asiatic pennywort (*Centella asiatica* L Urb.) and banana (*Musa sapientum* L), against *Candida albicans* and *Cllytococcus neoformans* by broth microdilution method. The extracts were determined for minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC). Antifungal activities of all of the ethanolic extracts were higher than those of the water extracts. The MFCs of ethanolic extracts of medicinal plants against *C. albicans* or *C. neoformans* were in the range of 3.13 - 12.5 mg/ml or 0.098 - 12.5 mg/ml, respectively, whereas MFCs of water extracts of medicinal plants were more than 50 mg/ml. The ethanolic extract of galangal minor showed the highest antifungal activity. Both fungi were more susceptible to the extract of galangal minor than galingale. *C. albicans* was equally susceptible to the extracts of asiatic pennywort, guava and banana, whilst *C. neoformans* was more susceptible to the extract of asiatic pennywort than guava or banana.

Witkowska-Banaszczak et al., (2005) have studied the antimicrobial activity of infusion, decoction, ethanol extract and fractions obtained by successive extraction of *Viola tricolor* herb with dichloromethane, ethyl acetate and methanol. The infusion, decoction and ethanol extract were found to be most effective against the tested microorganisms.

Dwarika Prasad (2014) have studied the whole plant of *Voila canescens* and *Bauhinia variegata*. These plants have been tested and gave effective antimicrobial activity against *E. coli*, *Staphylococcus aureus*, *Psudomonas* and *Bacillus subtilis*. 

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