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A Review: Plant Extracts a New Source of Antimicrobial Potential.

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ABSTRACT

Plant diseases create challenging problems in commercial agriculture and pose real economic threats to both conventional and organic farming systems. Disease management is complicated by the presence of multiple types of pathogens. Synthetic fungicides bring about the inhibition of pathogens by either destroying their cell membrane or its permeability or by inhibiting metabolic processes of the pathogens and hence are extremely effective but the flip side of this is that synthetic chemicals are harmful for human as well as soil health. Chemical fungicides are known to pollute the environment, soil and water besides causing deleterious effects on human health and biosphere. Hence there is a need to search for an environmentally safe and economically viable strategy for the control of diseases and to reduce the dependence on the synthetic agrochemicals. Use of plants as a source of medicine is as old as humanity that's why focus of the world is shifting towards natural products and analogues. These natural products or plant extracts can be exploited either as leads for chemical synthesis of new agrochemicals, or as commercial products in their own right, or as a source of inspiration to biochemists for the development of new bioassays capable of detecting other, structurally simpler, compounds with the same mode of action. Use of medicinal plants may thus offer a new source of antibacterial, antifungal and antiviral agents with significant activity against microorganisms.

Keywords: Plant extracts, Antimicrobial activity, Herbal formulation, Medicinal plants

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INTRODUCTION

Man is dependent on plants for almost every need and requirement. Hence, destruction of crop plants due to infection by fungal pathogens has always been an area of prime concern. Scientists all over the world are involved in finding methods of developing techniques for control of plant diseases. Chemical control is the most common and prevalent method of disease control. Synthetic fungicides bring about the inhibition of pathogens by either destroying their cell membrane or its permeability or by inhibiting metabolic processes of the pathogens and hence are extremely effective [1,2].

The negative side of the use of synthetic fungicides is that they are harmful for human and animal health as well as soil. They enter the food chain and cause several deleterious effects on biosphere, contributing to significant declines in populations of beneficial soil organisms, soil acidification and compaction, thatch accumulation, and diminished resistance to diseases [3]. The overzealous and indiscriminate use of most of the synthetic fungicides has created different types of environmental and toxicological problems [4-6].

Thus, current thinking about plant and environment protection suggests alternatives to pesticides and use of other strategies in addition to well known disease management methods such as crop rotation, use of resistant cultivars, planting disease free seeds, biological control etc. for control of fungal diseases [7]. Natural plant products are important sources of new agrochemicals for the control of plant diseases [8,9]. A search for an environmentally safe and economically viable strategy for the control of diseases has led to an increased use of plant based products in agriculture. Plant product preparations and bio–agents do not leave any toxic residues and therefore can effectively replace synthetic fungicides [10].

There are several traditional agricultural practices followed by farmers to control plant diseases. Formulation is a cheap, environmentally safe fungicide made by combining plant extracts and organic materials to control plant diseases. Many techniques of traditional agriculture require validation, such as use of organic materials (cow dung, oil cakes etc.) for control of plant disease [11]. Kautilya's Arthasastra was probably the oldest document, which described the use of organic materials to control the crop disorders. Use of cow dung for smearing the cuttings of fig before planting is mentioned in Dara Shikoh's documents [12].

Potato (*Solanum tuberosum* L.) is the fourth important crop worldwide by volume of production. It is high yielding, has a high nutritive value and is grown in about 140 countries [13]. The present area under potato cultivation in India is about 1.4 million hectares. India produces a total of about 25-28 million tones of potatoes every year. Potato is an economical food and a source of low cost energy to the human diet. It is also used for production of high quality starch, alcohol, etc. and its starch (farina) is used in laundries for sizing yarn in textile mills. It is also used for the production of dextrin and glucose. Potato tubers contain about 77.8% water, 22.6% carbohydrates, 2.1 % protein, 0.3% fat, 1.1% crude fibre, 0.9% ash, 40 IU vitamin A, 12 mg ascorbic acid per 100 g of edible portion etc. This crop is highly susceptible to early blight caused by *Alternaria solani* [14].

Foliar symptoms of early blight first appear small, irregular to circular dark brown spots on the lower (older) leaves, excessive defoliation may lead to death of the plant and consequent yield loss. The pathogen can also attack potato tubers and symptoms are circular to irregular lesions that are slightly sunken and often surrounded by a raised purple to dark brown border and produce a shallow, dry, corky rot [15-17]. Losses due to early blight typically are around 20-25%; however, there have been cases of 70-80% losses [18-22].

Herbal Formulation

India is considered to be a country having rich emporia of medicinal plants and where ancient systems of medicine such as Ayurveda, Siddha and Unani medicines have been in practice for many years. Ayurveda (4000-600 B.C.), Rigveda (4500-1600 B.C.) and Atharvaveda (1200 B.C.) are traditional indigenous systems of medicines. Ayurveda literally means "Science of life". According to Ayurveda, health is an indication of normal biological processes, which help to maintain mental and physical alertness and happiness of human being. Charak Samhita is the first recorded treatise on Ayurveda which was followed by Susharuta Samhita around 900 B.C. charak samhita dealt primarily with medicine while Sushruta Samhita was concerned with the advanced state of knowledge on the general principles and details of treatment [23].

The world is gradually turning to herbal formulations which are known to be effective against a large repertoire of diseases and ailments. More importantly, they are not known to cause any notable derogatory effects and are readily available at affordable prices [24, 25]. However, add a note of caution stating that plant remedies are effective and without side-effects, provided they are selected properly and taken under proper medical supervision. The active component, most often a secondary metabolite, varies in quality and quantity for a given plant species growing in different locations. The market value of such plants depends on their active content rather than merely their luxuriant growth. Reckon that close to 70,000 species of the plant kingdom have been used as herbal medicine at one time or other [26, 27]

Plant preparations have been used for centuries in medicine and pest control. Farmers in India use neem leaves to protect stored grain from insects. Herbs and spices, such as basil and clove possess antimicrobial properties and have been used to protect food from spoilage since time immemorial. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. Medicinal value of plants depends on these inherent substances that produce a definite physiological action on the human body [28]. In recent times, focus on plant research has increased all over the world and a large body of evidence has been collected to show immense potential of medicinal plants used in various traditional systems.

Morgenstern defines ethnobotany as the study of the indigenous uses of plants and the relationship between people and plants. Folk medicines of almost all civilizations of the world abound in herbal remedies [29]. Majority of the traditional medicines used in healthcare are obtained from plants [30]. In spite of several advancements in the field of synthetic drug

chemistry and antibiotics, plants continue to be one of the major raw materials for drugs treating various ailments of humans. Clinical and pharmaceutical investigations have in fact elevated the status of medicinal plants by identifying the role of active principles present in them and elaborating on their mode of action in human and animal systems [31, 32].

Primary healthcare systems involve use of medicinal plants as an effective source of both traditional and modern medicines. Any plant which possesses curative elements or properties in one or more of its organs may be termed as medicinal plant. Plant based medicaments have been employed since the dawn of civilization for prolonging the life of man and for combating various ailments [33]. World Health Organization (WHO) also advocates use of traditional medicines as safe remedies for ailments of both microbial and non-microbial origins. According to WHO, more than one billion people rely on herbal medicines. 21,000 plants all around the world have been listed for their medicinal uses and it has been estimated that as many as 80 percent of the world's population depends on plants for their primary healthcare needs [34].

Herbal remedies and alternative medicines are used throughout the world and in the past herbs often represented the original sources of most drugs [35]. The plant kingdom has provided an endless source of medicinal plants first used in their crude forms as herbal teas, syrups, infusions, ointments, liniments and powders [23]. An analysis of the soil around the human bones revealed extraordinary quantities of plant pollen of eight species. Seven of these are medicinal plants and still used throughout the herbal world [36]. With the development of chemistry and Western medicine, the active substances of many species have been isolated and in some cases duplicated in the form of synthetic drugs [37].

The synthetic preparation of some drugs is either unknown or economically impractical. For this reason, scientists continue to search for and test little-known plants and conserve those whose medicinal properties have become crucial in the fight against diseases [38]. Herbal-derived substances remain the basis for a large proportion of the commercial medications used today for the treatment of heart disease, high blood pressure, pain, asthma and other illnesses. For example, ephedra is an herb used in traditional Chinese medicine for more than 2000 years to treat asthma and other respiratory problems. Ephedrine, the active ingredient in ephedra, is used in the commercial pharmaceutical preparations for the relief of asthma symptoms and other respiratory problems. It helps the patient to breathe more easily. Today a great number of modern drugs are still derived from natural sources, and 25% of all prescriptions contain one or more active ingredients from plants [39]

Plants are naturally gifted at the synthesis of medicinal compounds. The extraction and characterization of active compounds from medicinal plants have resulted in the discovery of new drugs with high therapeutic value [40]. A classic example is aspirin, which was initially discovered as salicylic acid in willow bark and leaves, another noted example is taxol, recently proven to be effective against breast and ovarian cancers, which was initially discovered in bark of yew trees [41]. The use of medicinal plants (herbs) has a long history throughout the world and herbal preparations, including herbal extracts, can be found in the pharmacopoeias of

numerous countries. In recent years there have been renaissances of interest in natural or herbal remedies worldwide; partly because of the realization that modern medicine is not capable of providing a “cure-all” solution against human diseases and that the presence of unwanted side-effects is almost unavoidable [42].

Unlike modern drugs that invariably comprise a single active species, herb extracts and/or prescriptions contain multiple active constituents. Natural compounds contained in these “herbal cocktails” can act in a synergistic manner within the human body, and can provide unique therapeutic properties with minimal or no undesirable side-effects [43]. A key factor in the widespread acceptance of natural or alternative therapies by the international community involves the “modernization” of herbal medicine [41].

At present, however, quality-related problems (lack of consistency, safety, and efficacy) seem to be overshadowing the potential genuine health benefits of various herbal products, and a major cause of these problems seems to be related to the lack of simple and reliable analytical techniques and methodologies for the chemical analysis of herbal materials [43]. Sample preparation is the crucial first step in the analysis of herbs, because it is necessary to extract the desired chemical components from the herbal materials for further separation and characterization. Thus, the development of “modern” sample-preparation techniques with significant advantages over conventional methods (e.g. reduction in organic solvent consumption and in sample degradation, elimination of additional sample clean-up and concentration steps before chromatographic analysis, improvement in extraction efficiency, selectivity, and/or kinetics, ease of automation, etc.) for the extraction and analysis of medicinal plants is likely to play an important role in the overall effort of ensuring and providing high quality herbal products to consumers worldwide [40].

Plant diseases create challenging problems in commercial agriculture and pose real economic threats to both conventional and organic farming systems. Disease management is complicated by the presence of multiple types of pathogens. For any one crop the grower must deal with a variety of fungi, bacteria, viruses and nematodes. This situation is even more complicated for organic vegetable growers because they usually produce a wide array of vegetable crops and are prohibited from applying conventional synthetic fungicides. The world market continues to be extremely competitive and continues to require that growers supply high-quality disease free produce with an acceptable shelf life. Disease management is therefore a critical consideration in organic vegetable production.

Bavistin, mancozeb and thiram are the most commonly used plant fungicides. Such synthetic fungicides bring about the inhibition of pathogens by either destroying their cell membrane or its permeability or by inhibiting metabolic processes of the pathogens and hence are extremely effective [2]. The flip side of this is that synthetic chemicals are harmful for human as well as soil health. Chemical fungicides are known to pollute the environment; soil and water besides causing deleterious effects on human health and biosphere [4]. Inappropriate use of agrochemicals especially fungicides not only imposes adverse effects on ecosystems but also pocesses a possible carcinogenic risk higher than that of insecticides and

herbicides put together [44, 45]. Moreover, resistance by pathogens to fungicides has rendered certain fungicides ineffective [46].

Hence there is a need to search for an environmentally safe and economically viable strategy for the control of diseases and to reduce the dependence on the synthetic agrochemicals.

Recent trends favor the use of alternative substances derived from natural plant extracts to control pests [47-49]. The use of natural products for the control of fungal diseases in plants is considered as an interesting alternative to synthetic fungicides due to their less negative impacts on the environment [50]. These natural products or plant extracts can be exploited either as leads for chemical synthesis of new agrochemicals, or as commercial products in their own right, or as a source of inspiration to biochemists for the development of new bioassays capable of detecting other, structurally simpler, compounds with the same mode of action. Plant product preparations and bio-agents do not leave any toxic residues and therefore can effectively replace synthetic fungicides. Use of medicinal plants may thus offer a new source of antibacterial, antifungal and antiviral agents with significant activity against microorganisms [51].

Antimicrobial Activities

Use of plants as a source of medicine is as old as humanity. As the focus of the world is shifting towards natural products and analogues, the demand of herbal medicine is also increasing and several plants have been screened for activity. Antifungal activity of plant or their extracts as well as essential oil have been studied by several workers [52-65].

Antimicrobial screening of plant extracts is usually done with crude alcohol or aqueous extracts prepared either by cold or hot extraction methods. Crude or alcohol extract of several plants have been screened for their possible antimicrobial activities against pathogenic virus, bacteria, fungi and protozoa [66-81]. Pretorius *et al.*, tested crude extracts from thirty nine plant species for their antifungal potential against seven economically important plant pathogenic fungi [82].

All the active principles present in plants are usually aromatic or saturated organic compounds so they get extracted in ethanol or methanol [83]. Some proteins and glucosides etc. are soluble in water hence antimicrobial assay of anti microbial principle is usually done with aqueous, 50% alcohol or 100% alcohol extracts.

Mughal *et al.* observed that aqueous leaf extracts of *Allium sativum, Datura alba* and *Withania somnifera* inhibited the growth of *Alternaria alternata, Alternaria brassicola* and *Myrothecium roridum* [84]. According to Khan *et al.*, aqueous extract of *Allium cepa* exhibited antifungal activity against *Helminthosporium turcicum* and *Ascochyta rabiei* and that of *Calotropis procera* against *Alternaria redicina* [85]. Bajwa *et al.* assayed the antifungal activity of

aqueous extract of *Parthenium hysterophorous*, a herb, against *Drechslera hawaiiensis, Alternaria alternata* and *Fusarium monilifrome* [86].

Babu Joseph *et al.* observed that leaf extracts of *Azardiachta indica, Artemessia annua, Eucalyptus globules, Ocimum sanctum and Rheum emodi* inhibited the growth of *Fusarium solani* [87]. Hussain *et al.* reported the antifungal activity of methanolic, ethanolic and boiling water extracts of *Barringtonia racemosa* leaves, sticks and barks [88]. Pawar assayed the antifungal activity of stem extracts of 9 plants viz. *Azadirachta indica, Lantana camera, Callistemon rigidus, Capsicum annum, Datura inoxia, Terminalia thorelii, Citrus aurantifolia, Lawsonia inermis, and Santalum album* against 5 seed-borne pathogenic fungi viz. *Alternaria alternata*, *Aspergillus niger, Curvularia lunata, Fusarium moniliforme and Trichoderma viride* [79]. Antimicrobial activity of aqueous leaf extracts of *Ageratum conyzoides Boerhaavia diffusa*, *Dathura stramonium, Euphorbia hirta, Hyptis suaveolens, Jatropha gossypifolia, Phyllanthus niruri, Prosopis juliflora, Solanum nigrum, Tridex procumbens and Ziziphus jujube* against *Xanthomonas campestris*, *Agrobacterium rhizogenes* and *Aspergillus fumigatus* has been reported by Sheikh *et al*. [64]. Antimicrobial potential of crude extract of *Moringa oleifera* and *Allamanda cathartica* against multiple drug resistant clinical pathogens has been studied by Rajamanickam and Sudha [65].

Initial antimicrobial screening with crude extract is followed by screening of extracts prepared in various organic solvents. These extracts are studied to search for various phytochemicals, responsible for antimicrobial activity. Tatli and Akdemir reported antibacterial potential of methanolic extract of *Turkish Verbascum* spp against *Candida albicans, Cryptococcus neoformans, Staphylococcus aureus, S. aureus, Pseudomonas aeruginosa, Aspergillus fumigatus* and *Mycobacterium intracellulare* [89]. Saadabi studied the antimicrobial activity of aqueous, chloroform and methanol extract of *Lawsonia inermis* against six fungal pathogens (*Epidermophyton floccosum, Microsporum odouinii, Trichophyton rubrum, Trichophyton concentricum , Trichophyton tonsurans* and *Candida albicans*) and four human pathogenic bacteria (*Staphylococcus aureus, Bacillus subtilis, E. coli* and *Pseudomonas aeruginosa*) [90].

Jayaraman *et al.* studied antimicrobial activity of ethyl acetate, acetone, chloroform and water extract of *Stevia rebaudiana* leaves against *Staphylococcus aureus, Salmonella typhi, Escherichia coli, Bacillus subtilis, Aeromonas hydrophila, Vibrio cholerae* and *Candida albicans, Cryptococcus neoformans, Trichophyton mentagrophytes, Epidermophyton* species [91]. Nguyen *et al.* studied antimycotic potential of Cinnamon extract against *R. solani* [92]. Ashraf *et al.* reported antimicrobial activity of methanol, chloroform and aqueous extracts of *Origanum vulgare* were determined against nine different gram negative and gram positive bacterial strains and three fungal stains. The bacterial strains were *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 29213), *Micrococcus luteus* (ATCC 9341), *Pseudo-monas aeruginosa* (ATCC 33347), *Escherichia coli* (ATCC 25922), *Salmonella typhi* (ATCC 19430), *Shig-ella flexneri* (ATCC 25929), *Salmonella para typhi A* (ATCC 9150) and *Proteus mirabilis* (ATCC 49565) and fungal strains were *Aspergillus flavus*, *Aspergillus niger and Aspergillus pterus* [63]. Nikolajeva *et al.* have reported antimicrobial activity of aqueous and ethanolic extracts of 11

Bryophyta species and 9 *Marchantiophyta* species collected in Latvia was tested against *Staphylococcus aureus*, *Escherichia coli* and *Bacillus cereus* [93]. Bhagwat and Datar observed that *in vitro* antifungal activity of extracts of leaves and rinds of *Garcinia indica*, rhizomes of *Curcuma aromatica*, roots of *Glycyrrhiza gahliae*, leaves of *Nyctanthes arbour-tristis* and seeds of *Vernonia anthelmintica* against *Rhizopus stolonifer, Botrytis cinerea* and *Colletotrichum coccodes* [94].

Obafemi *et al.* reported that hexane, ethyl acetate and methanol extracts of *Tithonia diversifolia* exhibit antimicrobial activity against *Staphylococcus aureus, Bacillus cereus* and others [95]. Aqil and Ahmad reported antibacterial properties of ethyl acetate, acetone and methanol extract of traditionally used Indian medicinal plants [96]. Bobbarala *et al.* reported antifungal activity of some medicinal plants against phytopathogenic *fungi Aspergillus niger* [61]. Goyal *et al.* reported antibacterial activity of methanol, ethanol, ethyl acetate and aqueous leaf extract of *Ocimum sanctum* were determined against *Staphylococcus aureus, Bacillus subtilis,*and *Escherichia coli* [97].

Plant extracts also exhibit antiviral, trypanocidal, leishmanicidal and antimalarial activity. Tatli and Akdemir observed antimalarial activity of secondary metabolites from some *Turkish Verbascum* species [89]. Filho *et al.* investigated the antiviral activity of *Sorghum bicolor* against HSV-1, Bovine herpes virus 1 [98].

Antimicrobial activities of the crude ethanolic extracts of five plants were screened against multidrug resistant (MDR) strains of *Escherichia coli, Klebsiella pneumoniae and Candida albicans.* ATCC strains of *Streptococcus mutans, Staphylococcus aureus, Enterococcus faecalis, Streptococcus bovis, Pseudimonas aeruginosa, Salmonella typhimurium, Escherichia coli, Klebsiella pneumoniae* and *Candida albicans* were also tested. The strains that showed resistance against the maximum number of antibiotics tested were selected for an antibacterial assay. The MDR strains were sensitive to the antimicrobial activity of *Acacia nilotica, Syzygium aromaticum and Cinnamum zeylanicum*, whereas they exhibited strong resistance to the extracts of *Terminalia arjuna* and *Eucalyptus globulus*. Community-acquired infections showed higher sensitivity than the nosocomial infections against these extracts. The most potent antimicrobial plant was *A. nilotica* (MIC range 9.75-313µg/ml), whereas other crude plant extracts studied in this report were found to exhibit higher MIC values than *A.nilotica* against community acquired as well as nosocomial infection. This study concludes that *A. nilotica, C. zeylanicum and S. aromaticum* can be used against multidrug resistant microbes causing nosocomial and community acquired infections [99]. Extract of locally available plant *Cassia fistula* will be screened for antimicrobial activity. The antimicrobial activities of *Cassia fistula* plant parts have been studied earlier by many scientists [100-105].

Plant extracts have also been described for their anticancerous and antimutagenic activity. Khanum *et. al.* reported anticarcinogenic potential of curry leaves (*Murraya koenigii*) in dimethylhydrazine-treated rats [106]. Extracts prepared from *Gymnocladus dioicus, Holodiscus discolor,* and *Stephanandra tanakae, Ligustrum delavayanum, Ligustrum vulgare* and *Staphylea pinnata* were investigated for cytotoxic activity against Hela cell lines [107]. Qari evaluated

antimutagenic potential of aqueous extract of Origanum majorana in colchicine treated *Vicia faba* root meristem cells [108]. Jayaraman *et al.* evaluated antitumour activity of *Stevia rebaudiana* extract using human laryngeal epithiloma cell line (HEp2) via MTT assay [91].

Several reports are also available on pesticidal activity of plant extracts. Tewary *et al.*, reported pestcidal activity of *Berberis lycium, Hedera nepalensis, Acorus calamus, Zanthoxylum armatum* and *Valeriana jatamansi* against *Aphis cracccivora* , *Tetranychus urticae* and larvae of *Spodoptera litura, Plutella xylostella* and *Helicoverpa armigera* [109]. Kouninki *et al.* studied the toxicity of some terpenoids of essential oils of *Xylopia aethiopica* from Cameroon against *Sitophilus zeamais Motschulsky* [110]. Mondal *et al.* described the toxicity of chloroform extracts of *Derris indica* Bennet against *Callosobruchus maculates* [111].

Medicinal plants have generated the interest of man for therapeutic values chiefly because of the presence of secondary metabolites. It is obvious that plants have their own built in defense mechanism against infection by almost all microorganisms. The plants upon recognizing the invading pathogen synthesize several toxic chemicals such as phenolics, phytoalexins, and lignin and try to ward off the pathogen [112, 113]. The antimicrobial properties of plant extracts therefore are a result of presence of secondary metabolites such as alkaloids, phenols, flavanoids, terpenoids, essential oils etc. [114]. Several workers have reported antimicrobial activity of these secondary metabolites [115-122].

Ellof reported that tannins, saponins polypeptides and reducing sugars are soluble in water whereas terpenoids, flavonoids, alkaloids, and fatty acids are soluble in organic solvents [123-126]. Tannins and reducing sugars are soluble in both water as well as organic solvents but their solubility is more in organic solvents as compared to water. Harborne [114], Kokate *et al.*, [127] suggested that extraction of secondary metabolites from plant material by hot extraction with petroleum ether separates sterols, waxes and fatty acids leaving behind residue containing the defatted plant materials. Subsequently extraction of this residue with benzene separates out sterols and flavonoids. Terpenoids and flavonoids get extracted with chloroform. The last solvents i.e. alcohol removes alkaloids, flavonoids, polyphenols, tannins and reducing sugar from residue. Finally extraction with water yields remaining water-soluble metabolites such as anthocyanin, starch, tannins, saponins, reducing sugar and polypeptides [124]. All the active principles present in plants are saturated organic compound so they get extracted in ethanol or methanol [83].

Flavonoids have existed since one billion years and survived in vascular plants throughout evolution indicating their importance in nature. The association between plant flavonoids and various animal species, a wide range of biological activities of flavonoids has been reported [128]. They are known to be synthesized by plants in response to microbial infection. The inhibitory activity is due to formation of complexes with extracellular and soluble proteins and bacterial cell wall and disruption of microbial membranes [129]. Flavonoids are low molecular weight, polyphenolic compounds available in practically all dietary plants [130]. They represent a widespread group of water-soluble phenolic derivatives, which are mostly, brightly coloured. Over 4000 structurally unique flavonoids have been identified in plants.

Polyphenols, phenolic acids and flavonoids are powerful antioxidants and have been reported to demonstrate antibacterial, antiviral, anticarcinogenic, anti-inflammatory and vasiodilatory actions [131-133].

Phenols and polyphenol group of compounds consist of thousands of diverse molecules with heterogenous structure with common feature of having one or more phenol ring. They are synthesized in plants by shikimic acid pathway. Alkaloids are heterocyclic nitrogen compounds. Alkaloids are synthesized by decarboxylation of amino acids. Cinchona alkaloids present in the bark of Cinchona sp. have quinine as their main constituent, which is known since 1630 for its antimalarial activity. Diterpenoid alkaloids isolated from the family *Ranunculaceae* are commonly found to have antimicrobial properties [134].

Tannin refers to polymeric phenolic substances capable of tanning leather or precipitating gelatin from solution, known as astringency. Tannins are divided in two groups: hydrolysable and condensed tannins. Condensed tannins, which are generally known as proanthocyanidins are derived from flavonoid monomers [129]. Their molecular weight ranges from 500 to 3000 and they are found in almost every plant part: bark, wood, leaves, fruits and roots. Tannins work by stimulation of phagocytic cells, host-mediated tumor activity and a range of anti-infective actions as well as the ability to form complexes with proteins [135] .Their mode of antimicrobial action may be related to their ability to inactivate microbial adhesions, enzymes, cells envelop transport proteins etc. Scalbert reported the antimicrobial properties of tannins. According to his studies, tannins can be toxic to filamentous fungi; yeasts and bacteria [124]. Condensed tannins have been reported to bind with cell walls of ruminal bacteria, preventing their growth and protease activity [136]. Several workers have reported antimicrobial activity of tannins [137,138].

Saponins are naturally occurring surface-active glycosides. They are mainly produced by plants, but lower marine animals and some bacteria are also known to produce these compounds [139,140]. Saponins consist of a sugar moiety usually containing glucose, galactose, glucuronic acid, xylose, rhamnose or methylpentose, glycosidically linked to a hydrophobic aglycone (sapogenin) which may be triterpenoid in nature. A large number of the biological effects of saponins have been ascribed to their action on membranes. In fact, their specific ability to form pores in membranes has contributed to their common use in physiological research [141-144]. Saponins have been described for hypocholesterolaemic potential [145,146], anticarcinogenic activity [147,148], antifungal potential [149,150], antiviral and antiprotozoanal potential [151,152].

The fragrance of plant is due to presence of quinta essentia or essential oil fractions. These oils are highly enriched secondary metabolites that are based on isoprene units. They are also called as terpenes. Their general chemical structure is C10H16 and they occur as diterpenes, triterpenes and tetraterpenes (C20, C30 and C40) as well as hemiterpenes (C5) and sesquiterpenes (C15). When the compounds contain additional elements usually oxygen, they are termed as terpenoids [83].

Antimicrobial terpenoid were isolated from *Pterocarpus indicus* by Ragasa *et al.* [153]. Ten sesquiterpenes and six diterpenes were isolated and screened for antimicrobial activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Rhizoctonia solani* etc. [117]. Plant extracts also exhibit trypanocidal, leishmanicidal and antimalarial activity. The antimicrobial property of plant extracts is a result of presence of combinations of secondary metabolites such as phenols, flavonoids, terpenoids, essential oils, alkaloids etc. [114]. These secondary metabolites do not play any vital role in building and maintaining processes of plant cells.

The biological and molecular action of secondary metabolites induces various morphological and cytological changes in microorganisms. These changes can be studied at microscopic as well as macroscopic level. Macroscopic changes include change in colony colour, shape, size etc. Changes in cell number, cell size, cell shape, number of reproductive structure can be observed at microscopic level. Effect of extract on cytomorphology i.e. cell size; cell shape and cell number of different organisms has been studied by several workers. Burt and Reinders reported that oregano essential oil brings about extensive morphological changes in treated cells [154]. The cell structures appeared to be empty of contents and remains were flaccid while control cells were found to be whole. Zeylastral and demethylzeylastral, two phenolic compounds isolated from the roots of *Maytenus blepharodes* (*Celastraceae*), showed inhibition of synthesis of DNA, RNA, protein and cell wall [155]. Complete inhibition in the incorporation of the N-aceylyl-D-I-14C glucosamine suggests that the phenolic compounds compromise the cell wall synthesis and/or cytoplasmic membrane. Zhang *et al.* isolated steroid saponin from *Tribulus terrestris* L. and these steroid saponins showed significant *in vitro* and *in vivo* antifungal activity, weakening the virulence of *Candida albicans* and killing fungi through destroying the cell membrane [126].

Apart from this, plant extracts also have the ability to affect the protein, carbohydrate and lipid content of plasma membrane as well as their permeability. Plasma membrane of fungi consists of bilayer of protein and lipids. Inhibition of synthesis of DNA, RNA protein, lipid and carbohydrate may be due to presence of secondary metabolites. These secondary metabolites are target specific and their biochemical and molecular targets are mainly proteins such as receptors, enzymes and polynucleotides like DNA and RNA [156].

There are several methods available to assay antimicrobial sensitivity, Poison food technique [157] and Disc or Agar well diffusion method [158] is commonly used to determine antimicrobial sensitivity test. Agar well diffusion method depends on the inhibition of bacterial growth as an indication of activity, magnitude of which is measured as a function of the diameter of inhibition zone. The activity of extract is always compared with that of the currently used antibiotic in parallel line assay. Sensitivity of microbes against plant extracts by agar well method has been studied by several workers [159,103]. Antimicrobial activity by disc diffusion method has also been studied by several workers [160-163].

Although the diffusion method is commonly used in preliminary susceptibility testing but it is not always an accurate method to assay antimicrobial activity because there is a high

degree of interference with this method, arising from drug diffusion problems. A more generally accurate method of assessment is the broth dilution technique [158]. Therefore the broth dilution method was used to determine antimicrobial activity measured as MIC. In the diffusion methods there is the limited diffusion of the less polar active compound in solid media, which shows the lack of inhibition zone while in the broth dilution method the compounds in solution come in direct contact with the organisms [164,165]. Okore *et al.* assayed anticandidal activity of crude aqueous pod extract of *Lecaniodiscus cupanoides* by broth dilution technique [159]. Antimicrobial sensitivity by broth dilution technique has been reported by several workers [166,167].

Antifungal activity by poison food technique depends on 1 ml of stock solution mixed with 9 ml molten sterile culture medium and this mixture poured into pre-sterilized petri-plates (9 cm diameter) and allowed to solidify at room temperature, prepared petri plates inoculated aseptically with 5 mm disc of test cultures.. Antifungal activity was measured as a function of increase in growth of 5 mm disc of inoculums [157]. Several workers have studied sensitivity of microbes against plant extracts by poison food technique [168].

Plant products have been used as soil amendment, bare root dipping, seed dressing etc. for management of various crops. Organic matter influences physical characters of soil such as pore size, aeration, temperature, water retention capacity etc. which help in better solubilization of minerals together with the nutrients released by decomposing organic matter. Hamid *et al.* used soil amendment with organic manure, soil exposure to sunlight (solarization) and soil flooding for the management of *Rhizoctonia* disease in Potato [169].

Matsuzaki *et al.* reported that soil with cow manure amendments is best treatment for reducing the severity of the disease and improving the final tubers yield of potato [170]. Reduction in the stem infection has also been observed when oats preceded potato as a green manure crop [171]. Solarizing soils plus use of suitable organic materials have also been reported to actuate a chain reaction of chemical and microbial degradation, which enhance toxicity against soil flora and fauna, especially soil borne plant pathogens. These probably contributed to the higher nutrient contents, which could be found with organic manure amendment [172].

Al-Mughrabi *et al.* conducted field trials against foliar and tuber disease of potato by using three types of organic material including thermal compost, static wood chips and vermin castings [173]. Infection of *R.solani* afflicted the normal growth and yield of potato tubers. However treatment with extract was been helpful in resuming normal yield [174].

Several physiological processes in the host are stimulated due to activation of host enzymes. Enzymes like peroxidase, polyphenol oxidase, and chitinase and phenylalanine ammonia lyase are found to be involved in defense mechanism. Estimation of these biochemical markers and the mechanisms providing resistance against pathogens is therefore essential. Polyphenol oxidase oxidizes the phenols to highly toxic quinines and hence is considered to play an important role in disease resistance [175]. Among the various enzymes

studied, peroxidase has been correlated with plant defense mechanism by catalyzing the condensation of phenolic compound into lignin. It has been demonstrated earlier that peroxidase plays an important, early and specific role in hypersensitive containment of the pathogen [176].

Antimicrobial activity of plant extracts depends on chemical nature of compounds present in them. Various physical factors such as pH, temperature, and exposure to sunlight may bring about a change in chemical nature of these compounds. Commercial viability of any herbal formulation or plant extract depends on its ability to maintain stability at varying physical conditions. Several workers have studied the effect of various physical factors on efficacy of extract. Jeong *et al.* investigated the effect of heat treatment on antioxidant activity of extracts from citrus peels [177]. The antioxidant activity of citrus peels extract increased as heating temperature increased. Lee *et al.* investigated effect of heat and pH on Chinese leek extract and found that heat treatment above 15oC reduced the inhibitory activity while inhibitory activity is stable between pH 2.0 to 8.0 [178].

Instruments and Techniques

Solid and liquid media used for the study were sterilized in an autoclave (Yorco Scientific Instruments, India) at 121°C, 15 lb for 15 min. pH of the medium was set by digital pH meter (Systronics 335, India). Hot air oven (Yorco Scientific Instruments, India) was used for sterilizing glassware like petridishes, test tubes etc. Sterilization was accomplished by exposure of items to 150º C - 180º C for 2 to 4 hours. Hot plate (Remi) was used for dissolve the solid material. Inoculation was done with nichrome wire loop (calibrated to deliver 10 (l approximately) under aseptic condition of stericlean horizontal laminar flow bench (Deepak Meditech, India, Model No. DMI 88). Aerobic incubation was done in Biological oxygen demand (BOD) incubator (Super Deluxe, Yorco Scientific Industries Pvt. Ltd.) at optimum temperature and time required. Digital balance (Sartorius Model number GE 412) was used for weighing of material.

Microbial cells were counted by haemocytometer (Rohem Instruments, India Model No. B5 748 Silverlite ISI 0269) and microbial cell size was measured by ocular micrometer (Sigma Inc., Japan) calibrated previously with stage micrometer. Microscopy was done by monocular microscope (Olympus, Germany) as well as binocular microscope whereas trinocular microscope (Olympus, Germany) was used for microscopic photography work. Photography work was done by digital camera (Olympus, Japan, Model No. BX51).

Partially purified fractions in different organic solvents were prepared in glass soxhlet assembly. The extract was vaccum dried in JSWG vacuum evaporator.

Precoated silica gel 60 F254 TLC plates (E-merck) of uniform thickness (20mm x 20mm) and column chromatography was used for separation of secondary metabolites according to colour of bands. UV Fluorescence Analysis Cabinet (Macro Scientific works(R), New Delhi) was used for observed of thin layer chromatography plates. The O.D. measurements were taken with the help of digital spectrophotometer (Systronics 106, India) and digital colorimeter

(Range 400 to 700 nm, Naina Solaris Ltd., India).

Leaf area was measured with the help of Leaf Area Meter-211 (Range 1cm2 to 200 cm2, Systronic, India). Water bath (Yorco Universal Water Bath, Yorco Scientific Indus. Pvt. Ltd.) was used for maintained the temperature.

Secondary metabolites are widely present in medicinal herbs and plants (Harborne, 1984). Sandhu and Arora (2000) have reported that these secondary metabolites are responsible for antimicrobial activity of plant extracts. These secondary metabolites such as phenols, flavonoids, quinones, essential oils, alkaloids, sterols, thymol, coumarines and triterpenoids are untapped reservoirs of various valuable chemicals (Lefevre *et al.*, 2008). Natural plant-based remedies are used for both acute and chronic health problems, from treating common colds to controlling blood pressure and cholesterol. Herbal plant formulations also have preventive effect against plant pathogenic microbes. Scientists all over the world are involved in screening plant extracts for antimicrobial activity in search of novel compounds which can be used to control bacterial and fungal diseases of humans and plants.

There are two basics methods of preparing plant extracts; cold extraction methods which include preparation of infusion and tinctures and hot extraction methods which involve formulation of decoction and standard infusion. Water is most universally used solvent for preparation of extracts. Crude infusion is prepared by steeping dried plant material in cold or hot water for 24 hr. For preparation of decoction dry powder of plant material is boiled for 10-20 minutes in water and strained for use as a decoction. Tinctures are prepared by using water and alcohol in various ratios.

Antimicrobial screening is usually done with crude alcohol or aqueous extracts. Both cold and hot extraction methods are used for preparing the extracts. For cold extraction dried powdered plant material is suspended in cold solvent for 24-48 h. After which decoction is filtered and solvent is evaporated [179]. Dried residue is used as crude extract. Several workers have been screened the antimicrobial activity of crude extracts prepared by this method [180]. Hot extraction process involves boiling of dried plant material with the solvent in soxhlet assembly till complete extraction takes place. In this process a single solvent or a series of solvents ranging from non polar to polar in nature can be used [114,127].

Use of different solvents ensures complete extraction of all kinds of primary as well as secondary metabolites present in the plant or its parts. Petroleum ether, benzene, chloroform, hexane, methanol, ethanol and hydro alcohol have been used by several workers for successive extraction of active compounds in plant extracts [181].

Each plant species in this universe has its own specific set of secondary metabolites. Combined knowledge of biological activity and chemical constituents of the plant is desirable for discovery of new class of compounds. Each fraction prepared by successive extraction carries a specific set of secondary metabolites as the solubility of secondary metabolites differs in different organic solvents depending on polarity of solvent as well as compound used for

extraction. Phytochemical tests are performed for the detection of phytoconstituents present in individual fractions. Khadikar *et al.* investigated phytochemical properties of water extract of *Boerhaavia diffusa* and *Azadirachta indica* [182]. Jager *et al.* reported the plants are rich sources for a new group of multi potent extracts and observe the presence of pentacyclic triterpenes in various plants [183].

Materials and Methods

Plant material was collected from local gardens, roadsides and adjoining areas of city and dried at room temperature and then ground in an electrical grinder. The ground material was passed through sieve of mesh size 60 to obtain a fine powder which was used to prepare the extract. Cold extractions as well as hot extraction procedures were followed to procured crude and partially purified fractions respectively.

Cold Extraction

Crude extract was prepared by according to the modified cold extraction method suggested by Shadomy and Ingraff [179]. Cold extraction was done in water, 50% hydro alcohol as well as absolute alcohol. 20 gm dried and powdered plant material was suspended in 100 ml of solvent (alcohol/water and 50% hydro alcohol) for 24-48 hrs. The suspension was filtered through Whatman filter paper no.1 then vacuum dried with the help of rotary vacuum evaporator. The dried residue was used as extract and solvent was recycled.

Hot Extraction

Reflux method of solvent extraction was used for successive separation of different partially purified organic constituents present in dried plant material [114,127]. Solvent series used for successive separation was as follows:

Pet. ether → Benzene → Chloroform → Acetone → Alcohol →Methanol →Water

This method involves continuous extraction of powdered dried plant material in soxhlet apparatus with a series of organic solvents. Each time before extracting with next solvent the plant material was dried in an oven below 50°C. 40 gm dry plant powder was kept in Soxhlet extraction unit and extracted with 280 ml petroleum ether till all petrol soluble fractions was extracted. Residue was dried and used for extraction with next solvent. Same procedure was repeated with each solvent and finally residue was macerated with chloroform water to obtain aqueous fraction.

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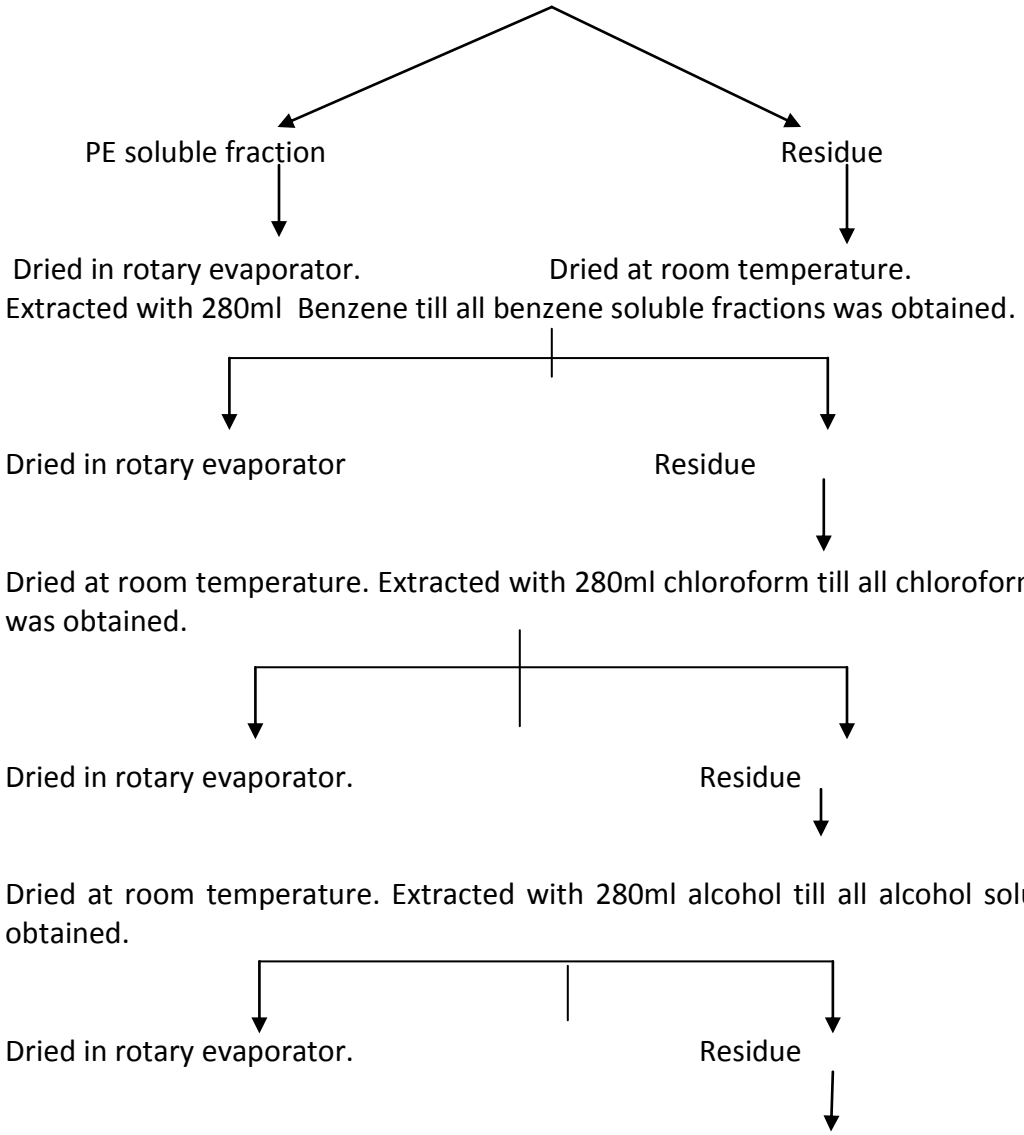
The outline of successive extraction was as follows:

40 gm dry plant powder was placed into 200 ml Soxhlet extraction unit and extracted with 280 ml petroleum ether (40^0-60^0 C) till all PE extract was obtained.



Crude extract and fractions obtained at every step including aqueous fraction were vacuum dried in a rotary evaporator. The dried extract and fractions were weighed and their percentage in terms of the dry weight of the plant material was estimated by the following formula given below.

$$\text{Percent extractive} = \frac{\text{Weight of dried extract}}{\text{Weight of dried plant material}} \times 100$$

Phytochemical Study of *Cassia fistula* fruit pulp extract

Qualitative methods will be used for the identification of different secondary metabolites or phytochemicals present in the plant extracts according to methods suggested by Kokate *et al.* [127].

Tests for Detection of Secondary Metabolites

Alkaloids

Alkaloids are compounds having one or more nitrogen containing heterocyclic ring. Presence of alkaloids in the partially purified fractions was tested by performing Mayer's test or Wagner's test or Hager's test. Reaction with Mayer's reagent produces a cream coloured precipitate; Hager's reagent gives yellow precipitate while Wagner's reagent results in formation of reddish brown precipitate.

Small amount of extract was stirred with few drops of dilute HCl and filtered. The filtrate was tested with various alkaloid reagents and observed for development of coloured precipitate.

Volatile Oils

The odorous volatile chemical constituents of plants are known as volatile or essential oils. Sudan III test was used to detect presence of volatile oils. Development of red colour on mixing with Sudan III indicates presence of volatile oils.
Small amount of extract was mixed with Sudan III dye and observed for development of red colour.

Tannins

Chemically, tannins contain the mixture of complex organic substances in which polyphenols are present. Development of green colour indicates presence of condensed tannins whereas blue colour indicates presence of hydrolysable tannins.

Small amount of extract was taken and treated with alcohol $FeCl_3$ solution and observed for colour development.

Saponin

Saponins are complex glycoside compounds in which the aglycone is triterpenoid or steroidal in nature. Foam test was used to detect presence of saponins.
Small amount of extract was diluted with 20 ml of distill water; then shaken in graduated cylinder for 15 minutes. Formation of a layer of foam at surface indicates presence of saponin.

Carbohydrates

Carbohydrates are widely distributed in plants and can be detected by Molish's test and Fehling's test. Small amount of extract was dissolved in 5 ml distilled water and filtered. Development of purple colour on addition of few drops of α-napthol and conc. H_2SO_4 to the filtrate indicates presence of sugars.

Similarly small quantity of filtrate was heated with equal amount of Fehling A and Fehling B solution. Development of brick red colour indicates presence of carbohydrates.

Flavonoids

Flavonoids usually occur in plants as glycosides in which one or more of phenolic hydroxyl groups are combined with sugar residues. Alkaline reagent test was used to detect flavonoids.
Small amount of extract was mixed with aqueous NaOH. Development of reddish brown colour shows presence of flavonoids.

Sterols

Sterols are triterpenes which are based on cyclopentane perhydroxy phenanthrene ring system. They are also called as phytosterols, Liebermann's Burchard test was used for detection of phytosterols.

Small amount of extract was mixed with 2 ml $CHCl_3$ and 1 ml of acetic anhydride. Subsequently concentrated H_2SO_4 was added gradually through the side of the test tube. Formation of brown coloured ring at junction of two layers indicates presence of sterols.

Description of Pathogen (*Alternaria solani*)

About 85 percent of plant diseases are caused by fungi. Fungi are not plants. Fungi are multicelled and, during certain stages of their life cycles, may be seen without a microscope. They have no chlorophyll and though they do have cell walls, the walls of many species are not made of cellulose as in true plants. Many species of fungi produce spores, which are reproductive structures that aid in dispersal and survival. Most fungi can be identified by the structure of these microscopic spores. Wind is important in the dispersal of most fungal pathogens. Spores can be carried for miles in the wind. Fungi can penetrate directly through the cuticle of plants. Therefore, they do not need natural openings in the plant or wounds for access. There are several groups of fungi that are important in causing plant diseases in the nursery. The most significant sub-division is the Deuteromycotina, which contain the serious pathogen *Alternaria solani.*

Alternaria solani is a fungal pathogen that produces a disease in tomato and potato plants called early blight. The pathogen produces distinctive "bulls eye" patterned leaf spots

and can also cause stem lesions and fruit rot on tomato and tuber blight on potato. Foliar symptoms usually occur on older leaves. If uncontrolled, early blight can cause significant yield reductions. Primary methods of controlling this disease include preventing long periods of wetness on leaf surfaces and applying fungicides.

Geographically, *Alternaria solani* is problematic in tomato production areas east of the Rocky Mountains and is generally not an issue in the less humid Pacific or inter-mountain regions. *Alternaria solani* is also present in most potato production regions every year but has a significant effect on yield only when frequent wetting of foliage favors symptom development [184].

Disease cycle

Alternaria solani belongs to the sub-division Deuteromycotina, class Hyphomycetes, family Dematiaceae , with a polycyclic life cycle. *Alternaria solani* reproduces asexually by means of conidia. The life cycle starts with the fungus over wintering in crop residues or wild members of the Solanaceae family, such as black nightshade. In the spring, conidia are produced. Multicellular conidia are splashed by water or by wind onto an uninfected plant. The conidia infect the plant by entering through small wounds, stomata, or direct penetration. Infections usually start on older leaves close to the ground. The fungus takes time to grow and eventually forms a lesion. From this lesion, more conidia are created and released. These conidia infect other plants or other parts of the same plant within the same growing season. Every part of the plant can be infected and form lesions. This is especially important when fruit or tubers are infected as they can be used to spread the disease. In general, development of the pathogen can be aggravated by an increase in inoculum from alternative hosts such as weeds or other solanaceous species. Disease severity and prevalence are highest when plants are mature [17].

Environment

Alternaria solani spores are universally present in fields where host plants have been grown. Free water is required for *Alternaria* spores to germinate; spores will be unable to infect a perfectly dry leaf. *Alternaria* spores germinate within 2 hours over a wide range of temperatures but at 26.6-29.4°C (80-85°F) may only take 1/2 hour. Another 3 to 12 hours are required for the fungus to penetrate the plant depending on temperature. After penetration, lesions may form within 2–3 days or the infection can remain dormant awaiting proper conditions [15.5°C (60°F) and extended periods of wetness]. *Alternaria* sporulates best at about 26.6°C (80°F) when abundant moisture (as provided by rain, mist, fog, dew, irrigation) is present. Infections are most prevalent on poorly nourished or otherwise stressed plants [185].

Hosts and symptoms

Alternaria solani infects stems, leaves and fruits of potato (*S. tuberosum*), tomato (*Solanum lycopersicum L.*), eggplant (*S. melongena L.),* bell pepper and hot pepper (*Capsicum*

spp.), and other members of the Solanum family. Distinguishing symptoms of *A. solani* include leaf spot and defoliation, which are most pronounced in the lower canopy. In some cases, *A. solani* may also cause damping off.

On potatoes

In potato, primary damage by *A. solani* is attributed to premature defoliation of potato plants, which results in tuber yield reduction. Initial infection occurs on older leaves, with concentric dark brown spots developing mainly in the leaf center. The disease progresses during the period of potato vegetation, and infected leaves turn yellow and either dry out or fall off the stem. On stems, spots are gaunt with no clear contours (as compared to leaf spots). Tuber lesions are dry, dark and pressed into the tuber surface, with the underlying flesh turning dry, leathery and brown. During storage, tuber lesions may enlarge and tubers may become shriveled. Disease severity due to *A. solani* is highest when potato plants are injured, under stress or lack proper nutrition. High levels of nitrogen, moderate potassium and low phosphorus in the soil can reduce susceptibility of infection by the pathogen [17].

On tomatoes

On tomato, foliar symptoms of *A. solani* generally occur on the oldest leaves and start as small lesions that are brown to black in color. These leaf spots resemble concentric rings - a distinguishing characteristic of the pathogen - and measure up to 1.3 cm (0.51 inches) in diameter. Both the area around the leaf spot and the entire leaf may become yellow or chlorotic. Under favorable conditions (e.g., warm weather with short or abundant dews), significant defoliation of lower leaves may occur, leading to sunscald of the fruit. As the disease progresses, symptoms may migrate to the plant stem and fruit. Stem lesions are dark, slightly sunken and concentric in shape. Basal girdling and death of seedlings may occur, a symptom known as collar rot. In fruit, *A. solani* invades at the point of attachment to the stem as well as through growth cracks and wounds made by insects, infecting large areas of the fruit. Fruit spots are similar in appearance to those on leaves – brown with dark concentric circles. Mature lesions are typically covered by a black, velvety mass of fungal spores that may be visible under proper light conditions [185].

Management

Cultural control

- Clear infected debris from field to reduce inoculum for the next year.
- Water plants in the morning so plants are wet for the shortest amount of time.
- Use a drip irrigation system to minimize leaf wetness which provides optimal conditions for fungal growth.
- Use mulch so spores in soil cannot splash onto leaves from the soil.
- Rotate to a non-Solanaceous crop for at least three years.

- If possible control wild population of Solanaceae. This will decrease the amount of inoculum to infect your plants.
- Closely monitor field, especially in warm damp weather when it grows fastest, to reduce loss of crop and spray fungicide in time.
- Plant resistant cultivars.
- Increase air circulation in rows. Damp conditions allow for optimal growth of *A. Solani* and the disease spreads more rapidly. This can be achieved by planting farther apart or by trimming leaves.

Chemical control

There are numerous fungicides on the market for controlling early blight. Some of the fungicides on the market are azoxystrobin, pyraclostrobin, chlorothalonil, copper products, hydrogen dioxide, mancozeb, potassium bicarbonate, and ziram. Specific spraying regiments are found on the label. Labels for these products should be read carefully before applying.

Economic significance

A. solani is one of the most important foliar pathogens of potato. In the U.S., yield loss estimates attributed to foliar damage, which results in decreased tuber quality and yield reduction, can reach 20-30%. In storage, *A. solani* can cause dry rot of tubers and may also reduce storage length, which both of which diminish the quantity and quality of marketable tubers.

Because *A. solani* is one of numerous tomato/potato pathogens that are typically controlled with the same products, accurately estimating both the total economic loss and the total expenditure on fungicides for control of early blight is difficult. Best estimates suggest that total annual global expenditures on fungicide control of *A. solani* is approximately $77 million: $32 million for tomatoes and $45 million for potatoes [186].

Alternaria solani is the causal agent early blight of potato that leads to major damages to potato crop. It is a major foliar disease of potato and causes 20-50% yield loses. It produces small, darkened lesions on the plants that spread into growing black spots of dead tissue. *A. solani* overwinters as mycelium or conidia in plant debris, soil, infected tubers or on other host plants of the same family. The disease is controlled primarily through the use of cultural practices such as resistant cultivars and foliar fungicides, crop rotation, tillage, removal and burning of infected plant debris, and eradication of weed hosts helps reduce the inoculums level for subsequent plantings [187].

The most common and effective method for the control of early blight is through the application of foliar fungicides. Protectant fungicides recommended for late blight control (e.g. maneb, mancozeb, chlorothalonil, and triphenyl tin hydroxide) are also effective against early blight. But on the other hand the pesticide treatment is not protected as chemicals pollute environment, effect health vulnerability in humans and when these harmful chemicals enter

into the food chain become hazardous to all living entities [188]. Botanical derivatives like phenols, tannins, and flavonoids are environmentally safe and may be used as an alternative to commercial fungicides for controlling pathogenic fungi [189,190].

Description of Plant *(Cassia fistula Linn.*)

Cassia fistula (Linn.) belongs to family Fabaceae and Sub–family Caesalpinioideae is a very common plant known for its medicinal properties are a semi-wild in nature. It is distributed in various regions including Asia, South Africa, China, West Indies and Brazil. It is commonly known as Amaltas and in English popularly called "Indian Laburnum" has been extensively used in Ayurvedic system of medicine for various ailments. It is deciduous and mixed-monsoon forests throughout greater parts of India, ascending to 1300 m in outer Himalaya, is widely used in traditional medicinal system of India [191].

Geographical distribution

In deciduous and mixed monsoon forests throughout greater parts of India, ascending to 1300 m in outer Himalaya. In Maharashtra, it occurs as a scattered tree throughout the Deccan and Konkan. The plant is cultivated as an ornamental throughout India [192].

Morphology

It is a deciduous tree with greenish grey bark, compound leaves, leaf lets are each 5-12 cm long pairs. A semi-wild tree known for its beautiful bunches of yellow flowers and also used in traditional medicine for several indications. A fruit is cylindrical pod and seeds many in black, sweet pulp separated by transverse partitions. The long pods which are green, when unripe, turn black on ripening after flowers shed. Pulp is dark brown in colour, sticky, sweet and mucilaginous, odour characteristic, and somewhat disagreeable. Drug occurs in flat or curved thick pieces; outer surface smooth to rough with warty patches; greenish grey to red; inner surface rough, reddish with parallel striations; fracture, laminate; odour, sweet and characteristic; taste, astringent [193].

A tree 6-9 m high; trunk straight; bark smooth and pale grey when young, rough and dark brown when old; branches spreading, slender. Leaves 23-40 cm long; main rhachis pubescent; stipules minute, linear-oblong, obtuse, pubescent. Leaflets 4-8 pairs, ovate or ovate-oblong, acute, 5-12.5 by 3.8-9.5cm, bright green and glaborous above, paler and silverypubescent beneath when young, the midrib densely pubescent on the underside, base cuneate; main nerves numerous, close, conspicuous beneath; petiolules 6-10 mm long, pubescent or glaborous. Flowers in lax racemes 30-50 cm. long; pedicels 3.8-5.7 cm. long, slender, pubescent and glaborous. Calyx 1 cm long divided to the base, pubescent; segments oblong, obtuse. Corolla 3.8 cm across, yellow; stamens all antheriferous [194].

The pods are pendulous, cylindric, nearly straight, smooth, shining, brown-black, indehiscent, with numerous (40-100) horizontal seeds immersed in a dark coloured sweetish

pulp. Seeds broadly ovate, 8mm. long, slightly less in breadth, and 5mm thick. The fruit pods are 40-70 cm long and 20-27mm in diameter, straight or slightly curved, smooth but finely striated transversely, the striations appearing as fine fissures. The rounded distal ends bear a small point marking the position of the style. The dorsal suture appears as a single vascular strand and the ventral suture as two closely applied strands. Internally the pod is divided by thin, buff coloured, transverse dissepiments at intervals of about 0.5cm. Each compartment contains one seed which is flat, oval, reddish brown with a well marked raphe. The seed contains a whitish endosperm in which the yellowish embryo is embedded [195].

Traditional Medicinal Uses

The root is prescribed as a tonic, astringent, febrifuge and strong purgative. Extract of the root bark with alcohol can be used for back wart fever. The roots are used in chest pain, joint pain, migraine and blood dysentery. The extract of the root lowered the blood sugar level up to 30 per cent. Root is useful in fever, heart diseases, retained excretions and biliousness. The aqueous extract of the root bark exhibits anti-inflammatory activity. The root is used in cardiac disorders biliousness, rheumatic condition, haemorrhages, wounds, ulcers and boils and various skin diseases. *Cassia fistula* leaves are crushed to prepare a thick paste and mixed with coconut oil. This paste is applied over the burnt skin twice a day [196].

The stem bark is used against amenorrhoea, chest pain and swellings. The bark possess tonic and antidysentric properties, it is also used for skin complaints, the powder or decoction of the bark is administered in leprosy, jaundice, syphilis and heart diseases. The leaves extract reduced mutagenecity in *E. coli*. The leaves are laxative and used externally as emollient, a poultice is used for chilblains, in insect bites, swelling, rheumatism and facial paralysis. Leaves posses anti periodic and laxative properties, the leaves are used in jaundice, piles, rheumatism ulcers and also externally skin eruptions, ring worms, eczema [197].

The leaves and bark mixed with oil are applied to pustules, insect bites. Juice of leaves is used in skin diseases. Juice of leaves is useful as dressing for ringworm, relieving irritation and relief of dropsical swelling. The pulp of the fruit around the seeds is a mild purgative. Leaves and flowers are both purgative like the pulp. Ashes from burnt pods mixed with little salt are used with honey taking 3- 4 times to relieve cough. Fruits are used as catharatic and in snake bite. Flowers and pods are used as purgative, febrifugal, biliousness and astringent. The ethanolic 50% extract of pods show antifertility activity in female albino rats. The heated pods are applied to swellings on the neck due to cold. The fruits are reported to be used for asthma. Pulp is given in disorders of liver [198].

The drug is used as analgesic as an antipyretic, it is a remedy for malaria and fever. It is also applied in blood poisoning, anthrax and antidysentric, leprosy and antidiabetic, for the removal of abdominal obstruction. The extract of the flower inhibits the ovarian function and stimulate the uterine function in albino rats. Fruits are used in the treatment of diabetes , antipyretic, abortifacient, demulcent, lessens inflammation and heat of the body; useful in chest complaints, throat troubles, liver complaints, diseases of eye and gripping. The fruit pulp

is used for constipation, colic, chlorosis and urinary disorders. The seeds are emetic, used in constipation and have cathartic properties. The seeds are slightly sweet and possess laxative, carminative, cooling, improve the appetite and antipyretic activity. They are useful in jaundice, biliousness, skin disease and in swollen throat. Seed powder is used in amoebiasis [199].

Description of Crop Plant (Potato)

Vegetables are the fresh and edible portions of herbaceous plants. They are important food and contribute minerals, vitamins, and fiber to the diet and highly beneficial for the maintenance of health and prevention of diseases. They may be edible roots, stems, leaves, fruits or seeds. Each group contributes to diet in its own way [200]. Vegetables due to their high nutritive values constitute the most important and inexpensive component of a balanced diet and are indispensable for the body. India is the second largest vegetable producer in the world, next only to China with an annual production of 81 million tons from 5.1 million hectares of land [201].

Vegetables belonging to family solanaceae are important due to their nutritional as well as economical values. However, farmers face heavy losses both in the quality and quantity of these crops due to various diseases. Early blight disease caused by fungal pathogen *Alternaria spp*. Inflict serious damage to these crops [202]. Potato (*Solanum tuberosum L.*) is the fourth important crop worldwide by volume of production; it is high yielding and having a high nutritive value and grown in about 140 countries [203].

Potato is the world's fourth important crop after wheat, rice and maize because of its great yield potential and high nutritive value. It constitutes almost half of the world's annual output of all root and tuber crops. With an annual global production of about 300 million tonnes, potato is an economically important staple crop in major countries. The major producers in the world are China, Russia, and India followed by US, Ukraine, Poland, Germany, Belarus, Netherlands and France. These together contribute about 70% of the total production. India ranks third contributing around 7.5% to the world's production. Production wise India has always remained in the top ten since last twenty years. The present area under potato cultivation in India is about 1.4 million hectares. India produces a total of about 25-28 million tons of potatoes every year [204].

Soil

Potato prefers sandy or medium loam soils rich in organic matter contents. The soil of the seed bed should be loose, friable, well aerated with good drainage. Light textured soils having friable soil structure and 'high humus content tend to promote more uniform soil temperature and better aeration which favour tuber development and harvesting becomes easier. Most favourable soil pH should be between 5-7.0 as the crop does not grow well in saline-alkali soil and under highly acidic conditions it suffers from the scab disease.

Climate

Potato is a temperate or cold loving crop which needs low temperature, low humidity, less windy and bright sunny days. The crop needs fertile soil with high moisture content but it must not be wet because wet soils become compact which is not good for tuber growth and development. Potato needs about 25°C at the time of germination, around 200C for vegetative growth but between 17-200C for tuberization and tuber development. It is noticed that higher temperature has an adverse effect on the tuber growth whereas a temperature above 30"C stops tuber formation completely. It is probably because at higher temperature the rate of respiration increases and the carbohydrate formed by the process of photosynthesis is consumed rather than stored in tubers. Under higher temperature the plants are elongated, leaves become wrinkled and plants have sickly appearance. Tubers do not develop under such conditions. Cloudy days, rains and high humidity are very congenial for spread of fungi and bacterial diseases, hence, such conditions are not good for the crop. In various parts of the country, it is grown under conducive climatic condition. However, in the Nilgiri hills, potato is cultivated throughout the year.

Seasonality

In India, more than 80% of the potato crop is raised in the winter season (Rabi) under assured irrigation from October to March. About 8% area lies in the hills during summer from April to October. Rainy season (Kharif) potato production is taken in Karnataka, Maharashtra, HP, J&K and Uttaranchal.

Nutrition

The potato contains vitamins and minerals, as well as an assortment of phytochemicals, such as carotenoids and natural phenols. Chlorogenic acid constitutes up to 90% of the potato tuber natural phenols. Others found in potatoes are 4-O -caffeoylquinic acid (crypto-chlorogenic acid), 5-O-caffeoylquinic (neo-chlorogenic acid), 3,4-dicaffeoylquinic and 3,5-dicaffeoylquinic acids. A medium-size 150 g potato with the skin provides 27 mg of vitamin C (45% of the Daily Value (DV)), 620 mg of potassium (18% of DV), 0.2 mg vitamin B6 (10% of DV) and trace amounts of thiamin, riboflavin, folate, niacin, magnesium, phosphorus, iron, and zinc. The fiber content of a potato with skin (2 g) is equivalent to that of many whole grain breads, pastas, and cereals [205].

The potato is best known for its carbohydrate content (approximately 26 grams in a medium potato). The predominant form of this carbohydrate is starch. A small but significant portion of this starch is resistant to digestion by enzymes in the stomach and small intestine, and so reaches the large intestine essentially intact. This resistant starch is considered to have similar physiological effects and health benefits as fiber. It provides bulk, offers protection against colon cancer, improves glucose tolerance and insulin sensitivity, lowers plasma cholesterol and triglyceride concentrations, increases satiety, and possibly even reduces fat storage. The amount of resistant starch in potatoes depends much on preparation methods.

Cooking and then cooling potatoes significantly increases resistant starch. For example, cooked potato starch contains about 7% resistant starch, which increases to about 13% upon cooling [206].

The cooking method used can significantly affect the nutrient availability of the potato. Potatoes are often broadly classified as high on the glycemic index (GI) and so are often excluded from the diets of individuals trying to follow a low-GI diet. In fact, the GI of potatoes can vary considerably depending on type (such as red, russet, white, or Prince Edward), origin (where it was grown), preparation methods (i.e., cooking method, whether it is eaten hot or cold, whether it is mashed or cubed or consumed whole, etc.), and with what it is consumed i.e., the addition of various high-fat or high-protein toppings [207].

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