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Efficacy of plant extracts in plant disease management

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ABSTRACT

The overzealous and indiscriminate use of most of the synthetic fungicides has created different types of environmental and toxicological problems. Recently, in different parts of the world, attention has been paid towards exploitation of higher plant products as novel chemotherapeutants in plant protection. The popularity of botanical pesticides is once again increasing and some plant products are being used globally as green pesticides. Pyrethroids and neem products are well established commercially as botanical pesticides and recently some essential oils of higher plants have also been used as antimicrobials against storage pests because of their relatively safe status and wide acceptance by the consumers. Some of the volatile oils, which often contain the principal aromatic and flavouring components of herbs and spices, have been recommended as plant based antimicrobials to retard microbial contamination and reduction in spoilage of food commodities. In the context of agricultural pest management, botanical pesticides are best suited for use in organic food production in industrialized countries but can play a much greater role in the production and post harvest protection of food products in developing countries.

Keywords: Botanical Pesticides; Plant Protection; Chemotherapeutants; Organoleptic

1. INTRODUCTION

The ultimate aim of recent research in this area has been the development of alternative control strategies to reduce dependency on synthetic fungicides. Plants have ability to synthesize aromatic secondary metabolites, like phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins [1]. The components with phenolic structures, like carvacrol, eugenol, and thymol, were highly active against the pathogen. These groups of compounds show antimicrobial effect and serves as plant defence mechanisms against pathogenic microorganisms [2]. The volatile antimicrobial substance allicin (diallyl thio sulphinate) is synthesized in garlic when the tissues are damaged and the substrate alliin (S-allyl-L-cysteine Sulphoxide) mixes with the enzyme alliin-lyase. Allicin is readily membrane-permeable and undergoes thiol-disulphide exchange reactions with free thiol groups in proteins. Allicin effectively controlled seed-borne *Alternaria* spp. in carrot, Phytophthora leaf blight of tomato and tuber blight of potato as well as *Magnaporthe* on rice and downy mildew of *Arabidopsis thaliana* [3]. Application of plant products especially essential oils is a very attractive method for controlling post harvest diseases. Essential oil extracted from lemon grass (*Cymbopogon* spp.) post harvest anthracnose of mango fruit [4]. The anti viral protein (AVP) extracts from *Bougainvillea spectabilis* and *Prosopis chilinesis* were found to be effective in reducing the sunflower necrosis virus (SFNV) infection both in cowpea and sunflower plants [5]. At present, scientists are investigating for plant products of antimicrobial properties. It would be advantageous to standardize methods of extraction and *in vitro* antimicrobial efficacy testing so that the search for new biologically active plant products could be more systematic. Thousands of phyto chemicals which have inhibitory effects on all types of microorganisms *in vitro* should be subjected *in vivo* testing to evaluate the efficacy in controlling the incidence of diseases in crops, plants, and humans [2].

2. BOTANICALS

Some plant contains components that are toxic to pathogens. When extracted from the plant and applied on infested crops, these components are called botanical pesticides or botanicals.

Commonly used botanicals:

Plant extracts: Neem (*Azadirachta indica*, A. Juss), Garlic (*Allium sativum*, Linn., Eucalyptus (*Eucalyptus globulus*, Labill., Turmeric (*Curcuma Longa*, Linn., Tobacco (*Nicotiana tabacum*, Linn., Ginger (*Zingiber officinale*, Rosc.

Essential oils: Nettle oil (*Urtica* spp.), Thyme oil (*Thymus vulgaris*, Linn.), Eucalyptus oil *Eucalyptus globulus*, Labill. Rue oil (*Ruta graveolens*, Linn.), Lemon grass oil (*Cymbopogon flexuosus* (Steud.) Wats. and Tea tree oil (*Melaleuca alternifolia*).

Gel and latex: *Aloe vera* (Tourn. Ex Linn.).

Why Consider Botanicals?

- Sustainable solutions in agriculture
- Reduce crop losses
- Eco-friendly
- Easily bio-degradable
- Organic farming
- Cheaper
- Integrated Diseases Management

3. PLANT EXTRACTS

3.1. Methods of Plant Extract Preparation

Extraction methods involve separation of medicinally active fractions of plant tissue from inactive/inert components by using selective solvents and extraction technology (**Table 1**). Solvents diffuse into the solid plant tissues and solubilize compounds of similar polarity. Quality of plant extract depends on plant material, choice of solvents and the extraction methods.

3.2. Plant Material

Plants are collected either randomly or by following leads supplied by local healers in geographical areas where the plants are found. Fresh or dried plant materials can be used as a source for the extraction of secondary plant components. Many authors had reported about plant extract preparation from the fresh plant tissues [6]. The logic behind this came from the ethno medicinal use of fresh plant materials among the traditional and tribal people. But as many plants are used in the dry form (or as an aqueous extract) by traditional healers and due to differences in water content within different plant tissues, plants are usually air dried to a constant weight before extraction. Other researchers dry the plants in the oven at about 40˚C for 72 h [7]. In most of the reported works, underground parts (root, tuber, rhizome, bulb etc.) of a plant were used extensively compared with other above ground parts in search for bioactive compounds possessing antimicrobial properties.

3.3. Choice of Solvents

Successful determination of biologically active compound from plant material is largely dependent on the type of solvent used in the extraction procedure. Properties of a good solvent in plant extractions include low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action and inability to cause the extract to complex or dissociate. As the end product in extraction will contain traces of residual solvent, the solvent should be non-toxic and should not interfere with the bioassay [8]. The choice will also depend on the targeted compounds to be extracted. Initial screening of plants for possible antimicrobial activities typically begins by using the crude or alcohol extractions and can be followed by various organic solvent extraction methods. Water is universal solvent, used to extract plant products with antimicrobial activity. Though traditional healers use primarily water but plant extracts from organic solvents have been found to give more consistent antimicrobial activity compared to water extract [9]. Also water soluble flavonoids (mostly anthocyanins) have no antimicrobial significance and water soluble phenolics only important as antioxidant compound a study reported that extraction of tannins and other phenolics were better in aqueous acetone than in aqueous methanol. In another study, among the twenty different solvents evaluated, chloroform was found to be the best solvent for the extraction of non-polar biological active compounds [10]. Since nearly all of the identified antimicrobial compounds from plants are aromatic or saturated organic compounds, they are most often obtained through initial ethanol or methanol extraction. Thus the most commonly used solvents for preliminary investigations of antimicrobial activity in

Table 1. Solvents used for active component extraction [1].

Water	Ethanol	Methanol	Chloroform	Dichloro-methanol	Ether	Acetone
Tannins	Alkaloids	Terpenoids	Terpenoids	Terpenoids	Alkaloids	Flavonols
Saponins	Tannins	Saponins	Flavonoids	-	Terpenoids	-
Terpinoides	Terpinoides	Tannins	-	-	Coumarins	-
-	Flavonol	Flavones	-	-	-	-

plants are methanol, ethanol and water. The other solvents used by researchers are dicholro-methane, acetone, and hexane. Some authors use a combination of these solvents to obtain the best solvent systems for extraction. [11] examined a variety of extractants for their ability to solubilize antimicrobials from plants, rate of extraction, ease of removal, toxicity in bioassay and acetone received the highest overall rating. Though there is a wide diversification in the usage of solvents, it is necessary to focus on a standardized extraction method and solvent system for a wide variety of researchers working in diverse settings to minimize the variability in the antimicrobial efficacy reports.

3.4. Extraction Methods

Variation in extraction methods are usually depend on the length of the extraction period, solvent used, pH of the solvent, temperature, particle size of the plant tissues and the solvent-to-sample ratio. The basic principle is to grind the plant material (dry or wet) finer, which increases the surface area for extraction thereby increasing the rate of extraction. In the study by [12], 5 min extractions of very fine particles of diameter 10 μm gave higher quantities than values obtained after 24 h in a shaking machine with less finely ground material. Earlier studies reported that solvent-to-sample ratio of 10:1 (v/w) solvent to dry weight ratio has been used as ideal [13]. The extraction method that has been widely used by researchers is plant tissue homogenization in solvent [9]. Dried or wet, fresh plant parts are ground in a blender to fine particles, put in a certain quantity of solvent and shaken vigorously for 5 - 10 min or left for 24 h after which the extract is filtered. The filtrate then may be dried under reduced pressure and re-dissolved in the solvent to determine the concentration. Some researchers however centrifuged (approximately 20,000 × g, for 30 min) the filtrate for clarification of the extract. Another common method is serial exhaustive extraction which involves successive extraction with solvents of increasing polarity from a non polar (hexane) to a more polar solvent (methanol) to ensure that a wide polarity range of compound could be extracted [13]. Other researchers employ soxhlet extraction of dried plant material using organic solvent. This method cannot be used for thermolabile compounds as prolonged heating may lead to degradation of compounds.

4. ANTIMICROBIAL SECONDARY METABOLITES

Plants have limitless ability to synthesize aromatic secondary metabolites, most of which are phenols or their oxygen-substituted derivatives. Important subclasses in this group of compounds include phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins (**Figure 1**). These groups of compounds show antimicrobial effect and serves as plant defense mechanisms against pathogenic microorganisms (**Table 2**). Simple phenols and phenolic acid are bioactive phytochemicals consisting a single substituted phenolic ring. Phenolic toxicity to microorganisms is due to the site(s) and number of hydroxyl groups present in the phenolic compound. Quinones are characteristically highly reactive, colored compounds with two ketone substitutions in aromatic ring. Flavones, flavonoids and flavonols are phenolic structure with one carbonyl group. They are synthesized by plants in response to microbial infection and are often found effective *in vitro* as antimicrobial substance against a wide array of microorganisms (**Table 3**). Tannins are polymeric phenolic substances possessing the astringent property. These compounds are soluble in water, alcohol and acetone and give precipitates with proteins. Coumarins are phenolic substances made of fused benzene and α-pyrone rings. The crude sap, volatile and essential oil extracted from whole plant or specialised plant parts like roots, stem, leaves, flowers, fruits and seeds are widely used in preparing the antimicrobial compounds which are significantly used against the different plant pathogens/diseases (**Table 4**).

Six broad chemical groups are as follows.

- Flavonoides and isoflavonoides
- Saponins
- Steroides
- Tannins
- Phenolic and phenolic acids—Chlrogenic acid, protocatechuic acid, ferulic acid, caffeic acid
- Coumarins and Pyrones

Table 2. Mode of action of phyto chemicals [1].

Class	Sub-class	Mechanism
Phenolics	Simple phenols	Membrane disruption, substrate deprivation
Phenolic acids	Phenolic acids	Bind to adhesins, complex with cell wall, inactivate enzymes
Terpenoids, essential oils		Membrane disruption
Alkaloids		Intercalate into cell wall
Tannins		Bind to proteins, enzyme inhibition, substrate deprivation
Flavonoids		Bind to adhesins, complex with cell wall, Inactivate enzymes
Coumarins		Interaction with eucaryotic DNA
Lectins and polypeptides		Form disulfide bridges

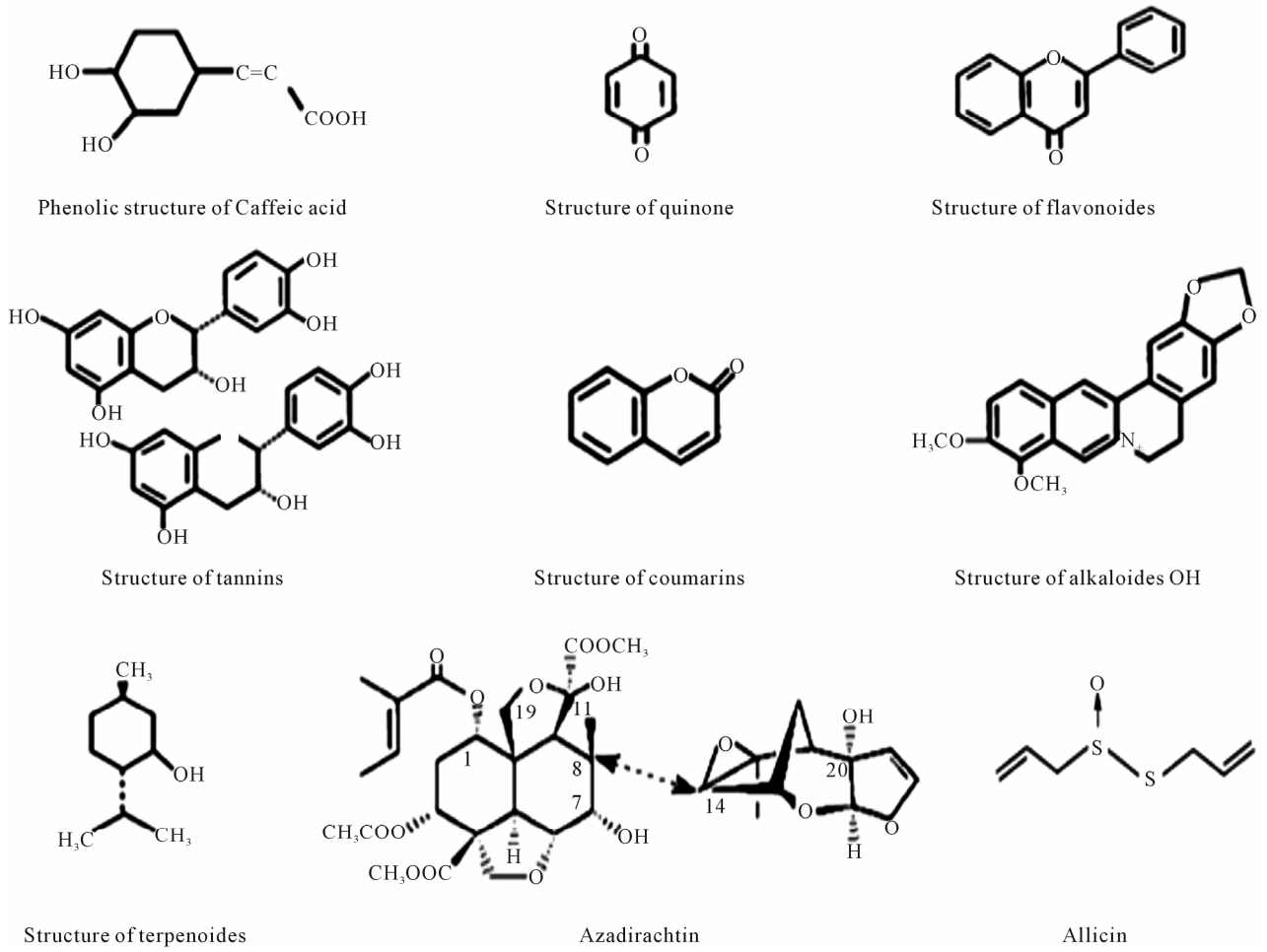

Figure 1. Structure of antimicrobial compounds [14].

Table 3. Botanicals produced by plants having antimicrobial activity.

Common name	Scientific name	Compound	Class	Activity
Apple	*Malus pumila* Mill.	Phloretin	Flavonoid derivative	General
Ashwagandha	*Withania somnifera* Dunal.	Withafarin A	Lactone	Bacteria, fungi
Bael tree	*Aegle marmelos* Linn.	Essential oil	Terpenoid	Fungi
Blue gum tree	*Eucalyptus globulus* Labill.	Tannin	Polyphenol	Fungi, Bacteria, Viruses
Onion	*Allium cepa* Linn.	Allicin	Sulfoxide	Fungi, Bacteria
Thyme	*Thymus vulgaris* Linn	Caffeic acid	Terpenoid	Fungi, Bacteria, viruses
Turmeric	*Curcuma longa* Linn.	Curcumin	Terpenoids	Fungi, Bacteria, protozoa
Thorn apple	*Datura stramonium* Linn.	Hyoscymine Scopolamine	Alkaloids	Fungi
Black pepper	*Piper nigrum* Linn.	Piperine	Alkaloid	Fungi
Castorbean	*Ricinus communis* Linn.	Ricinine Ricininoleic	Alkaloids	Fungi
Neem/Margosa tree	*Azadirachta indica* A.Juss.	Azadirachtin	Terpenoides	Fungi, Bacteria
Garlic	*Allium sativum* Linn.	Allicin	Solfoxide	Fungi, Bacteria

Table 4. Botanicals.

Plant	Part used	Preparations	Diseases/pathogen	References
Datura/thorn apple (*D.stamonium*) *Calotropis procera* (Ait.) R. Br. *Oscimum* spp.	Root, stem, Leaf, flowers	Crude extract	*Curvularia lunata*	[15]
Turmeric (*Curcuma longa* Linn.), Ginger (*Zingiber officinale* Rosc.)	Rhizome	Crude extract	*Phytopthora infestans*, *Fusarium solani*, *Pyricularia oryzae*	[16]
Perslane (*Portulaca olaracea* Linn.)	Leaf	Crude extract	*Helminthosporium maydis*	[17]
Hena (*Lowsonia inermis* Linn.)	Leaf	Crude extract	*Dreshslera oryzae*	[18]
Neem/Margosa (*Azadirachta indica* A.Juss.), Sugar apple (*Annona squamosa* Linn.), Holy basil (*Oscimum sanctum* Linn.)	Leaf, Stem Bark, root	Crude extract	Anthracnose of pepper	[19]
Neem/Margosa (*Azadirachta indica* A.Juss.),	Seed kernel	Oil	*A. alternata*	[20]
Ambrosioides Linn., *Oscimum* spp.	Leaf	Essential oils	*Aspergillus flavus*	[21]
Garlic (*Allium sativum* Linn.), Datura (*D. stramonium* Linn.)	Bulb, Leaf	Ethanol extracts	*Curvularia lunata*	[22]
Spearmint (*Mentha spicata* Linn.), Greek Sage (*Salvia fruticosa* Mill.), *Thymbra* spp.	Leaf	Essential oils	*Rhizoctonia solani*, *Sclerotium sclerotiorum*	[23]
Spanish flag (*Lantana camara* Linn.)	Leaf	Crude Extracts	Castor grey rot (*Botrytis ricini*)	[24]
Neem/Margosa (*Azadirachta indica* A.Juss.),	Seed, Leaf	Crude Extracts	Early blight of tomato	[25]
Madar (*Calotropis procera* (Ait.) R.Br.	Leaf	Crude Extracts	Tikka leaf spot disease of groundnut	[26]
Neem/Margosa (*Azadirachta indica* A.Juss.),	Seed	NSKE	Powdery mildew of pea	[27]
Spanish flag (*L.camara* Linn.), Pongam (*Pongamia pinnata* L.Pierre.)	Leaf	Crude extracts	Leaf blight of onion	[28]
Holy basil (*Oscimum sanctum*Linn.), peach (*Prunus persica* Linn.) Stokes.	Leaf	Essential oil	Grey mould (*Botrytis cinerea*) of grapes	[29]
Neem/Margosa (*Azadirachta indica* A.Juss.),	Leaf	Achook formulations (azadirachtina)	Sheath blight of rice	[30]
Neem/Margosa (*Azadirachta indica* A.Juss.),	Seed kernel	Neem oil	Rice tungro virus	[31]
Neem/Margosa (*Azadirachta indica* A.Juss.),	Leaf, Seed	Achook, Neemazal,	Bacterial blight of rice	[32]
Oregano (*Origanum hercleoticum* (weed species)	Leaf	Essential oils	*Fusarium oxysporum*, *Phoma tracheiphila*	[4]
Neem/Margosa (*Azadirachta indica* A.Juss.), Black cumin (*Nigelia sativa* Linn. Asfetida (*Ferula asafoetida* Linn.)	Seeds	Essential oils	*Fusarium oxysporum*, *A.niger*, *A.flavus*	[33]
Strawberry (*Fragaria* spp.)	Fruit	Volatile compounds	Anthracnose of strawberry	[34]
Raspberry (*Rubus* spp.) and Strawberry (*Fragaria* spp.)	Fruit	Volatile compounds	Post harvest decay fungi	[35]
Garden croton (*Codiaeum variegatum* Linn.)	Leaf	Phenolic compounds	*Alternaria alternate*, *Fusarium oxysporum*	[36]
Oleander (Nerium *oleander* Linn.)	Leaf	Crude extracts	Brown spot of rice (*Bipolaris oryzae*)	[37]
Indian aloe (*Aloe barbadensis* Mill.) Neem/Margosa (*Azadirachta indica* A.Juss.), Tobacco (*Nicotiana tabacum* Linn.)	Leaf	Crude extracts	Dry rot of yam *F. oxysporum*, *A.nizer*	[38]
Black pepper (*Piper nigrum* Linn., Clove (*Syzygium aromaticum* (Linn.) Merr. & Perry, Geranium (*Pelargonium graveolens* L'Herit), Nutmeg (*Myristica fragrans* Houtt.), (*Origanum vulgare* spp. *hirtum* (Link) Letsw. and thyme [*Thymus vulgaris* Linn.	Leaf	Volatile oil	Anti bacterial (gram positive and gram negative)	[39]

Continued

Metasequoia glyptostroboides	Leaf	Essential oil	*Fusarium oxysporum*, *Fusarium solani*, *Phytophthora capsici*, *Colletotrichum capsici*, *Sclerotinia sclerotiorum*, *Botrytis cinerea* and *Rhizoctonia solani*	[40]
Clove (*Syzigium aromaticum* Linn.), Turmeric (*Curcuma longa* Linn.), garlic (*Allium sativum* Linn.) and Holy Basil (*Ocimum Sanctum* Linn.)	Leaf, seed, fruit	Crude extract	*Aspergillus flavus*	[41]
Aspilia africana, *Chromolaena odorata*, *Musa paradisiaca* and *Tithonia diversifolia*	Leaf	Aqueous extract	*Cercospora* leaf spot of Seasame (*Sesamum indicum*) L.)	[42]
Lemon grass (*Cymbopogon* spp.), Thyme (*Thymus vulgaris* Linn.)	Leaf, root	Volatile compound	Black Mould Disease on Onion Bulbs (*Aspergillus niger*)	[43]
Brassica napus and Tomato (*Lycopersicon esculentum* Mill.)	Leaf, stem	Water extract	Bacterial disease on Onions	[44]
Ginger (*Zingiber officinale* Rosc.), aloe (*Aloe ve*ra), bitter kola (*Garcinia cola*) and neem (*Azadirachta indica* A.Juss.)	Leaf, Fruit and Seed	Crude	Root rot disease of cow pea (*Vigna unguiculata* L.)	[45]
Chloranthus japonicas, *Paulownia coreana*	Roots, stem	Crude extract	rice blast, rice sheath blight, and wheat leaf rust	[46]
Urtica dioica L., thyme *Thymus vulgaris* L., *Eucalyptus* spp., *Ruta graveolens* L. and *Achillea millefolium* L.	Leaf	Volatile oil	*Aletrnaria alternate*	[47]
Acacia nilotica, *Achras zapota*, *Datura stramonium*, *Emblica officinalis*, *Eucalyptus globules*, *Lawsonia inermis*, *Mimusops elengi*, *Peltophorum pterocarpum*, *Polyalthia longifolia*, *Prosopis juliflora*, *Punica granatum* and *Sygigium cumini*	Leaf, Seed, fruits	Aqueous extract	*Aspergillus* spp.	[48]
Eugenia aromatica , *Piper betle*), *Alpinia galanga* and *Sphaeranthus indicus*	Leaf	Crude	Stem rot disease of Vanilla	[49]
Ocimum gratissimum, *Aframomum melegueta*	Leaf	Crude	Post harvest yam (*Diascorea* spp.) rot	[50]
Neem (*Azadirachta indica* A.Juss.)	Leaf	Crude	Protozoan, Bacteria, Antifertility	[51]

Methods for Evaluation of Efficacy of Plant Extract

In vitro antimicrobial susceptibility testing (AST)
1) Diffusion test
a) agar well diffusion
b) agar disk diffusion
c) poison food technique
d) bio autography
2) Dilution methods
a) agar dilution
b) broth micro dilution assay
c) broth macro dilution assay

5. VOLATILE OILS

- Volatiles are small molecular weight organic compounds having appreciable vapour pressure at ambient temperature
- Play role in defense system
- Inhibit the growth of pathogen
- Generally Recognized As Safe (GRAS)
- Isolated by hydro distillation or steam
- Terpinoides—monoterpenes (C10), ses quiterpenes (C15), diterpines (C20)

Examples: Black pepper (*Piper nigrum* Linn.), Clove (*Syzygium aromaticum* Linn.) Merr. & Perry, Nutmeg (*Myristica fragrans* Houtt.), Oregano (*Origanum vulgare* Linn.) and Thyme (*Thymus vulgaris* Linn.)

6. ESSENTIAL OILS

- The potential essential oils used against post harvest pathogens.
- Fungistatic
- Terpenoides and aromatic compounds
- Component—carvacrol, thymol, cymene, terpine, phenylpropene derivatives, eucalyptol and anisol

Examples: Nettle oil (*Urtica* spp.), Thyme oil (*Thymus vulgais* Linn.), *Eucalyptus* oil (*Eucalyptus globules* Labill., Rue oil (*Ruta graveolens* Linn.), Lemon grass oil (*Cymbopogon flexuosus* (Steud.) Wats.) and Tea tree oil (*Melaleuca alternifolia* Maiden & Betche).

7. LIMITATIONS OF BOTANICALS FOR PLANT DISEASE MANAGEMENT

- Extraction methods are not standardized
- Rapid degradation
- Most studies are *in vitro* efficacy
- Need the development of formulations
- Some chemical compounds are harmful to human and plants
- Less effective
- Less availability formulations

8. CONCLUSION

Plants contain thousands of constituents and are valuable sources of new and biologically active molecules possessing antimicrobial property (**Table 4**). The ethnobotanical study of plant is important for modern day medicine but its usefulness cannot be overemphasized if methods are not standardized to obtain comparable and reproducible results. At present, scientists are investigating for plant products of antimicrobial properties. It would be advantageous to standardize methods of extraction and *in vitro* antimicrobial efficacy testing so that the search for new biologically active plant products could be more systematic and interpretation of results would be facilitated. Thousands of phytochemicals which have inhibitory effects on all types of microorganisms in vitro should be subjected *in vivo* testing to evaluate the efficacy in controlling the incidence of disease in crops, plants, and humans. Efficient collaborations with pharmacologists and medical doctors, plant pathologists and microbiologists are crucial to see the complete development of an interesting lead compound into an exploitable product.

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