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NATURAL RESOURCES: AN ECOFRIENDLY AND SAFER ALTERNATE TO CONTROL PLANT DISEASES

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ABSTRACT: 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 antifungal activity. Review of literature reports that plant extracts have been mostly screened for activity against human pathogenic bacteria and fungi. Compared to this, there are very few reports regarding inhibitory activity against plant pathogenic fungi. Although there are several reports of antifungal activity of plant products yet not much work has done to develop herbal bio-control agents or formulations using plant products or extracts. Herbal Products are cheaper than chemicals with minimal or practically no adverse side effects on hosts. *Eucalyptus globulus* extract has been screened for antifungal activity against human pathogenic fungi and bacteria but very few reports are available regarding plant pathogenic fungi especially early blight caused by *Alternaria solani*. Antifungal activity of organic substances has been reported by some workers but no study till date has been reported on preparation of herbal formulation by combining plant powder, extracts and organic substances. The present work proposes to develop a formulation by combining plant extracts/ plant powder with these traditionally used organic substances for control of early blight of tomato caused by *Alternaria solani*. This type of formulation will provide a cheap and environmentally safe herbal fungicide that can be further used as a commercial herbal bio-control agent.

INTRODUCTION: Inappropriate use of agrochemicals especially fungicides not only impose adverse effects on ecosystems, it also possess a possible carcinogenic risk higher than that of insecticides and herbicides put together¹⁻⁴. Moreover, resistance by pathogens to fungicides has rendered certain fungicides ineffective⁵. 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.

Nature has been a source of bio-control agents for thousands of years and an impressive number of antimicrobial compounds have been isolated from natural sources⁶⁻⁸. 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 environment⁹.

In addition to well known disease management methods, there are several traditional agricultural practices followed by farmers to control plant diseases such as crop rotation, use of resistant cultivars, planting disease free seeds, biological control, land preparation, pest control, storage, plant nutrients, grafting, soil selection, plant propagation, mixed cropping, crop rotation, intercropping, shifting cultivation, terrace farming and use of organic materials like cow dung, oil

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cakes etc.¹⁰ for control of fungal diseases¹¹⁻¹³. Kautilya's Arthashastra was probably the oldest document, which described the use of organic materials to control the crop disorders¹⁴⁻¹⁷.

Among all natural sources plants are the main and most popular source of natural products of medicinal interest. Since antiquity, man has used plants to cure common diseases. Plants have formed the basis of sophisticated traditional medicinal systems that have been in existence for thousands of years and continue to provide mankind with new remedies. The various traditional medicinal systems have been extensively reviewed by¹⁸. He described the African traditional medicinal systems, American traditional medicine, Australian and south east medicine, Ayurvedic medicine (Indian traditional medicine), Chinese traditional medicine, European medicine and classical Arabic or North African traditional medicines. African traditional medicine is the most oldest and diverse of all medical systems. Ayurveda is well known form of medicine originating from Asia.

Reports available on green plants represent a reservoir of effective chemo-therapeutants, these are non-phytotoxic, more systemic and easily biodegradable¹⁹⁻²⁰ that 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 action²¹. Plant product preparations and bio-agents do not leave any toxic residues and therefore can effectively replace synthetic fungicides. Tomato is very important solanaceous crops in India either for local consumption and export. Tomato is considered as one of the highest nutritional crops because of its high contents of Vitamin C²². It is susceptible to infection by the blight disease caused by *Alternaria solani* during fruiting period²³⁻²⁴. Which causes great reduction in the quantity and quality of fruit yield. It is well known that tomato fruits are mostly consumed freshly, thereby spraying fungicides just before harvesting resulted in high fungicide residue in the fruits, which cause great hazard to the human health²⁵.

Therefore, the object of this study is to test the efficiency of herbal formulations in reducing the infection of early blight caused by *Alternaria solani* in tomato.

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 rot²⁶⁻²⁷. Losses due to early blight typically are around 20-25%; however, there have been cases of 70-80% losses²⁸⁻²⁹.

Eucalyptus globulus (Labill) also called Tasmanian bluegum, is one of the world's best known eucalyptus trees. One of the first tree species introduced to other countries from Australia, it is now the most extensively planted eucalyptus in the world. It is now primarily used in line plantings along roads and as windbreaks, but formerly, extensive plantations were established. Although bluegum eucalyptus has great climatic adaptability, the most successful introductions worldwide have been to locations with mild, temperate climates, or to high, cool elevations in tropical areas³⁰. Due to isoprenoid accumulation eucalyptus plant has great antifungal, antibacterial, antitumor, antiviral, antimalarial and antioxidant properties. *Eucalyptus* essential oil is also used as natural pesticide³¹. The chemical compositions of the leaf oils of various *Eucalyptus* species had been reported³². Various organic substances such as cow dung, neem oil cake, mustard oil cake, coconut oil cake etc. are used by farmers as organic manure³³⁻³⁴. Some workers also reported the significant antifungal properties of the organic substances.

Bioformulation: Plants are rich in a wide variety of secondary metabolites such as tannin, terpenoids, alkaloids, flavonoids, phenols, steroids glycosides and volatile oils etc. These secondary metabolites possess antimicrobial property as well as play an important role in defense against attack by insects and herbivores³⁵. Investigation of antimicrobial properties of plants identifies them as but for developing a medicinal formulation from plants.

It is essential to identify the phytochemical constituents present in plant extracts with antimicrobial properties. Fresh or dried plant materials can be used as a source for the extraction of secondary plant components.

Many authors had reported plant extract preparation from the fresh plant tissues³⁶⁻³⁹. The logic behind this came from the ethno-medicinal use of fresh plant materials among the traditional and tribal people. But, mostly researchers preferred dried plant material for extraction of secondary plant metabolites⁴⁰. Due to some problems associated with use of fresh plant material during antimicrobial screening of plants. The dried material can be used for large scale extraction of antimicrobial metabolites but fresh material cannot be used. Dried plant material contains only stable secondary metabolic components hence use of dried material is preferred during antimicrobial screening⁴¹⁻⁴².

Indiscriminate use of fungicides may lead to toxic residues, development of fungicide resistance, environmental contaminations and carcinogenic, teratogenic and mutagenic effects in humans, animals and plants⁴³. In this context, biocontrol approaches may help to develop an eco-friendly control strategy for management of serious plant diseases.

Folk medicines of almost all civilizations of the world abound in herbal remedies. Majority of the traditional medicines used in healthcare are obtained from plants⁴⁴. Some workers noticed that the neem seed oil has more effective than the powder formulation in reducing egg-laying and adult emergence of the bruchid⁴⁵. The excessive misuse of a wide range of fungicides has led to it being harmful to the environment and increases the resistant pathogen populations⁴⁶.

As a matter of fact, it has been estimated that about 25% of all prescribed medicines today are substances derived from plants⁴⁷⁻⁴⁸. The used of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed. Furthermore, increasing reliance of the medicinal plants in the industrialized countries has been traced to the extraction and development of

several drugs and chemotherapeutic from these plants as well as from traditionally used rural herbal remedies.

Various organic substances such as cow dung, neem oil cake, mustard oil cake, coconut oil cake etc. are used by farmers as organic manure.

Antimicrobial Activity: 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 workers⁴⁹⁻⁶⁰.

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 protozoa⁶¹⁻⁷⁵.

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 activity⁷⁶ 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*.

The antimicrobial activity of plants is due to the presence of aromatic secondary metabolites such as alkaloids, flavonoids, sterols and tannins etc. which may act as phytoalexins, toxins, inhibitors etc.⁷⁷.

Several workers have reported the antimicrobial activity of various plant extracts, plant derived products and essential oils and other natural products against a wide range of food spoiling microorganisms, depending upon their concentration, testing methods and active constituents present⁷⁸⁻⁸².

A study reported that extraction of tannins and other phenolics was better in aqueous acetone than in aqueous methanol. Similar findings have been

reported by several workers⁸³. Some authors use a combination of these solvents for complete extraction of secondary metabolites from plants⁸⁴⁻⁸⁷. In recent years the interest in the possible use of natural alternatives to food additives to prevent fungal growth has notably increased. Plants and plant parts represent a source of natural alternatives to improve the shelf life and safety of foods. Plant extracts of many higher plants have been reported to exhibit *in vitro* antibacterial, antifungal and insecticidal properties⁸⁸, due to presence of antimicrobial metabolites like flavonoids, coumarins and phenols. Essential oils from *Azadirachta indica* and *Morinda lucida* were found to inhibit the growth of toxigenic.

To find out which group of compounds is responsible for antimicrobial activity, initial screening of plants typically begins by using the crude aqueous or alcohol extracts prepared either by cold or hot extraction methods. Water and alcohol are universal solvent used to extract plant products with antimicrobial activity as most of the antimicrobial compounds are soluble in these solvents. Since nearly all of the identified antimicrobial compounds from plants are aromatic or saturated organic compounds, they are most often obtained through initial alcoholic extraction⁸⁹. Crude extract contains different type of secondary metabolites with different polarity. Thus the most commonly used solvents for preliminary investigations of antimicrobial activity in plants are water and alcohol (usually methanol and ethanol)⁹⁰ have demonstrated the antifungal effect of methanol extracts of nine medicinal plants against *Candida albicans*, *C. glabrata*, *C. tropicalis*, *Cryptococcus luteolus*, *C. neoformans*, *Trichosporan beigelli*, *A.flavus*, *A. parasiticus* and *A. niger*. Hexane, ethyl acetate and methanolic extracts of dried powdered leaves of *Eucalyptus globulus* were screened for basic secondary metabolite and antibacterial activity⁹¹. Antifungal and antibacterial activity of plant base gums & resins have been reported by some workers⁹². Plant based bioformulations also have preventive effect against plant pathogenic microbes. Scientists all over the world is 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

Methodology:

Isolation of *Alternaria solani*:

The test fungus will be isolated from infected leaf and fruit of tomato by Potato dextrose Agar plate method⁹³ and single spore technique⁹⁴. Identification of fungus will be done by standard keys. V8 medium is also used by some worker for isolation of *Alternaria solani*⁹⁵. Isolation of pathogen associated with leaf of tomato showing leaf blight symptoms shows the presence of *Alternaria* conidia under microscopic detection. These will be subjected to isolation of the associated pathogen on Potato-Dextrose-Agar medium by using single spore technique.

Extract Preparation: There are several methods for the extraction, purification and screening of the antifungal compound of plant origin. In this view, the use of efficient system of materials and methods is essential for evaluating the efficacy of medicinal plants as antimicrobial agents. Selection of plants is the first step. In the present study both plants were selected according to the literature available on their medicinal properties.

The next step after selection of plant material is the extraction. Extraction is the separation of medicinally active portion of plant tissue using selective solvents through standard procedures. Such extraction technique separates the soluble secondary metabolite from plant and leave behind the insoluble cellular marc. The basic parameters influencing the quality of extract are the plant parts used as starting material, the solvent used for extraction and the extraction method. The use of appropriate extraction technology, plant material and solvent is necessary during the screening of plant material for their antimicrobial activity.

The extraction is usually done from the fresh as well as dry plant material. Some workers used the fresh plant material for the extraction during antimicrobial screening but use of dry plant material is preferred due to three reasons. The first reason is there are fewer problems associated with the large scale extraction of dried plant material than with the fresh material. Secondly, the time delay between collecting plant material and processing makes it difficult to work with the fresh material.

The differences in water content during the collection and processing period may affect solubility of the material and also affect subsequent separation by liquid-liquid extraction. The third reason for using dried plant material during antimicrobial screening is the secondary metabolic plant component should be relatively stable especially if it should be used as antimicrobial agent. Hence in most of the studies of antifungal screening extract was prepared with dried plant material although some examples of using fresh plant material is also available. Essential oil is generally extracted from fresh plant material for obtaining high percent value, as these are aromatic substances, thus major part can be lost during the drying of the material. In some cases dried plant material was also used for the extraction of essential oil ⁹⁶.

Several authors have used water and alcohol extract for screening against various pathogens ⁹⁷⁻¹⁰⁰. Initial screening with crude extract is followed by screening of extracts prepared in various organic solvents. Crude or partially purified extracts is the mixture of all secondary metabolites present in plant part. The purpose of using organic solvents is the separation of these metabolites according to their polarity and solubility. Different workers have used a variation of solvents for the purpose. Dichloromethane ¹⁰¹, acetone ¹⁰², hexane ¹⁰³, DMSO, chloroform ¹⁰⁴ are generally used solvents. Some authors use a combination of these solvents to obtain the best solvent system for the extraction ¹⁰⁵. Though there is a wide diversification in the usage of solvents, it is necessary to focus on a standardized solvent system for efficacy of antimicrobial screening; hence in the present study various organic solvents were studied and standardized prior to antifungal screening.

Cold Extraction: This extraction was done in two universal solvents i.e. water and alcohol. Cold extract was prepared according to modified method of ¹⁰⁶. 100% alcoholic, 50% alcoholic as well as 100% aqueous extract of leaf of *Eucalyptus globulus* was prepared by dissolving 20 g dried and powdered plant material in 100 ml of solvent (alcohol/ water) for 24 h. The mixture was then filtered and supernatant was evaporated under reduced pressure using a rotary evaporator. The dried residue was used as extract, which was stored

in an airtight jar in refrigerator. Several workers have been screened the antimicrobial activity of crude extracts prepared by this method ¹⁰⁷.

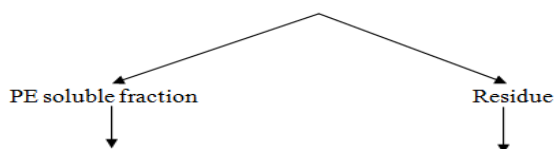
Decoction requires boiling the plant material with water and/or organic solvent for a specific time period. Dried plant parts can also be used externally in form of mixed with oils and petroleum jelly.

(a) Hot Extraction: Hot extraction method is serial exhaustive method which involves successive extraction with solvents for the separation of different phytochemical constituents from plant parts ¹⁰⁸⁻¹⁰⁹. Leaves were used for hot extraction as these parts showed best antifungal activity against test fungi. Solvent series used for successive separation was non-polar to polar i.e.

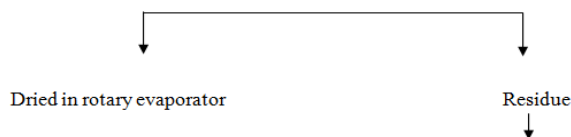
Pet. ether → Benzene → Chloroform → Acetone
→ Alcohol → Methanol → Water

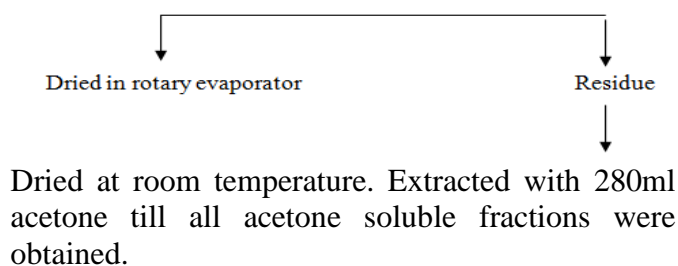
This method involves continuous extraction of powdered dried plant material in soxhlet apparatus with the above series of organic solvents. Extraction with next solvent was done each time after the plant material was dried in an oven below 50°C. 40 gm dry plant powder was kept in soxhlet extraction unit and extracted with 280ml 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 water to obtain aqueous fraction. The outline of successive extraction process used was as follows:

40 gm dry plant powder was extracted with 280 ml petroleum ether (40⁰ -60⁰ C) till all PE extract was obtained.



Dried at room temperature. Extracted with 280ml Benzene till all benzene soluble fractions were obtained.

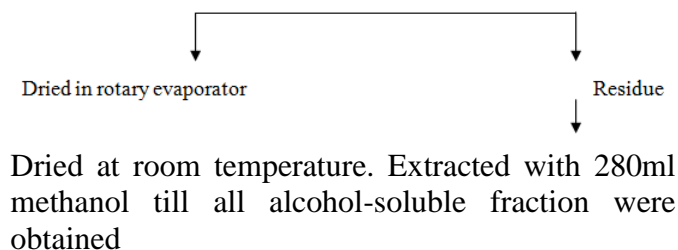




Dried in rotary evaporator

Residue

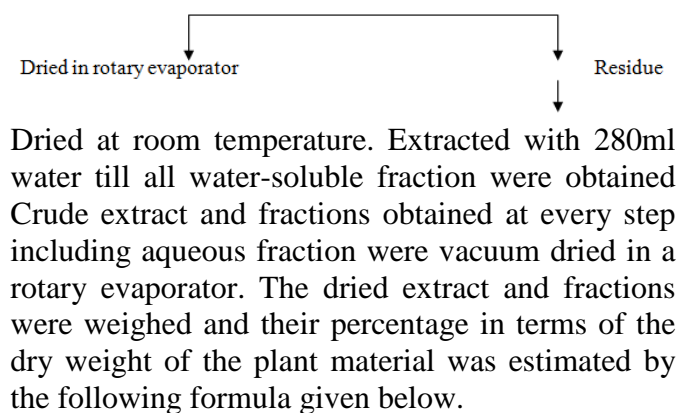
Dried at room temperature. Extracted with 280ml acetone till all acetone soluble fractions were obtained.



Dried in rotary evaporator

Residue

Dried at room temperature. Extracted with 280ml methanol till all alcohol-soluble fraction were obtained



Dried in rotary evaporator

Residue

Dried at room temperature. Extracted with 280ml water till all water-soluble fraction were 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.

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

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 alcohol have been used by several workers for successive extraction of active compounds in plant extracts¹¹⁰⁻¹¹¹. 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 phyto-constituents present in individual fractions.

In vitro and *In vivo* assay of antifungal activity of plant extracts, various elicitors and binders like oil cakes, cow dung, guar gum individually and in combination etc., against *Alternaria solani*.

Antifungal susceptibility of the extract is done by various methods. Broadly these methods are classified into diffusion and dilution. The diffusion tests include agar well diffusion, disk diffusion and bio-autography whereas dilution methods include poison food technique (agar dilution) and macro or micro broth dilution¹¹². Diffusion methods against *Aspergillus* spp. were previously used by¹¹³⁻¹¹⁵. Dilution methods are generally used for antimicrobial screening as well as MIC and MFC determination. MIC is the minimum inhibitory concentration which is the lowest concentration that will inhibit the visible growth of micro organisms after optimum incubation period for the growth. MFC is the minimum fungicidal concentration on which fungal strain is completely killed.

Although diffusion methods are commonly used for the susceptibility testing but the dilution methods are more appropriate¹¹⁶, as in the diffusion methods there is limited diffusion of less polar active compounds in solid media whereas in broth dilution method the compounds in solution easily in contact with the organism¹¹⁷⁻¹¹⁸ used poison food and macro broth dilution method for the determination of antifungal activity, Some workers also reported the significant antifungal properties of the organic substances.

The present work proposes to develop a formulation by combining plant extracts/ plant powder with these traditionally used organic substances for control of early blight of tomato caused by *Alternaria solani*. This type of formulation will provide a cheap and environmentally safe herbal fungicide that can be further used as a commercial herbal bio-control agent. Several plant extracts have shown the antimicrobial activity against fungal pathogens under *in vitro* and *in vivo* conditions¹¹⁹. The effect of neem kernel cake powder in controlling the plant pathogenic fungi has been reported. Use of various oilcakes and fertilizers were also evaluated and the effectiveness of various groups of fungicides for controlling early blight (*Alternaria solani*) as well as their effect on tomato fruit yield, following early blight severity in leaf lets and stems; percentage of leaf drop; incidence of healthy, infected and sun-damaged fruits; yield and the percentages of large, medium and small sized fruits were observed.

Description of Pathogen (*Alternaria solani*):

Blight disease is caused by *Alternaria solani* belongs to the sub-division Deuteromycotina, class Hyphomycetes, family Dematiaceae. Species of the genus are cosmopolitan, surviving both as saprophytes as well as weak parasites. The characteristics feature of the genus is the production of obclavate or beaked, pigmented conidia with relatively thin transverse and longitudinal septa (Muriform). The conidia are porospores produced from simple, dark, septate conidiophores in simple and borne singly¹²⁰.

The pathogen produces distinctive 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 regions¹²¹.

Disease symptoms caused by *Alternaria*: Among the different diseases caused by the genus *Alternaria*, blight disease is one of the most dominant one that causes average yield loss in the range of 32-57%. Symptoms of this disease include presence of irregular, often circular brown to dark brown colour leaf spots on the leaves with concentric lines inside the spots. Often the circular spots coalesce to form large patches resulting in the leaf blight. In several cases, small dark coloured spots are also formed on pods and tender twigs¹²².

Disease cycle: Under free moisture or near-saturated humidity at a wide range of temperatures (8°–32°C), conidia germinate to produce one or more germ tubes. These subsequently penetrate the host epidermal cells directly by means of appressoria or they enter through stomata or wounds by hyphal growth¹²³. Penetration can occur at temperatures between 10°C and 25°C. Host colonization is facilitated by enzymes (cellulases, pectin methyl galacturonase) that degrade the host cell wall and by a number of toxins that kill host cells and enable the pathogen to

derive nutrients from the dead cells¹²⁴. Lesions become visible 2–3 days after infection, and spore production occurs 3–5 days later. This relatively short disease cycle allows for polycyclic infection. The fungus survives between crops as mycelia or conidia in soil, plant debris, and seed. Therefore, the life cycle of *A. solani* includes soil- and seed- as well as air-borne stages, making the pathogen difficult to control by means of rotation and sanitation. The main hosts of *A. solani* are solanaceous crops including tomato, potato, eggplant, and pepper¹²⁵⁻¹²⁶. *Alternaria solani* reproduces asexually by means of conidia with polycyclic life cycle. The life cycle starts with the fungus overwintering in crop residues or wild members of the Solanaceae family. Every part of the plant can be infected and form lesions.

This is especially important when fruits 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 mature¹²⁷. *Alternaria solani* spores are universally present in fields where host plants have been grown.

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 conditions¹²⁸.

Description of Plant (*Eucalyptus globulus*):

Eucalyptus globulus Hook, the economically important genera belongs to family Myrtaceae order Myrtales. It is commonly known as lemon scented tree due to lemon type smell of aromatic substances in leaves and gum. *Eucalyptus* oil has great medicinal values due to its anti-inflammatory, antispasmodic, decongestant and antiseptic prop- In addition, it is also has anti-diabetic activity¹²⁹. Antibacterial and antifungal properties of *Eucalyptus* extract and oil are also reported by some authors¹³⁰⁻¹³³.

Geographical Distribution: *Eucalyptus globulus* Labill belonging to the family Myrtaceae is a fast-growing species native to Australia and widely distributed in southern China, such as Guangdong, Guangxi, Sichuan and Yunnan. The genus name *Eucalyptus* comes from the Greek word *Eucalyptus*, meaning "well-covered," and refers to its flowers that, in bud, are covered with a cup-like membrane¹³⁴. *Eucalyptus globulus* (Labill) also called Tasmanian bluegum, is one of the world's best known eucalyptus trees. It is the "type" species for the genus in California, Spain, Portugal, Chile, and many other locations. One of the first tree species introduced to other countries from Australia, it is now the most extensively planted eucalyptus in the world.

Morphology: *Eucalyptus* is an evergreen tree of 24-40 m in height with tall straight, solid cylindrical woody and white shining stem. Leaf is petiolated exstipulate simple, lanceolated ovate smooth aromatic with oil glands. Inflorescence is umbellate in cluster of three. The flower of eucalyptus is pedicillate, complete actinomorphic and epigynous. Placentation is axile. Seasonality of rainfall is not of critical importance to the species. Bluegum *eucalyptus* is much used for pulpwood, particularly so because its bark, acceptable in most pulping processes, adds greatly to the yield. It is used mostly for bleached products made by sulfate, sulfite, or bisulfate processes. Other uses include the extraction of essential oils from the leaves, honey production from the flowers (that are also good pollen sources), plantings for erosion control,

and roadside plantings to provide a noise and headlight buffer¹³⁵. The wood is heavy and shrinks greatly in drying so that it is unsuitable for lumber.

Traditional Medicinal Uses: The essential oil extracted from the leaves of *Eucalyptus globulus* Labill is known to be a rich source of traditional medicines with a variety of biological activities. It is widely used to treat pulmonary tuberculosis¹³⁶ diabetes¹³⁷⁻¹³⁸, asthma and also used as disinfectant¹³⁹⁻¹⁴⁰, antioxidant agent and antiseptic agent especially in the treatment of upper respiratory tract infections and certain skin diseases. Ointments containing eucalyptus oil have been used in traditional aboriginal medicines to heal wounds and fungal infections. The essential oil extracted from the leaves of *Eucalyptus globulus* Labill is known to be a rich source of traditional medicines with a variety of biological activities. It is widely used to treat pulmonary tuberculosis diabetes, asthma and also used as disinfectant antioxidant agent¹⁴¹ and antiseptic agent especially in the treatment of upper respiratory tract infections and certain skin diseases.

Eucalyptus leaves and inflorescence have anti-inflammatory, antispasmodic, decongestant and antiseptic properties¹⁴². Due to its medicinal properties, it is often used in preparation of medicinal drugs for rashes, inhalers, liniments, creams and mouthwashes. *Eucalyptus* oil is generally used as a stimulant and as an antiseptic gargle. It helps in treating a number of respiratory problems like cold, cough, running nose, asthma and bronchitis. In addition, it is also has anti-diabetic activity¹⁴³. Antibacterial and antifungal properties of *Eucalyptus* extract and oil are also reported by some authors¹⁴⁴⁻¹⁴⁷.

Antimicrobial activities of *Eucalyptus globules*:

Eucalyptus leaves contain the essential oil which are used for medicinal purpose. Due to isoprenoid accumulation eucalyptus plant has great antifungal, antibacterial, antitumor, antiviral, antimalarial and antioxidant properties (Kumar and Laxmidhar, 2011). *Eucalyptus* essential oil is also used as natural pesticide.¹⁴⁸ The chemical compositions of the leaf oils of various *Eucalyptus* species had been reported¹⁴⁹. Few studies have been reported on the chemical constituents of the essential oil obtained from the leaves of *Eucalyptus globulus* Labill¹⁵⁰.

Description of Crop Plant (Tomato): 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 crops¹⁵¹. Tomato is very important solanaceous crops in India either for local consumption and export. Tomato is considered as one of the highest nutritional crops because of its high contents of Vitamin A, C, potassium, minerals and fibers.¹⁵²

It is susceptible to infection by the blight disease caused by *Alternaria solani* during fruiting period¹⁵³⁻¹⁵⁴ which causes great reduction in the quantity and quality of fruit yield. It is well known that tomato fruits are mostly consumed freshly, thereby spraying fungicides just before harvesting resulted in high fungicide residue in the fruits, which cause great hazard to the human health¹⁵⁵. Therefore, the object of this study is to test the efficiency of herbal formulations in reducing the infection of early blight caused by *Alternaria solani* in tomato.

Physiology of tomato: Tomato (*Lycopersicon esculentum*) belongs to the genus *Lycopersicon* under Solanaceae family. Tomato is a herbaceous sprawling plant growing to 1-3 m in height with weak woody stem. The flowers are yellow in colour and the fruits of cultivated varieties vary in size from cherry tomatoes, about 1–2 cm in size to beefsteak tomatoes, about 10 cm or more in diameter. Most cultivars produce red fruits when ripe. Tomato is a native to Peruvian and Mexican region. It is one of the most versatile vegetable with wide usage in Indian culinary tradition. Tomatoes are used for soup, salad, pickles, ketchup, puree, sauces and in many other ways it is also used as a salad vegetable. Tomato has very few competitors in the value addition chain of processing.

They contain the carotene lycopene, one of the most powerful natural antioxidants. In some studies, lycopene, especially in cooked tomatoes, has been found to help prevent prostate cancer, but other research contradicts this claim. Lycopene has also been shown to improve the skin's ability to protect against harmful UV rays. The tomato crop

is cultivated during winter and summer seasons. The crop cannot withstand severe frost.

It grows well under an average monthly temperature range of 21 -23 °C but commercially it may be grown at temperatures ranging from 18 °C to 27 °C. Temperature and light intensity affect the fruit-set, pigmentation and nutritive value of the fruits

The crop will be ready for harvest in about 2- 3 months after planting. The harvesting of the tomatoes is done as per the requirement of the market and in a typical season 8 to 10 harvesting is done to feed the market as per its requirement.

Tomato is mainly grown as Rabi crop in the plains of India. However in the hilly region it can also be grown as a summer and rainy season crop.

CONCLUSION: Review of literature reports that plant extracts have been mostly screened for activity against human pathogenic bacteria and fungi. Compared to this, there are very few reports regarding inhibitory activity against plant pathogenic fungi. Although there are several reports of antifungal activity of plant products yet not much work has done to develop herbal bio-control agents or formulations using plant products or extracts. Especially, plant extracts have not been used to significant extent in development of fungicides to protect the economically important crop like tomato. *Eucalyptus globulus* extract has been screened for antifungal activity against human pathogenic fungi and bacteria but very few reports are available regarding plant pathogenic fungi especially early blight caused by *Alternaria solani*. Antifungal activity of organic substances has been reported by some workers but no study till date has been reported on preparation of herbal formulation by combining plant powder, extracts and organic substances.

Therefore, present study will investigate the protective action of herbal formulation prepared by combining *Eucalyptus globulus* leaf extract/ dry powder suitable binders such as cow dung, guar gum, gum acacia etc. and elicitors (organic substances such as neem oil cake, mustard oil cake, coconut oil cake, pongamia oil cake etc.) to control early blight disease in tomato.

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