



Evaluating the Antifungal Activity of Some Traditional Medicinal Plant Extracts against *Alternaria solani* (Tomato Early Blight Pathogen) in Ethiopia

Meseret Tadelo¹, Tamirat Wato², Tilahun Negash²

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ABSTRACT

Background: Tomato (*Lycopersicon esculentum* Mill.) belongs to the family Solanaceae. In Ethiopia, control of early blight is largely dependent on fungicidal application. There is a research need to identify effective botanical extracts to control *Alternaria solani* that cause early blight of tomato and for evaluation of plant extracts through different solvents on the target pathogen.

Methods: *In vitro* experiment was conducted to evaluate the effectiveness of crude extracts of 16 selected medicinal plants against *Alternaria solani*. Thus, crude extracts were extracted from medicinal plants with different solvents (methanol, ethanol and petroleum at (25%, 50% and 100%) concentrations. The *Alternaria solani* was isolated from infected tomato leaves showing early blight symptoms. Evaluation of plant extracts was carried out against *Alternaria solani* using food poisoned technique on PDA.

Result: Results showed that most of the methanolic extract plants were showed significant inhibition of the mycelial growth as compared to ethanolic and petroleum ether extracts. A higher rate of mycelial reduction was recorded by ethanol extracts of *Allium sativum* at all concentrations (100%) followed by methanol extracts of *Allium sativum* at 25%, 50%, 100% concentration (90.02%, 97.01%, 100 % respectively). The effectiveness of extracts against *Alternaria solani* depends on use at the higher concentrations and various solvents. For crude extracts that have shown higher inhibitory effects against *Alternaria solani* in vitro conditions, actual chemical compounds should be identified. Furthermore, it is also important to evaluate these plants on other microbes, study to test in vivo and to assess their real potential field condition wherever early blight is an important disease of tomato.

Key words: *Alternaria solani*, Disease intensity, *In vitro* test, *Lycopersicon esculentum*, Medicinal plants.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) belongs to the family Solanaceae (Isah *et al.* 2014). In Ethiopia tomato is one of the most important and widely grown vegetable crops, both during the rainy and dry seasons for its fruit by smallholder farmers, commercial state and private farms (Ambecha *et al.* 2012; Emana *et al.* 2014; Ketema *et al.* 2015; Tsehay *et al.* 2020). The total production of tomato in Ethiopia has shown a marked increase since it became the most profitable crop providing a higher income to small-scale farmers compared to other vegetable crops (Lemma 2002; Desta and Yesuf 2015). The national average yield of tomatoes in Ethiopia is very low which is around 9.3 tons/ha and less than 50% of the current world average yield of about 27 tons/ha (CSA 2017).

Tomato production and productivity are very low due to many biotic and abiotic factors. Among biotic factors, early blight caused by *Alternaria solani* (Ellis and Martin) is the important foliage and fruit disease causing yield losses of about 79% in the world and 53 % in Ethiopia (Chaerani and Voorrips 2006; Somappa *et al.* 2013; Desta and Yesuf 2015). *Alternaria solani* is a soil-inhabiting air-borne pathogen responsible for leaf blight, stem collar, fruit rot and can damage during all stages of plant development disseminated by fungal spores (Abada *et al.* 2008; Creswell 2014; Desta and Yesuf 2015).

¹Department of Plant Science, College of Agriculture and Environmental Sciences, Debark University, P.O. Box 90, Debark, N/Gondar, Ethiopia.

²Department of Plant Science, College of Agriculture and Natural Resource, Bonga University, P.O. Box 334, Bonga, Ethiopia.

Corresponding Author: Tilahun Negash, Department of Plant Science, College of Agriculture and Natural Resource, Bonga University, P.O. Box 334, Bonga, Ethiopia. Email: tilatanejash211@gmail.com

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In Ethiopia, control of early blight is largely dependent on fungicidal application. Because of the polycyclic nature of the disease, several applications of fungicides are required to offer an adequate protection of the tomatoes from early blight attacks.

However, the application of fungicide is generally associated with issues of environmental pollution, high production costs (Abuley *et al.* 2018) and the risk of fungicide resistance that may arise due to excessive application of fungicides (Abdel-fattah *et al.* 2011; Alwathnani and Perveen 2012).

The use of resistant cultivars in the control of early blight offers an economical and environmentally friendly alternative consistent with the objective of integrated pest management (Abuley *et al.* 2018). Unfortunately, no tomato cultivar has been reported to be completely resistant to early blight in Ethiopia (Holley *et al.* 1983; Ainhwa 2016). Because of the continued cultivation of tomatoes in major growing areas of Ethiopia, early blight is a constant threat throughout the year. Recent efforts have focused on developing environmentally safe, long-lasting and effective biocontrol methods for the management of plant diseases (Meena *et al.* 2021). So, plant products (botanicals) are used as an alternative to synthetic chemicals as they are cheap, non-photo toxic, systemic, environmentally friendly and necessary to minimize these diseases.

They are easily biodegradable, hence considered safe for the environment and human health compared to synthetic fungicides and cheaper (Koul 2008; Nerio *et al.* 2010 and Gurjar *et al.* 2012). Plant extracts are important sources of antimicrobial products for the available new chemotherapeutic agent of the control plant diseases (Mousavi *et al.* 2009; Safaray *et al.* 2009). Several plant products have shown antimicrobial activity against fungal pathogens under *in vitro* and *in vivo* conditions (Abo-Elyousr and Nashwa 2012; Bahraminejad *et al.* 2015; Ahmad *et al.* 2017). Among these, plant extract has proved effective in inhibiting the growth and reproduction of fungal plant diseases like *Alternaria solani*, *Parthenium hysterophorus*, *Vernonia amygdalina*, *Eucalyptus camaldulensis*, *Nerium oleander*, *Lantana camara* and *Ocimum sanctum* (Singh *et al.* 2014); *Pongamia piñata*, *Aegle marmelos*, *Azadirachta indica*, *Brassica campestris*, *Piper nigrum*, *Euphorbia tirucalli*, *Vitex negundu*, *Ageratum conyzoides*, *Tagetes patula* and *Zigiphus jujube* (Pattnaik *et al.* 2012); *Ocimum tenuiflorum*, *Azadirachta indica*, *Pongamia pinnata*, *Datura stramonium*, *Withania somnifera*, *Calotropis gigantia*, *Allium crispum*, *Lepidium sativum* and *Mentha requienii* (Sahu *et al.* 2014); garlic extracts (Abo-Elyousr and Nashwa 2012); *Eucalyptus globules* (Patel and Jasrai 2015) were shown to have inhibitory activity against different plant pathogens.

There is no scientific study conducted and no efforts have been made to utilize locally available botanicals for the control of *Alternaria solani* causative agents of early blight of tomato in Ethiopia. There is a research need to identify effective botanical extracts to control *Alternaria solani* that cause early blight of tomato and for evaluation of plant extracts through different solvents on the target pathogen. Therefore, the study was undertaken with the following specific objectives.

- To evaluate the efficacy of different plant extracts against *Alternaria solani* *in vitro* conditions.
- To determine the yield of the crude extracts of antifungal compounds from medicinal plants by using different solvents.

MATERIALS AND METHODS

An experiment was conducted Haramaya University, Ethiopia in the Laboratory of Plant Pathology for evaluating

the antifungal activity of traditional medicinal plant extracts with different solvents at three concentration levels.

Collection of plant materials

Samples of fresh plant materials with potential antifungal activity were collected from the South-Eastern part of Ethiopia based on previous reports on their antifungal activity and traditional knowledge in the societies (Table 1). These plant materials have been published by many reports on the antimicrobial activity of plant extracts. Collected plant materials were identified at Addis Ababa University National Herbarium, Ethiopia. The collected plant materials were washed with tap water and air-dried under shade (Baris *et al.* 2006) to a constant weight. The dried plant was chopped and ground to a fine powder using a coffee grinder and stored in a sealed plastic bag at room temperature until needed.

Treatment and experimental design

The experiment was arranged in a factorial randomized complete design (CRD) combination of 16 plant material x 3 solvents (ethanol, methanol and petroleum) with three concentrations (25%, 50% and 100%) in three replications positive and negative control was including.

Isolation and identification of the pathogen

Diseased tomato leaves showing typical symptoms of early blight were collected. The infected leaf parts were cut into small pieces and surface sterilized with 0.1 percent mercuric chloride (HgCl₂) solution for 30 seconds and washed three times with distilled water, then cultured on Potato Dextrose Agar (PDA) and incubated at 25°C for 3 days. The cultured fungus was observed under microscopy and identified based on morphological features of the colony, spore characteristics and referring the relevant literature. Pure culture of the pathogen was maintained on PDA Petri plates at 4 °C. Pathogenicity test was done by spray method under greenhouse conditions (Simmmons 2007).

Preparation of plant extracts

Fifty grams of each powdered material were macerated separately in 250 ml of (ethanol, methanol and petroleum ether) contained in a sterile conical flask covered with cotton wool plug and wrapped with aluminum foil (Kulkarni *et al.* 2011). The extraction was done using an electrical flask shaker at maximum speed (300rpm) with continuous shaking for 48 hrs. The extracts were taken out by filtering using a clean muslin cloth (Wokocha and Okereke 2005) followed by filtration using a Whatman number 1 filter paper to avoid a fibrous portion of the plant completely (Bekele *et al.* 2015). The debris was discarded while the filtrate was transferred to an evaporating dish and kept in a dry oven at 30°C-40°C until the extract materials were concentrated. The extracts were then stored in air-tight bottles at 4°C in a refrigerator until use in bioassay (Naduagu *et al.* 2008). Finally, the gram yield of the dried residue of each plant extracts was calculated using the formula (Kigundu *et al.* 2009).

$$\text{Percentage yield (\%)} = \frac{\text{Weight of extract}}{\text{Weight of plant material}} \times 100$$

In vitro* evaluation of efficacy plant extracts against *Alternaria solani

Different concentrations of the extracts were prepared by diluting the crude extracts with Dimethyl Sulfoxide (DMSO) to get 25%, 50% and 100% concentration. One ml of the different concentrations from each plant extract (*i.e.*, stock solution) was mixed thoroughly in melted serialized PDA (18 ml) medium in a serialized conical flask, just before pouring in sterilized 9 cm diameter Petri plates. Then after solidification, 6 mm diameter of actively growing mycelium disc from seven days old culture of the test pathogen by the help of sterilized cork borer was placed at the center of medium and three replications were maintained for each treatment. Plates containing PDA medium with fungicide tilt (25%, 50% and 100%) served as a positive control and plates with medium added with distilled water served as negative controls. The plates were then sealed with Parafilm and incubated at 25°C; three replicates were maintained for each treatment. The mycelia growth was recorded six days after inoculation. The experiments were repeated twice and the mean of readings was taken for calculations. The inhibitory activity of each treatment was expressed as the percent growth inhibition as compared to the negative control (0%) using the following (Pandey *et al.* 1982).

$$\text{Growth inhibition (\%)} = \frac{\text{DC} - \text{DT}}{\text{DC}} \times 100$$

Where

DC = Diameter mycelium growth of fungus (mm) in control,
DT = Diameter mycelium growth of fungus (mm) in treatment

Data analysis

The data collected were subjected to analysis of variance (ANOVA) using Statistical Analysis System (SAS) version

9.2 Software. The mean comparison of different treatments was performed using Tukey's post-test at $P \leq 0.05$ level of significance.

RESULTS AND DISCUSSION

Percentage yield of plant extract

The extracts obtained by using three solvents were compared for the efficiency of eluting solvents by calculating the percentage yield of extracted materials. In methanol extract, the maximum yield was from *Eucalyptus globules* (44.92%) followed by *Vernonia amygdalina* (40.52%), *Ricinus communis* (36.76%), *Solanum nigrum* (29%) and *Solanum incanum* (29%), However, *Allium cepa* (9.36%) and *Allium sativum* (9.36%) gave the least amount of yield as compared to other extracts. In ethanol extract, the maximum yield was due to *Eucalyptus globules* (36.1%) followed by *Hagenia abyssinica* (28.28%), *Ricinus communis* (27.40%), *Vernonia amygdalina* (26.76%) and *Zehneria scabra* (22.44%). The least amount of yield plant extract was recorded from *Rumex nepalensis* (9.24%). Using petroleum as solvent of extraction, the highest yield was obtained from *Eucalyptus globules* (20.38%), *Justicia schim* (15.46%), *Coriandrum sativum* (8.86%), *Hagenia abyssinica* (7.32%) and *Rumex nepalensis* (7.06%). The lowest percentage yields recorded were the petroleum extract of the *Melia azedarach* (3.64%) (Table 2).

Efficacy test of the plant extracts on *Alternaria solani* disease

The effect of interaction among sixteen medicinal plant extracts was studied by using three solvents at three levels of concentrations in the laboratory (Fig 1-3) against *Alternaria solani* showed significant ($P < 0.001$) variation on the percent of growth inhibition (Table 3). In this investigation, the inhibitory activity of the methanol, ethanol and petroleum ether extracts at the three concentrations (25%, 50% and

Table 1: Botanicals and their parts used in the experiment.

Scientific name	Common name	Family	Local name	Plant parts
<i>Allium cepa</i>	Onion	Alliaceae	Key shinkurt	Bulb
<i>Allium sativum</i>	Garlic	Amaryllidaceae	Nech shinkurt	Bulb
<i>Coriandrum sativum</i>	Coriander	Apiaceae	Dimbilal	Fruit
<i>Eucalyptus globules</i>	Eucalyptus	Alliaceae	Bahirzaf	Leaf
<i>Justicia schim Periana</i>		Acanthaceae	Smiza	Leaf
<i>Hagenia abyssinica</i>	Hagenia	Rosaceae	Kosso	Leaves
<i>Malva parviflora</i>		Malvaceae	Lenkuata	Leaf
<i>Melia azedarach</i>	Chinaberry	Meliaceae	Neem	Leaf
<i>Ricinus communis</i>	Castor	Euphorbiaceae	Yegulo zeyit	Leaf
<i>Rumex abyssinicus</i>	Spinach Rhubarb	Polygonaceae	Mokemoko	Leaf
<i>Rumex nepalensis</i>	Sorrel	Polygonaceae	Tult	Leaf
<i>Rumex nervosus</i>		Polygonaceae	Ambacho	Leaf
<i>Solanum incanum</i>	Sodom apple	Solanaceae	Embuay	Leaf
<i>Solanum nigrum</i>	Black nightshade	Solanaceae	Alumina	Leaf
<i>Vernonia amygdalina</i>	Bitter leaf	Asteraceae	Girawa	Leaf
<i>Zehneria scabra</i>		Cucurbitaceae	Aregeressa	Leaf

Table 2: The percentage yield of plant extracts using methanol, ethanol and petroleum.

Medicinal plant species	Plant part used	Weight of sample (g)	The dry weight of the extract			Percentage yields (%) of extracts		
			Methanol	Ethanol	Petroleum	Methanol	Ethanol	Petroleum
<i>Melia azedarach</i>	Leaf	50	16.72	12.75	3.64	16.72%	12.75%	3.64%
<i>Zehneria scabra</i>	Leaf	50	27.00	22.44	4.60	27.00%	22.44%	4.60%
<i>Rumex nepalensis</i>	Leaf	50	13.24	9.24	7.06	13.24%	9.24%	7.06%
<i>Solanum incanum</i>	Leaf	50	29.00	13.34	5.40	29.00%	13.34%	5.40%
<i>Coriandrum sativum</i>	Seed	50	16.94	16.18	8.86	16.94%	16.18%	8.86%
<i>Rumex abyssinicus</i>	Leaf	50	16.14	13.56	5.54	16.14%	13.56%	5.54%
<i>Solanum nigrum</i>	Leaf	50	29.00	13.34	5.40	29.00%	13.34%	5.40%
<i>Justicia schim</i>	Leaf	50	20.98	13.68	15.46	20.98%	13.68%	15.46%
<i>Allium sativum</i>	Bulb	50	9.36	21.62	5.80	9.36%	21.62%	5.80%
<i>Malva parviflora</i>	Leaf	50	20.22	15.50	4.14	20.22%	15.50%	4.14%
<i>Hagenia abyssinica</i>	Leaf	50	15.90	28.28	7.32	15.90%	28.28%	7.32%
<i>Eucalyptus globules</i>	Leaf	50	44.92	36.10	20.38	44.92%	36.10%	20.38%
<i>Vernonia amygdalina</i>	Leaf	50	40.52	26.76	6.36	40.52%	26.76%	6.36%
<i>Allium cepa</i>	Bulb	50	9.36	21.62	5.80	9.36%	21.62%	5.80%
<i>Rumex nervosus</i>	Leaf	50	27.64	21.56	5.60	27.64%	21.56%	5.60%
<i>Ricinus communis</i>	Leaf	50	36.76	27.40	6.46	36.76%	27.40%	6.46%

Table 3: ANOVA table for mycelia growth inhibition from plates treated with different extracts of respective plant species with solvents and concentrations at different days after inoculation.

Sources	Df	Mean squares				
		6 DAI	7 DAI	8 DAI	9 DAI	10 DAI
Bot	15	16210.23***	917.38***	1450.16***	1167.91***	951.98***
Sol	2	27544.89***	11084.88***	13772.44***	18726.13***	17108.16***
Con	2	6376.96***	3358.85***	3188.48***	3036.90***	3249.87***
Bot*Sol	30	58156.36***	1870.89***	1938.54***	2128.91***	1988.4***
Sol*Con	4	111.89***	69.2***	27.97***	15.09***	9.58**
Bot*Con	30	1195.53***	49.32***	39.85***	53.44***	35.74***
Bot*Sol*Con	60	2221.32***	52.01***	39.85***	32.17***	27.69***
Error		3.64	7.12	3.64	2.64	1.85
CV		3.19	4.33	3.19	2.81	2.33
R2		99.14	98.17	99.14	99.45	99.57

DAI=Days after inoculation, DF = degree of freedom, Bot= botanical, Sol = solvent, Con = concentration, CV = Coefficient of variation, ***Very highly significant, (P<0.001) **highly significant, R2 = coefficient of determination.

100%) showed significant different (P<0.05) variations in the degree of inhibitory activity against *Alternaria solani* as compared to the negative control. However, none of the treatments except ethanol extract of *Allium sativum* (25%, 50% and with methanol extract (100%) was unequally inhibited the mycelial growth of *Alternaria solani* to that of positive control (tilt). The extract of *Allium sativum* with ethanol at all concentrations and with methanol extract at (100%) was completely inhibited against radial growth mycelium as compared to a positive control (Table 4).

In the present finding, methanol extracts were showed the highest percentage growth inhibition zone at 100% was recorded from *Allium sativum* (100%) followed by *Allium cepa* (82.39%), *Vernonia amygdalina* (81.44%), *Ricinus communis* (81.16%) and *Eucalyptus globules* (80.68%), while at the lowest percentage growth inhibition zone was recorded from methanol extracts of *Rumex nepalensis*

(64.96%). At 50% concentration, *Allium sativum*, *Vernonia amygdalina* and *Eucalyptus globules* resulted in a higher rate of reduction of mycelial growth of *A.solani* in the percentage of (97.01%), (80.11%) and (79.55%) respectively be an effective zone of inhibition at as compared to the other treatments. *Allium sativum* (90.02), *Vernonia amygdalina* (78.98%), *Hagenia abyssinica* (75.38%) and *Allium cepa* (75%) indicated the highest inhibition zone among methanol extracts at 25% of concentration level. Although the difference between *Allium cepa* and *Hagenia abyssinica* was not significantly varied (P>0.05). Similarly, the lowest percentage growth inhibition zone was recorded from methanol extracts *Rumex nepalensis* (49.43%) at a concentration level of 25% (Table 4).

From our result, the antimicrobial activity of the ethanol extracts at 100% concentration was showed the highest percent mycelial growth inhibition was recorded by *Allium*

sativum (100%), followed by *Ricinus communis* (71.59%), *Rumex nervosus* (59.85%) and *Allium cepa* (57.20%). The lowest percentage growth inhibition zone was recorded by *Solanum incanum* (37.69%) followed by *Rumex abyssinicus* (39.39%), but it was significantly ($P < 0.05$). The ethanol extract at 50% concentration produced the highest percent growth inhibition by *Allium sativum* (100%), *Eucalyptus globules* (81.82%) and *Rumex nervosus* (55.87%). The lowest percentage growth inhibition zone was recorded from *Coriander sativum* (34.66%) and *Solanum incanum* (35.04%) at a concentration of 50%. At 25% concentration, the highest mycelial inhibition of *Alternaria solani* was recorded from the ethanol extracts of *Allium sativum* (100%), *Eucalyptus globules* (79.73%) and *Allium cepa* (49.43%) against *Alternaria solani*. The ethanol extract showed less effective inhibition zone *Coriander sativum* (28.79%) and *Rumex nepalensis* (28.41%) at a concentration level of 25% (Table 4).

The petroleum ether extracts at 100% concentration showed significantly varied effects on mycelial growth inhibition of *A. solani* (Fig 3). Among the various plant extracts, *Solanum incanum* was recorded the highest percentage of growth inhibition (86.82%) followed by *Rumex nervosus* (83.52%), *Malva parviflora* (81.82%) and *Melia azedarach* (80.68%) at 100% concentration while the lowest percentage mycelial growth inhibition zone was observed from petroleum ether extracts *Eucalyptus globules* (40.15%), *Allium sativum* (44.67%) and *Hagenia abyssinica* (50.95%) at a concentration of 100%. At 50% concentration, the

petroleum ether extracts of *Rumex nervosus* (81.44%), *Malva parviflora* (79.73%) and *Melia azedarach* (78.41%) displayed a higher rate of mycelial inhibition over all the other extracts. The least inhibition of mycelial growth of *A. solani* was recorded in *Eucalyptus globules* (34.47%) followed by *Hagenia abyssinica* (44.13) at 50% percent concentration. The petroleum ether extracts of *Malva parviflora* (76.70%), *Rumex nervosus* (76.14%) and *Melia azedarach* (75.76%) were found to be best in inhibiting the mycelial growth of *A. solani* at 25% concentration. The lowest percentage growth inhibition zone was recorded from extracts *Eucalyptus globules* (27.65%) followed by *Hagenia abyssinica* (36.74%) at a concentration of 25% (Table 4).

For each medicinal plant extract, the highest rate of radial mycelial growth inhibition was recorded in a higher concentration level. Overall, the highest rate mycelial inhibition was recorded by ethanol extract of *Allium sativum*, followed by methanol extract with *Allium sativum* and petroleum extract of *Solanum incanum* (Table 2).

Tomato (*Solanum lycopersicum* Mill.) belongs to the family *Solanaceae* and is the world's largest important vegetable crop after potato. Tomato is known as a productive as well as protective food. Tomato production and productivity are very low due to a lack of proper disease, insect pest management and other agronomic practices (Fig 4).

Various results of yield extracts were obtained from different plant materials with different solvents. Most methanol extracts give maximum yields followed by ethanol,

Table 4: Effect of sixteen medicinal plant extracts were studied by using three solvents at three levels of concentrations.

	Per cent inhibition of mycelia growth (%) 10 days after inoculation								
	Methanol extracts			Ethanol extracts			Petroleum ether extracts		
	25%	50%	100%	25%	50%	100%	25%	50%	100%
<i>Zehneria scabra</i>	68.75 ^{GFE}	71.4 ^{FEGD}	75.76 ^{FE}	30.87 ^{IJ}	37.12 ^{HGI}	41.48 ^{ILKJ}	52.84 ^{DC}	57.58 ^C	59.85 ^C
<i>Rumex nepalensis</i>	49.43 ^{GH}	53.98 ^{HG}	64.96 ^{FHG}	28.41 ^J	39.39 ^G	45.46 ^{IH}	45.1 ^{HG}	53.79 ^{DE}	57 ^{DFCE}
<i>Melia azedarach</i>	71.59 ^{DGE}	77.7 ^{CBD}	78.41 ^{CED}	32.95 ^{IH}	39.02 ^G	43.18 ^{LKJ}	75.76 ^B	78.41 ^B	80.68 ^B
<i>Solanum incanum</i>	66.01 ^{GF}	72.92 ^{FCED}	73.86 ^{FHG}	33.71 ^H	35.04 ^I	37.69 ^L	49.1 ^{FE}	53.79 ^{DE}	86.82 ^{DFCE}
<i>Coriander sativum</i>	68.18 ^{GFE}	69.13 ^{FG}	73.67 ^{FHG}	28.79 ^J	34.66 ^{HI}	44.89 ^{IHJ}	46.78 ^{FG}	51.14 ^{FE}	54.55 ^{FGE}
<i>Rumex abyssinicus</i>	54.17 ^{IJ}	69.51 ^{CEBD}	75.57 ^{FEG}	32.39 ^{IH}	37.50 ^{HGI}	39.39 ^{LK}	54.55 ^C	56.82 ^{DC}	57.95 ^{DCE}
<i>Solanum nigrum</i>	73.3 ^{DCE}	74.05 ^{GEFD}	76.33 ^{FED}	38.83 ^{GF}	45.664 ^{FE}	54.55 ^{EF}	50.76 ^{DE}	55.11 ^{DC}	56.82 ^{DFCE}
<i>Justicia schim</i>	57.56 ^I	62.12 ^H	71.78 ^{HG}	36.74 ^G	38.83 ^{HG}	48.11 ^{GH}	45.83 ^{HG}	47.92 ^{FGH}	53.98 ^{FG}
<i>Allium sativum</i>	90 ^B	97 ^A	100 ^A	100 ^A	100 ^A	100 ^A	42.61 ^H	44.70 ^{IH}	44.67 ^H
<i>Malva parviflora</i>	58.9 ^{IH}	60.99 ^H	71.59 ^H	40.91 ^F	43.56 ^F	48.86 ^{GH}	76.70 ^B	79.73 ^B	81.82 ^B
<i>Hagenia abyssinica</i>	75.38 ^{DC}	78.6 ^{CB}	79.92 ^{CBD}	37.31 ^G	39.21 ^G	40.91 ^{LKJ}	36.74 ^I	44.13 ^I	50.95 ^I
<i>Eucalyptus globules</i>	72.73 ^{DE}	79.55 ^B	80.68 ^{CB}	79.73 ^B	81.82 ^B	83.14 ^B	27.65 ^J	34.47 ^J	40.15 ^I
<i>Vernonia amygdalina</i>	78.98 ^C	80.11 ^B	81.44 ^{CB}	46.59 ^D	48.86 ^{DE}	50.76 ^{GF}	42.80 ^H	47.54 ^{GH}	57 ^{DFCE}
<i>Allium cepa</i>	75 ^{DC}	79.17 ^{CB}	82.39 ^B	49.43 ^C	52.27 ^{DC}	57.20 ^{ED}	47.35 ^{FEG}	54.55 ^{DC}	59.09 ^{DC}
<i>Rumex nervosus</i>	63.83 ^I	66.29 ^I	73.49 ^I	43.75 ^E	55.87 ^C	59.85 ^D	76.14 ^B	81.44 ^B	83.52 ^B
<i>Ricinus communis</i>	55.68 ^I	75.76 ^{CEBD}	81.16 ^{CB}	48.30 ^{DC}	50.39 ^D	71.59 ^C	44.70 ^{HG}	48.86 ^H	56.1 ^{FG}
NC	0 ^K	0 ^J	0 ^J	0 ^K	0 ^J	0 ^M	0 ^K	0 ^K	0 ^J
PC	100 ^A	100 ^A	100 ^A	100 ^A	100 ^A	100 ^A	100 ^A	100 ^A	100 ^A
Tukey's (0.05)	2.27	3.11	2.19	1.4	2.1	2.2	1.93	1.8	1.99
CV (%)	2.15	2.75	1.8	1.9	2.5	2.5	2.28	1.97	2.04

NC: Negative control; PC: Positive control; CV: Coefficient of variability.

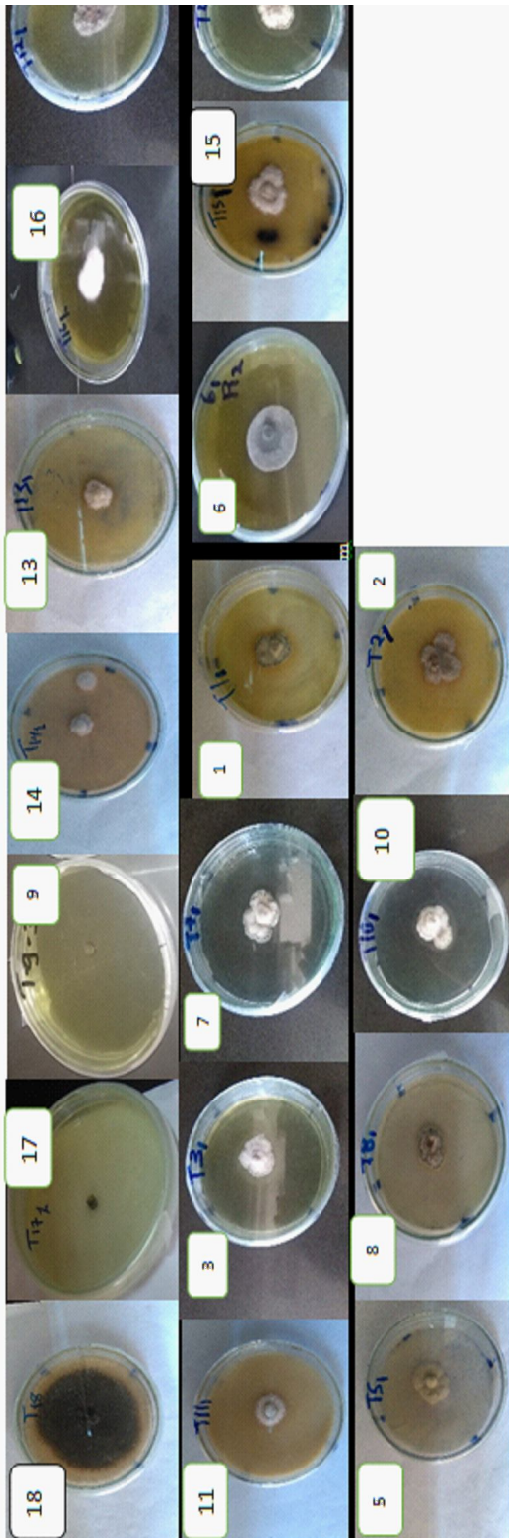


Fig 1: Growth 10 days of *Alternaria solani* on PDA medium with sixteen plant extracts at 100% concentrations by methanol extract.
Allium sativum (9), *Zehneria scabra* (1), *Rumex nervosus* (15), *Vernonia amygdalina* (13), *Rumex abyssinicus* (6), *Solanum nigrum* (7), *Solanum incanum* (4), *Rumex nepalensis* (2), *Ricinus communis* (16), *Melia azedarach* (3), *Malva parviflora* (10), *Eucalyptus globules* (12), *Coriandrum sativum* (5), *Allium cepa* (14), *Hagenia abyssinica* (11).



Fig 2: Growth 10 days of *Alternaria solani* on PDA medium with sixteen plant extracts at 100% concentrations by Ethanol extract.
Allium sativum (9), *Zehneria scabra* (1), *Rumex nervosus* (15), *Vernonia amygdalina* (13), *Rumex abyssinicus* (6), *Solanum nigrum* (7), *Solanum incanum* (4), *Rumex nepalensis* (2), *Ricinus communis* (16), *Melia azedarach* (3), *Malva parviflora* (10), *Eucalyptus globules* (12), *Coriandrum sativum* (5), *Allium cepa* (14), *Hagenia abyssinica* (11).

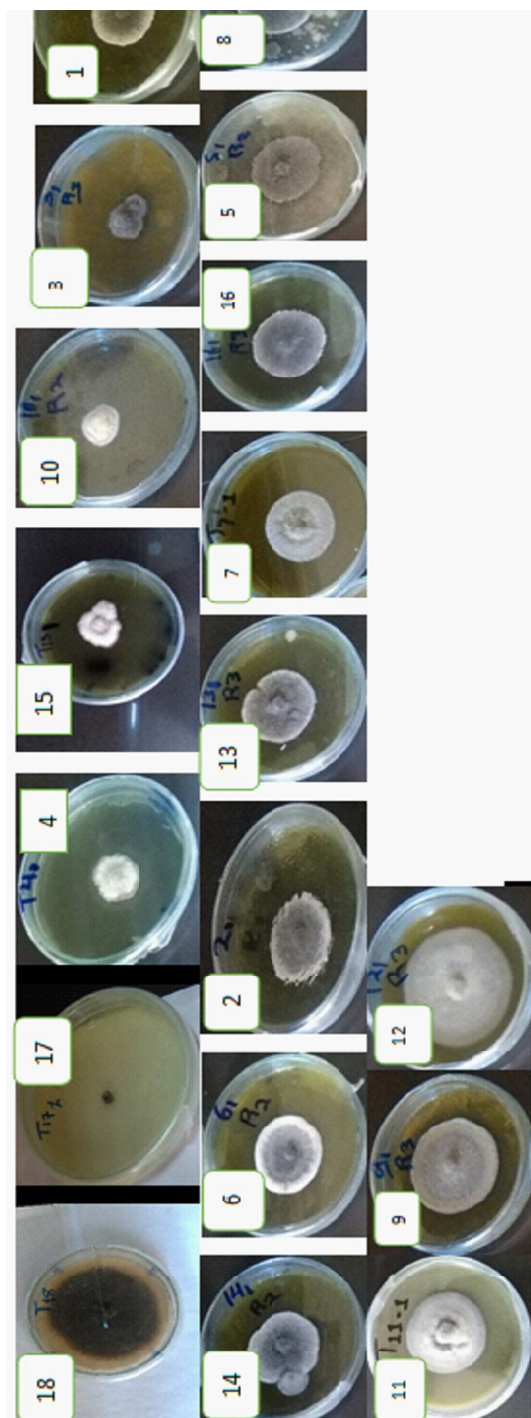


Fig 3: Growth 10 days of *Alternaria solani* on PDA medium with sixteen plant extracts at 100% concentrations by methanol extract. *Allium sativum* (9), *Zehneria scabra* (1), *Rumex nervosus* (15), *Vernonia amygdalina* (13), *Rumex abyssinicus* (6), *Solanum incanum* (4), *Rumex nepalensis* (2), *Ricinus communis* (16), *Melia azedarach* (3), *Malva parviflora* (10), *Eucalyptus globules* (12), *Coriandrum sativum* (5), *Allium cepa* (14), *Hagenia abyssinica* (11).

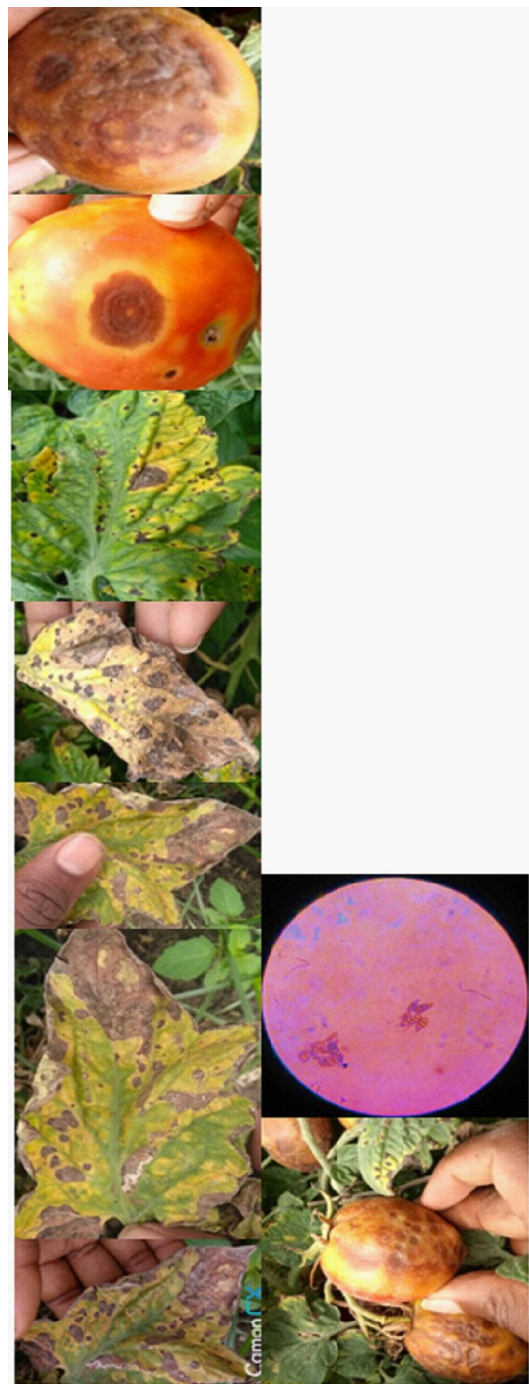


Fig 4: Symptom early blight of tomato on leaf and fruit and conidial of *Alternaria solani*.

while the minimum amount was obtained from petroleum ether extract. The percentage yield of the methanol extracts of *Eucalyptus globules* was obtained maximum yield followed by *Vernonia amygdalina* by three different solvents the minimum yield was recorded from petroleum ether extract. The previous work also studies (Djelloul *et al.* 2017) recorded that extracts of *Eucalyptus globules* with petroleum ether solvents were obtained maximum yield than *Rosmarinus officinalis* plant. The maximum gram yield of *Hagenia abyssinica* extracted was found to be in the order of ethanol, methanol and petroleum ether. However, opposite results were reported by Tesfaye *et al.* (2016) who recorded maximum yield found to be in the order of methanol, petroleum ether and ethanol.

The maximum amount of gram yield was obtained extracted increased from non-polar to polar solvents. The present study correlated with the previous studies, methanol extracts are higher solubility of extractable bioactive components to remaining used the other solvents (Dhawan and Gupta 2017). Petroleum ether is non-polar while methanol and ethanol are polar compounds. The efficiency of methanol was related to intermediate polarity its strong antimicrobial activity (Augusto *et al.* 2014; Nguyen *et al.* 2015). The yield of methanol extraction increases alongside the solubility of antimicrobial plant components increases (Merveill *et al.* 2017). Several workers have reported that a wide range of significant differences for the yields could be due to various factors such as time of extraction, type and part of plant materials used, extraction of solvent polarities, fineness of powder and extent of dryness (Maharjan *et al.* 2010; Ngeny 2012; Silva *et al.* 2014; Naima *et al.* 2015; Arakaki *et al.* 2016; Felhi *et al.* 2017).

The efficacy of the sixteen plant extracts against the tomato early blight fungi was tested *in vitro*. The results showed that the extracts exhibited significantly ($P < 0.05$) a varied percentage of inhibition of the mycelia growth of the fungal pathogens at three concentrations with three solvents tested. The maximum mycelial inhibition was recorded at 100% concentration while the lowest inhibition was at 25% concentration. Our results were similarly correlated works Abo-Elyousr *et al.* (2012) and Hubert *et al.* (2015) who reported that the effects of plant extracts increased with an increasing amount of concentration.

The present study showed that among sixteen plants most of the methanol extracts displayed higher antifungal activity against mycelium growth of *Alternaria solani* as compared to ethanol and petroleum ether extract. This agrees with the finding of some workers who reported that the methanol extracts for all of the tested plants showed significant antimicrobial activities against *E. coli* compared to the activity of the other solvents such as diethyl ether, ethyl acetate and chloroform, N- butanol, diethyl-ether, ethyl acetate (El Sayed and Aly 2014; Hussein 2016).

Ethanol extract of *Allium sativum* was highly preventing against mycelia growth of *Alternaria solani* in order of ethanol > methanol > Petroleum Ether extract. Closely in agreement

with our study Hajano *et al.* (2012) showed the extraction of garlic bulb was completely inhibitory against tested *M. oryzae*. The ethanol extract of *A. sativum* was the most effective plant that reduced the radial growth of *Colletotrichum kahawae* (Amsalu *et al.* 2011). The finding was also following Sesan *et al.* (2017) who reported that *Allium sativum* was the greatest inhibitory activity among nine plant species against *Fusarium oxysporum*. The use of *Allium sativum* has a great potential in suppressing several plant pathogenic fungi (Amin *et al.* 2009; Charimbu *et al.* 2009; Panchal and Patil 2009; Su and Cheng *et al.* 2008; Taskeen-Un-Nisa *et al.* 2011; Amsalu *et al.* 2011). The present finding of this study compares to other studies to become sometimes contrary for our study when used common materials that same plants may have different antimicrobial activity in same solvents on different organisms or same organisms. These reasons may be due to different extraction procedures used from one author to another author (Nasim *et al.* 2012).

A methanol extract of *Solanum incanum* was showed a higher degree of inhibition against *Alternaria solani* pathogen followed by petroleum ether and ethanol extracts. However, at a concentration of 100%, the PE of *Solanum incanum* exhibited the highest percentage of inhibition as compared to the methanol and ethanol extracts. A similar result was obtained by some workers; methanolic extract of *Solanum incanum* has better antimicrobial activity on *E.colli* (Hussein 2016). Ethanol extract of fruit *Solanum incanum* gives higher inhibition against pathogenic bacteria and fungus (Indhumathi and Mohandass 2014; Kipngeno *et al.* 2014). In our study, the ethanol extract of *Eucalyptus globules* exhibited higher antifungal activity compared to methanol and petroleum ether. Previous research work revealed that *Eucalyptus globules* leaf from methanol extracts was effective to inhibit the growth of *Alternaria brassicae* *in vitro* conditions (Ramezani and Mohammad 2015). Antifungal properties of *Eucalyptus globules* ethanol extracts against gladiolus wilt at different concentration was highly appreciated (Jan *et al.*, 2015).

The results obtained from methanol extracts of *Allium cepa* were a more inhibitory effect compared to the other two solvents. Alcohol extract of onion and garlic bulb extract showed the highest inhibition of *Alternaria alternata* growth at both 5% and 10% concentrations (Sanjeev *et al.* 2017). Aqueous extract of *Allium cepa* also inhibited against *Fusarium solani* (Ahmed *et al.* 2012), *F. oxysporum* f. sp. *Gladioli* (Chohan *et al.* 2011; Parvu and Parvu 2011).

The previous studies indicated that medicinal plants have different antimicrobial activities depending on extraction solvent, plant types and amount of the presence of bioactive compound (Sequeira *et al.* 2013; Stankovi *et al.* 2015; Bahraminejad *et al.* 2015; Arakaki *et al.* 2016). These plants have huge availability nearly our village and easy preparation method can be used by farmers. Among plant extracts, ethanol and methanol extract of *Allium sativum* was found highly effective in reducing the mycelial growth of

Alternaria solani as a positive control of tilt fungicide. Many researchers had also shown that the most potent antimicrobial activity by mostly total inhibition and intermediate inhibition.

CONCLUSION AND RECOMMENDATION

Tomato (*Solanum lycopersicum* Mill.) belongs to the family *Solanaceae* and is the world's largest important vegetable crop after potato. Tomato production and productivity are very low due to a lack of proper disease and insect pest management and other agronomic practices. Early blight of tomato is the most economically important disease was found widely distributed in all the surveyed areas. The findings of the present study indicated that early blight of tomato incidence and severity varies among districts, date of planting, crop density, weed management and environmental factors. The present study revealed that proper weeding practices, optimum plant density, crop rotation with non-solanaceous plants and other related farm practices should be carried out to reduce the yield loss of tomatoes due to early blight in the surveyed area. Moreover, extensive studies are required to investigate whether *Amaranthus hybridus*, *Datura stramonium*, *Commelina benghalensis*, *Cyperus esculentus*, *Ipomea ariocarpa* and *Chenopodium procerum* weeds are an alternative hosts for the early blight of tomato.

Since the study was additional *in vitro* experiments, this investigation demonstrates the potentials medicinal plants as potential alternatives to fungicides in the control of early blight of tomato. Our results revealed that most methanolic extracts showed significant inhibition of the mycelial growth of the test plant pathogen as compared to ethanolic and petroleum ether extracts. The Ethanol extract of *Allium sativum* at all concentrations and methanol extract at 100% concentration showed the complete reduction of the mycelial growth of *Alternaria solani*. The finding of this investigation suggested that the same plants have different antimicrobial activity in different solvents. Even if the rate of inhibition differed from one extract to the other, our results showed that the extracts of all evaluated solvents (methanol, ethanol and petroleum ether) were significantly inhibited the mycelia growth of the *Alternaria solani* at all different concentrations compared to untreated control.

Plant extracts were highly recommended for use at the higher concentrations in controlling *Alternaria solani*, the causative agent of early blight of tomato since they are locally available. The results of this study are also important steps towards developing natural plant-based fungicides which are eco-friendly for the management of early blight of tomato for the development of commercial formulations of botanicals. *Allium sativum* has a strong pungency smell and is volatile so that it can be kept away from the garden pest. The higher amount of gram yield was obtained extracted increase with increasing polarity. For crude extracts that have shown higher inhibitory effects against *Alternaria solani* *in vitro* conditions, their actual chemical compounds identified should be further studied to *in vivo* conditions. Furthermore,

it is also important to evaluate these plants on other microbes and to assess their real potential field conditions wherever early blight is an important disease of tomatoes.

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