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ANTIMICROBIAL EFFECT OF *ASPARAGUS OFFICINALIS* L. EXTRACTS

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Abstract

This experiment was carried out in the tissue culture laboratory, Agricultural Botany Dept., Faculty of Agriculture, Cairo University, Giza, Egypt and Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt, during the two years 2018 and 2019 with the aim of assessing the antimicrobial effect of different biomass extracts of *Asparagus officinalis* L. plant (Mary Washington 500 w cultivar) from family Asparagaceae such as callus, fresh shoot, fresh root, dry shoot and dry root using different solvents (methanol, ethyl acetate, acetone, chloroform and petroleum ether) and estimating the inhibition zones. The antifungal activity of the different extracts has been verified *in vitro* by antifungal assays against five phytopathogenic fungi (*Alternaria tenuissima*, *Botrytis cinerea*, *Fusarium oxysporum*, *Macrophomina phaseolina* and *Rhizoctonia solani*). The results showed that the inhibition percent of mycelial growth increased with increasing concentration of dry biomass extracts for all fungi used except *Botrytis cinerea*. *Macrophomina phaseolina* was less affected by different biomass extracts compared with all fungi used. The results indicated that dry biomass with ethyl acetate extracts showed better inhibition percent against all fungi used at most concentrations. Regarding the antibacterial activity, ethyl acetate extract was superior in inhibition of Gram-positive bacterial strains of all biomass types used. While dry shoot and root methanol extracts as well as fresh shoot and callus ethyl acetate extracts were the most inhibiting of Gram-negative bacteria strains.

Key Words: *Asparagus officinalis* L., plant extracts, antifungal activity, antibacterial activity, phytopathogenic fungi.

Introduction

Asparagus (*Asparagusofficinalis* L.) is one of the promising nontraditional horticultural crops in Egypt. However, it's considered one of the most important vegetable crops in some Asian, African, European and American countries (Hassan, 2001). Pathogenic fungi are one of the vital problems of crops (Xie *et al*., 2017 and Jain *et al.*, 2019). Fungal pathogens are proved to be a common and popular contaminant of agro-ecosystem that approximately causes 70–80% of total microbial crop loss (Moore *et al*., 2000 and Santra and Banerjee 2020). Common chemical fungicides have long been used to control fungal diseases. These chemical fungicides increase the risk of environmental pollution and adverse effects on biodiversity (Aktar *et al*., 2009 and Queyrel *et al*., 2016). Therefore, the search for new, more effective and environmentally friendly approaches and less toxic alternatives such as fungicides of fungal infections is very important to enhance resistance to common antifungal agents. Natural chemical strategies for controlling crop diseases are of considerable interest because of environmental and health concerns about the widespread use of chemical pesticides (Brauer *et al.*, 2019 and Zhang, *et al.*, 2020). The potential of secondary metabolites to protect plants can be used in a more modern and environmentally friendly alternative strategy (Pino *et al*., 2013 and Marutescu *et al*., 2017). Shrestha *et al*., (2018) stated that phytochemical screening revealed the presence of coumarin, flavonoid, catecholic tannin and reducing compounds in the alcoholic extract of *A. racemosus*. Çoban *et al*., (2009) observed that the ether extract of *A. officinalis* L., had an antimicrobial effect against ten pathogen bacteria and five yeasts. Patel and Patel (2013) and Kishor *et al*., (2019) pointed out that *Asparagus*

racemosus have potent antimicrobial activity against Gram-positive and Gram- negative bacteria.

Materials and methods

Collection of plant material

In vivo plant material of *A. officinalis* L. (Mary Washington 500 w cultivar) was collected from the experimental field in Fac. Agri., Cairo University, Giza, Egypt, in the growing season, 2018, as well as *in vitro* plant material of the same species was collected from Plant Tissue Culture Laboratory, Agricultural Botany Department, Faculty of Agriculture, Cairo University, Giza, Egypt.

Preparation of plant material

• The plant samples (*in vivo* and *in vitro* grown plants as well as callus tissues) were thoroughly washed well and weighed.

• The plant samples (*in vivo* grown plants) were dried completely at 50-60°C for 48 h in a hot air oven then crushed using a mechanical grinder.

Preparation of plant extracts

The plant material (15g) was soaked in 200 ml of the solvent at room temperature in the dark for one week before being filtered. Plant material extraction (*in vivo* and *in vitro* grown plants as well as callus tissues) was extracted with methanol, ethyl acetate, acetone, chloroform and petroleum ether. The filtered solutions were evaporated to dry by being placed in a water bath at 40°C overnight. Plant extracts were concentrated and preserved at 4°C until required for the experiments.

Antifungal activity assay

Antifungal screening: The five phytopathogenic fungi, including *Alternaria tenuissima*, *Botrytis cinerea*, *Fusarium oxysporum*, *Macrophomina phaseolina* and *Rhizoctonia solani* were isolated from infected samples. Samples of different plants, *i.e*. strawberry, tomato and cucumber were collected from Beheira and Qalyubia governorates, Egypt. The isolated fungi were purified out either by hyphal tip or single spore technique (Dhingra and Sinclair, 1985). Pure cultures were maintained during the experiments on potato dextrose agar (PDA 200g grated potato, 20g dextrose, 20g agar), *In vitro* antifungal assays of prepared against the fungus were performed with the poisoned plate technique (Das *et al*., 2010).

Antibacterial activity assay

Prior to the test, bacterial cultures *Bacillus subtilis* and *Ralstonia solanacearum* were prepared as follows: The bacterial cultures were sub-culture in nutrient broth 30°C for 24-27 h. The broth culture turbidity of was equilibrated. Then grown in nutrient agar, the test bacteria (0.1 ml) were streaked on nutrient medium plates using a sterile cotton swab. Agar was allowed to holes numbers were cut using a sterile cork borer and distribution of holes in petri dishes. In each hole, the different extract was loaded. Then, the petri dishes were left at room temperature for 2 h to allow diffusion of the test sample. Antibacterial activities of plant extracts were evaluated using a well diffusion method on nutrient agar (Das, *et al*., 2010). The inhibition zones were reported in millimeter (mm). *R. solanacearum* (-ve) and *B. subtilis* (+ve) were used as references for the antibacterial assay. Briefly, nutrient agar plates were inoculated with bacterial strain under aseptic conditions and wells (diameter=6mm) were filled with 25 µl or 50 µl of the test samples and incubated at 30°C for 24–72 hours. After the incubation period, the diameters of growth inhibition zones were measured and at three replicates for each test.

Statistical analysis

Data was subjected to appropriate statistical and conventional methods of analysis of variance according to Snedecor and Cochran 1989. The mean differences were compared by least significant difference test (LSD) at $P \leq 0.05$.

Result and Discussion

Antifungal activity

Effect of different dry biomass of *A. officinalis* L. on linear growth of some fungi on PDA medium by using different solvents

Effect of *A. officinalis* L. on the linear growth of *R. solani* on the PDA medium represented in table 1 and Fig. 1 showed that different extracts caused a significant inhibition on the linear growth of *R. solani* compared to the control. Different root extracts were more effective than shoot extracts at most concentrations used. Both shoot petroleum ether extract and root ethyl acetate extract at the concentration of 600 µg ml^{-1} were superior in inhibiting the linear growth of *R. solani* with inhibition percent of 100%.

Data in table 2 and Fig. 2 revealed that the different extracts caused a significant inhibition on the linear growth of *F. oxysporum* compared to the control. Different shoot and root extracts caused significant inhibition on the linear growth at all concentrations used compared to the control. Different root extracts significantly inhibited linear growth at all concentrations used compared to shoot extracts except chloroform and petroleum ether extracts. Shoot chloroform extracts caused non-significant inhibition on the linear growth at both used concentrations compared

Table 1: Effect of dry biomass extracts of (*A. officinalis* L.) on the linear growth of *R. solani* on the PDA medium by using different extraction solvents.

Bio-mass type	Solvent	Concentration µg ml^{-1}			
		400		600	
		Linear growth (cm)	Inhi-biti-on^{b}(%)	Linear growth (cm)	Inhi-biti-on^{b}(%)
Shoot	Methanol	4.95	45	2.05	77.22
	Ethyl acetate	5.09	43.44	3.69	59
	Petroleum ether	5.37	40.33	0	100
	Acetone	2.75	69.44	1.82	79.78
	Chloroform	5.83	35.22	3.87	57
Root	Methanol	2.38	73.56	1.35	85
	Ethyl acetate	2.43	73	0	100
	Petroleum ether	3.13	65.22	0.89	90.11
	Acetone	0.75	91.67	0.47	94.78
	Chloroform	5.74	36.22	3.78	58.00
Control		9	—	9	—
LSD(P ≤ 0.05) solvent(S)		0.29	—	1.36	—
LSD(P ≤ 0.05) Type (T)		0.17	—	0.79	—
LSD(P ≤ 0.05) S x T		0.41	—	1.93	—

[a] Mean of three replicates [b] Inhibition % = (Control-Treatment)/ control)*100)

Table 2: Effect of dry biomass extracts of (*A. officinalis* L.) on the linear growth of *F. oxysporum* on the PDA medium by using different extraction solvents.

Bio-mass type	Solvent	Concentration µg ml^{-1}			
		400		600	
		Linear growth (cm)	Inhi-biti-on^{b}(%)	Linear growth (cm)	Inhi-biti-on^{b}(%)
Shoot	Methanol	5.75	36.11	5.05	43.89
	Ethyl acetate	5.40	40	4.60	48.89
	Petroleum ether	3.05	66.11	1.10	87.78
	Acetone	4.00	55.56	2.85	68.33
	Chloroform	5.30	41.11	5.05	43.89
Root	Methanol	4.80	46.67	0	100
	Ethyl acetate	4.55	49.44	3.65	59.44
	Petroleum ether	4.60	48.89	4.35	51.67
	Acetone	3.80	57.78	1.00	88.89
	Chloroform	5.40	40	5.05	43.89
Control	—	9	—	9	—
LSD(P≤0.05) solvent (S)		1.42	—	0.66	—
LSD(P≤0.05) Type (T)		0.82	—	0.38	—
LSD(P≤0.05) S x T		2.01	—	0.93	—

[a] Mean of three replicates [b] Inhibition % = (Control-Treatment)/ control)*100).

with root chloroform extracted. Root methanol extract at the concentration of 600 µg ml^{-1} had the highest inhibition percent of 100%.

Regarding table 3 and Fig. 3, different extracts caused significant inhibition on the linear growth of *M. phaseolina* compared to the control. Different shoot and root extracts caused significant inhibition on the linear growth at all concentrations used compared to control. Root methanol and chloroform extracts resulted in a significant inhibition on the linear growth at all concentrations used in comparison with shoot extracts. There were no differences between values of shoot and root petroleum ether extracts and shoot chloroform extract at the low concentration. Shoot ethyl acetate extract at 400 µg ml^{-1} and 600 µg ml^{-1} recorded the highest inhibition value 73.89 and 82.22%, respectively in comparison with the other treatments.

Data in table 4 and Fig. 4 showed that different

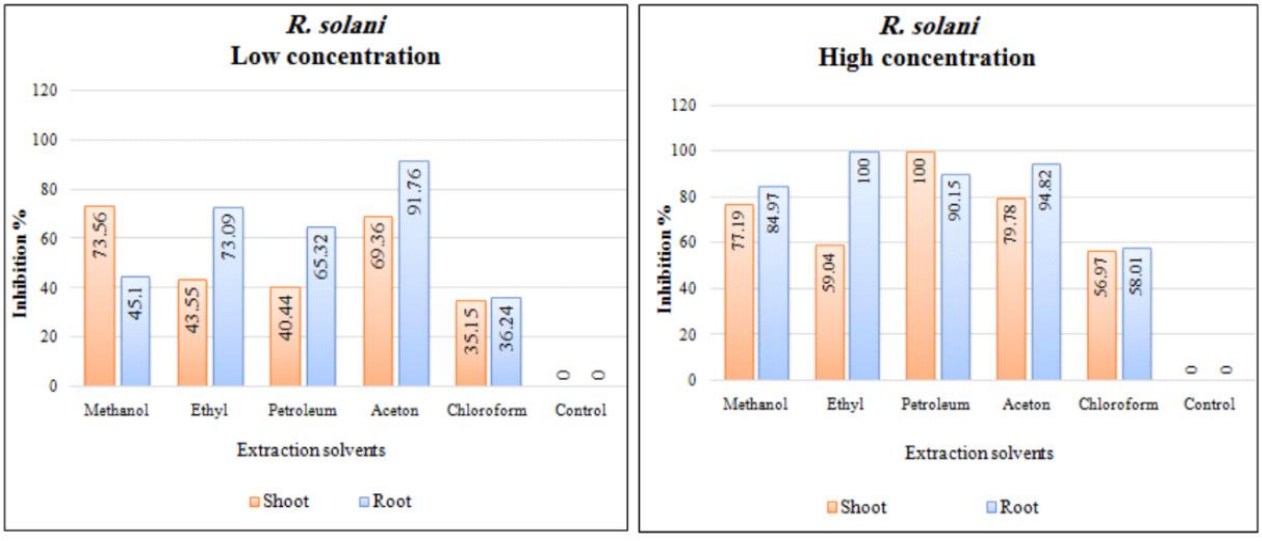


Fig. 1: Effect of dry biomass extracts of (*A. officinalis* L.) on inhibition zone of *R. solani* on the PDA medium by using different extraction solvents.

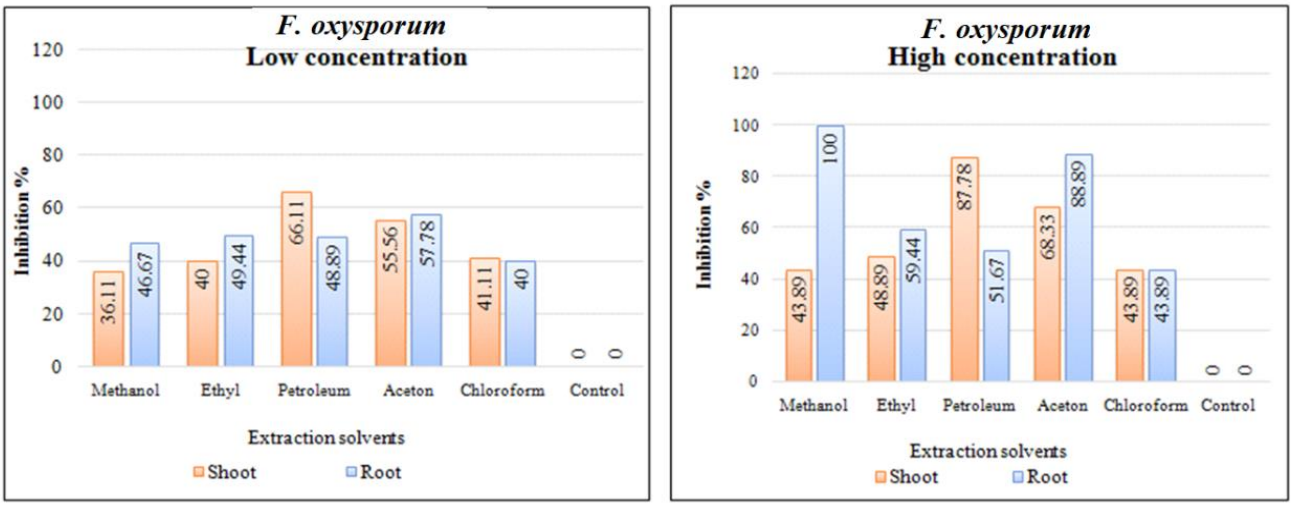


Fig. 2: Effect of dry biomass extracts of (*A. officinalis* L.) on inhibition zone of *F. oxysporum* on the PDA medium by using different extraction solvents.

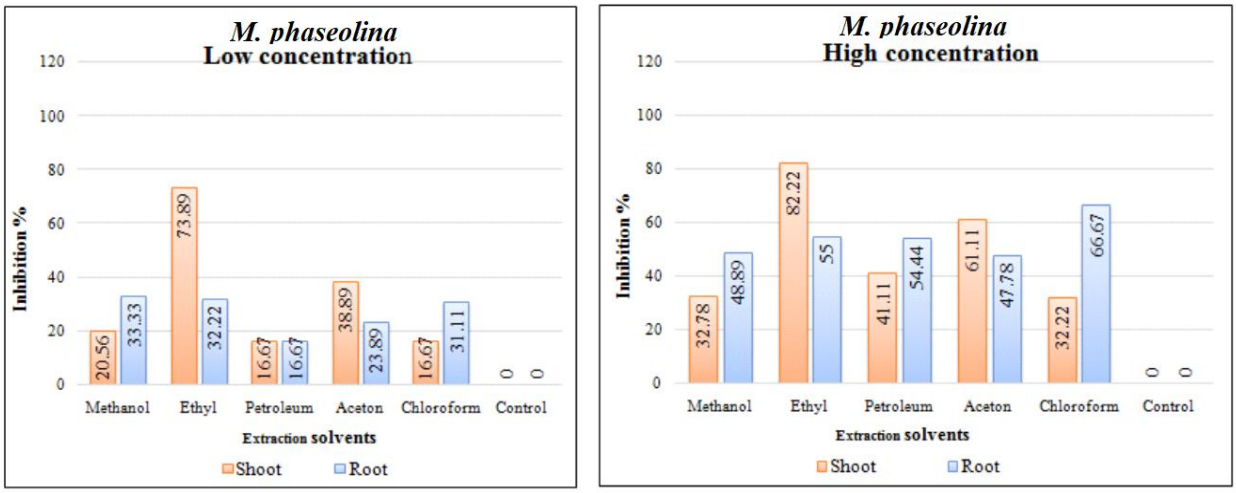


Fig. 3: Effect of dry biomass extracts of (*A. officinalis* L.) on inhibition zone of *M. phaseolina* on the PDA medium by using different extraction solvents.

extracts caused significant inhibition on the linear growth of *A. tenuissima* compared with control. Different shoot and root extracts caused a significant inhibition on the linear growth at all concentrations used in comparison with control. Different root extracts gave inhibition values more than shoot extracts on the linear growth at all concentrations used. The highest inhibition value (100%) was recorded with root chloroform extract at the concentration of 600 µg ml^{-1}.

Effect of *A. officinalis* L. on the linear growth of *B. cinerea* on the PDA medium in table 5 and Fig. 5 showed that different extracts caused a significant inhibition on linear growth. Root acetone extracts caused a significant inhibition on the linear growth at all concentrations used in comparison with shoot acetone extracts. Shoot chloroform extract was non-significant compared with root extract at low and high concentration used. Root acetone extract at 600 µg ml^{-1} as well as shoot ethyl acetate extract at 400 and 600 µg ml^{-1} gave the superior inhibition effect (100%) than the other treatments.

The different dry biomass types of *A. officinalis* L. were extracted by absolute methanol, ethyl acetate, acetone, chloroform and petroleum ether as solvents differ in their polarities. The purpose was to evaluate their bioactivity against some pathogenic fungi using the poisoned plate technique as found in *Asparagus racemosus*, Sangvikar (2012) and Parveen (2020). Shoot and root extracts tables 1 to 5 significantly inhibited all of the studied pathogenic fungi compared to control. Root extracts had a more inhibitory effect on the *A. tenuissima* than shoot extracts. Root and shoot extracts had a fluctuating inhibitory effect on *B. cinerea*, *F. oxysporum*, *M. phaseolina* and *R. solani*. Growth reduction percentages ranged between (36.22 to 91.67%) and (35.22

Table 3: Effect of dry biomass extracts of (*A. officinalis* L.) on the linear growth of *M. phaseolina* on the PDA medium by using different extraction solvents.

Bio-mass type	Solvent	Concentration µg ml^{-1}			
		400		600	
		Linear growth (cm)	Inhi-biti-on^{b}(%)	Linear growth (cm)	Inhi-biti-on^{b}(%)
Shoot	Methanol	7.15	20.56	6.05	32.78
	Ethyl acetate	2.35	73.89	1.60	82.22
	Petroleum ether	7.50	16.67	5.30	41.11
	Acetone	5.50	38.89	3.50	61.11
	Chloroform	7.50	16.67	6.10	32.22
Root	Methanol	6.00	33.33	4.60	48.89
	Ethyl acetate	6.10	32.22	4.05	55
	Petroleum ether	7.50	16.67	4.10	54.44
	Acetone	6.85	23.89	4.70	47.78
	Chloroform	6.20	31.11	3.00	66.67
Control		9	—	9	—
LSD(P≤0.05) solvent (S)		0.24	—	0.88	—
LSD(P≤0.05) Type (T)		0.14	—	0.51	—
LSD(P≤0.05) S x T		0.33	—	1.25	—

[a] Mean of three replicates [b] Inhibition % = (Control-Treatment)/ control)*100)

Table 4: Effect of dry biomass extracts of (*A. officinalis* L.) on the linear growth of *A. tenuissima* on the PDA medium by using different extraction solvents.

Bio-mass type	Solvent	Concentration µg ml^{-1}			
		400		600	
		Linear growth (cm)	Inhi-biti-on^{b}(%)	Linear growth (cm)	Inhi-biti-on^{b}(%)
Shoot	Methanol	5.55	38.33	5.00	44.44
	Ethyl acetate	6.50	27.78	5.35	40.56
	Petroleum ether	5.20	42.22	1.75	80.56
	Acetone	4.20	53.33	2.90	67.78
	Chloroform	6.20	31.11	0.80	91.11
Root	Methanol	4.10	54.44	3.20	64.44
	Ethyl acetate	5.35	40.56	5.25	41.67
	Petroleum ether	2.80	68.89	1.30	85.56
	Acetone	3.50	61.11	0.80	91.11
	Chloroform	5.00	44.44	0	100
Control		9	—	9	—
LSD(P≤0.05) solvent (S)		0.6	—	0.87	—
LSD(P≤0.05) Type (T)		0.34	—	0.5	—
LSD(P≤0.05) S x T		0.84	—	1.23	—

[a] Mean of three replicates [b] Inhibition % = (Control-Treatment)/ control)*100)

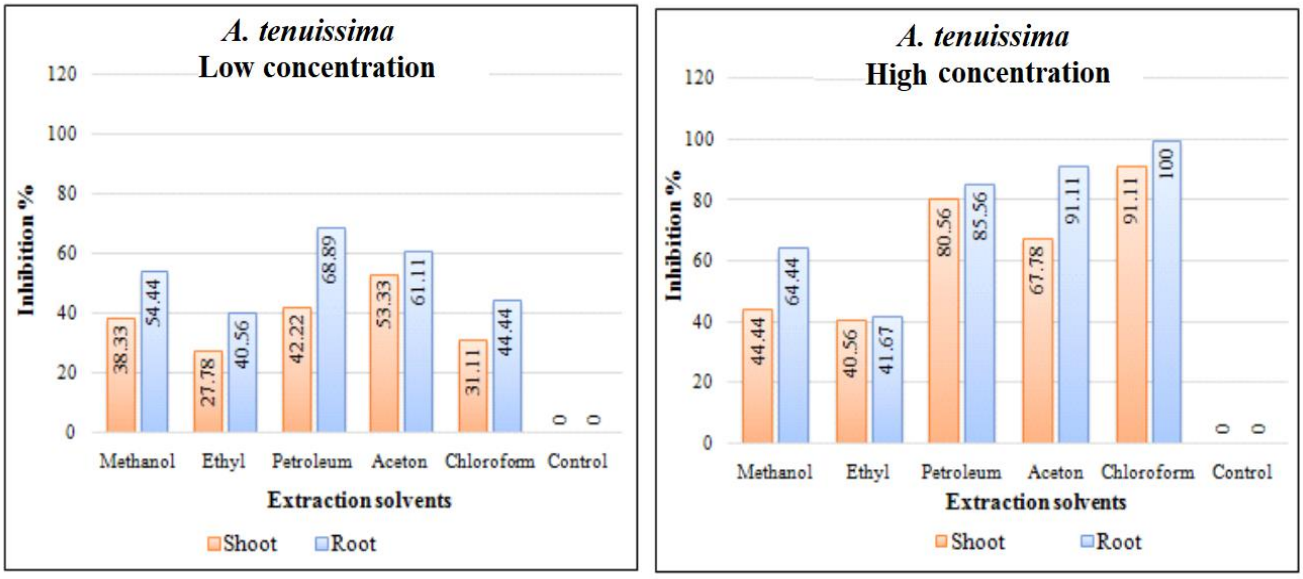


Fig. 4: Effect of dry biomass extracts of(*A. officinalis*L.) on inhibition zone of *A. tenuissima* on the PDA medium by using different extraction solvents.

to 69.44%) for *R. solani*; (40.56 to 68.89%) and (27.78 to 53.33%) for *A. tenuissima* and (40.00 to 57.78%) and (36.11 to 66.11%) for *F. oxysporum* in the case of root and shoot extracts, respectively, at the low concentration. On the other hand, the different dry biomass (root and shoot) extracts showed different patterns of effects on *M. phaseolina* and *B. cinerea* as inhibition percentages ranged between (16.67 to 33.33%) and (16.67 to 73.89%) for *M. phaseolina* and (16.67 to 86.67%) and (16.67 to 100%) for *B. cinerea* in the case of root and shoot extracts, respectively, at the low concentration. Battu and Kumar (2010) and Tinrat and Sila-asna (2017) found that biological activity may be due partly to the presence of various phytochemical compounds in *A. racemosus* like; cardiac glycosides, flavonoids, phenolics, saponins, steroids and terpenoids.

Inhibition percent caused significant or non-significant decreases in mycelial growth by increasing the concentration of the extracts. Shrestha *et al*., (2018)

Table 5: Effect of dry biomass extracts of (*Asparagus officinalis* L.) on the linear growth of *B. cinerea* on the PDA medium by using different extraction solvents.

Bio-mass type	Solvent	Concentration µg ml^{-1}			
		400		600	
		Linear growth (cm)	Inhi-biti-on[b](%)	Linear growth (cm)	Inhi-biti-on[b](%)
Shoot	Methanol	5.75	36.11	3.10	65.56
	Ethyl acetate	0	100	0	100
	Petroleum ether	6.85	23.89	6.10	32.22
	Acetone	5.60	37.78	2.30	74.44
	Chloroform	7.50	16.67	7.50	16.67
Root	Methanol	7.50	16.67	7.50	16.67
	Ethyl acetate	1.20	86.67	0.65	92.78
	Petroleum ether	7.50	16.67	0.45	95
	Acetone	1.40	84.44	0	100
	Chloroform	7.50	16.67	7.50	16.67
Control		9	—	9	—
LSD(P≤0.05) solvent (S)		0.2	—	0.28	—
LSD(P≤0.05) Type (T)		0.11	—	0.16	—
LSD(P≤0.05) S x T		0.28	—	0.40	—

[a] Mean of three replicates [b] Inhibition % = (Control-Treatment)/ control)*100)

stated that *A. racemosus* has shown selected antimicrobial effects against *S. cerevisiae* and *C. albicans* with inhibition zone of 25 mm in an average. Shukla (2018) mentioned that the antimicrobial activity of hydroalcoholic extract (80% ethanol) of *Glycyrrhiza glabra* root and *A. racemosus* leaves due to the presence of different phytochemicals with biological activity like saponin, phenol, carbohydrates, flavonoids, proteins and amino acids.

Effect of different fresh biomass types extracts of *A. officinalis* L. plant on the linear growth of some fungi on the PDA medium by using different extraction solvents

Table 6: Effect of different fresh biomass types extracts of (*A. officinalis* L.) plant on the linear growth of *R. solani* on the PDA medium by using different extraction solvents.

Bio-mass type	Solvent	Concentration µg ml^{-1}			
		400		600	
		Linear growth (cm)	Inhi-biti-on[b](%)	Linear growth (cm)	Inhi-biti-on[b](%)
Shoot	Methanol	5.5	38.89	1.3	85.56
	Ethyl acetate	1.35	85	0	100
	Petroleum ether	6.3	30	3.85	57.22
	Acetone	7.35	18.33	4.8	46.67
	Chloroform	6.5	27.78	5.3	41.11
Root	Methanol	6.3	30	4.5	50
	Ethyl acetate	1.55	82.78	0	100
	Petroleum ether	6.55	27.22	2.3	74.44
	Acetone	3.25	63.89	3.65	59.44
	Chloroform	6.95	22.77	5.45	39.44
Callus	Methanol	5.5	38.89	2.4	73.33
	Ethyl acetate	2.1	76.67	0	100
	Petroleum ether	4.05	55	3.1	65.55
	Acetone	6.1	32.22	5.55	38.33
	Chloroform	5.8	35.56	5	44.4
Control		9	—	9	—
LSD(P≤0.05) solvent (S)		0.7	—	0.86	—
LSD(P≤0.05) Type (T)		N.S	—	N.S	—
LSD(P≤0.05) S x T		1.2	—	1.5	—

[a] Mean of three replicates [b] Inhibition % = (Control-Treatment)/ control)*100)

Effect of different fresh biomass types extracts of *A. officinalis* L. plant on the linear growth of *R. solani* on the PDA medium are clarified in table 6. Different solvent extracts caused a significant inhibition on the linear

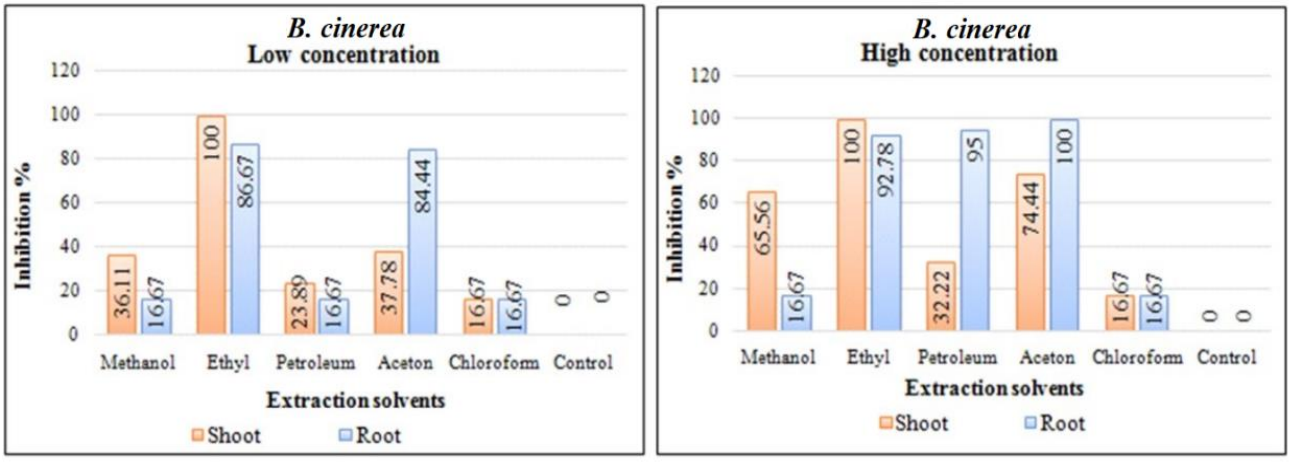


Fig. 5: Effect of dry biomass extracts of (*A. officinalis* L.) on inhibition zone of *B. cinerea* on the PDA medium by using different extraction solvents.

growth. Different types of extracts caused non-significant inhibition on the linear growth. It showed an interaction between fresh biomass type and solvent type. Ethyl acetate extracts of different fresh biomass types recorded the highest inhibition values on the linear growth being; (100, 100, 100%) and (85, 82.78, 76.67%) for shoot, root and callus at the concentration of 600 and 400 µg ml^{-1}, respectively.

Data in table 7 revealed that different extracts caused a significant inhibition on the linear growth of *F. oxysporum* compared to the control. Different shoot, root and callus extracts significantly inhibited linear growth in all concentrations used compared to the control. Different root extracts significantly inhibited linear growth in most concentrations used compared to the shoot and callus extracts. Callus methanol and chloroform extracts caused significant inhibition on the linear growth in all concentrations used compared to the shoot and root extracts except for shoot methanol extract at high concentration.

Regarding table 8, different extracts caused significant inhibition on the linear growth of *M. phaseolina* compared to the control. The inhibition percent ranged from 16.67 to 28.89% and 16.67 to 52.78% in the case of different fresh biomass types and solvents at low and high concentrations, respectively. Callus methanol extract caused significant inhibition (28.89%) on the linear growth at the low-use concentration in comparison with different fresh biomass types and solvents. The inhibition percent was the highest (52.78%) in the case of root acetone extract at high concentration compared to different fresh biomass types and solvents.

Data in table 9 indicated the major differences on the linear growth of *A. tenuissima* between different extracts within the fresh biomass type and the solvent. Different shoot, root and callus extracts significantly inhibited linear growth in all concentrations used compared to control. Callus chloroform extract showed the highest inhibition percent (69.44%), followed by shoot petroleum ether extract (58.33%), then shoot chloroform extract (49.44%) at low concentration. Different callus extracts showed the highest inhibition percent at high concentration

Table 7: Effect of different fresh biomass types extracts of (*A. officinalis* L.) plant on the linear growth of *F. oxysporum* on the PDA medium by using different extraction solvents.

Bio-mass type	Solvent	Concentration µg ml^{-1}			
		400		600	
		Linear growth (cm)	Inhi-biti-on[b](%)	Linear growth (cm)	Inhi-biti-on[b](%)
Shoot	Methanol	5.7	36.67	0.55	93.89
	Ethyl acetate	6	33.33	4.1	54.44
	Petroleum ether	5.65	37.22	4.05	55
	Acetone	5.85	35	4.6	48.89
	Chloroform	5.95	33.89	5.45	39.44
Root	Methanol	5.7	36.67	4.6	48.89
	Ethyl acetate	4.9	45.56	4.05	55
	Petroleum ether	4.75	47.22	3.5	61.11
	Acetone	5.3	41.11	4.2	53.33
	Chloroform	6.3	30	5.1	43.33
Callus	Methanol	5.25	41.67	4.8	46.67
	Ethyl acetate	5	44.44	4.35	51.67
	Petroleum ether	5.7	36.67	4.55	49.44
	Acetone	5.95	33.89	4.6	48.89
	Chloroform	3.65	59.44	0	100
Control		9	—	9	—
LSD(P≤0.05) solvent (S)		0.56	—	0.5	—
LSD(P≤0.05) Type (T)		0.4	—	0.36	—
LSD(P≤0.05) S x T		0.97	—	0.87	—

[a] Mean of three replicates [b] Inhibition % = (Control-Treatment)/ control)*100)

Table 8: Effect of different fresh biomass types extracts of (*A. officinalis* L.) plant on the linear growth of *M. phaseolina* on the PDA medium by using different extraction solvents.

Bio-mass type	Solvent	Concentration µg ml^{-1}			
		400		600	
		Linear growth (cm)	Inhi-biti-on[b](%)	Linear growth (cm)	Inhi-biti-on[b](%)
Shoot	Methanol	7	22.22	6.25	30.56
	Ethyl acetate	7.5	16.67	6	33.33
	Petroleum ether	6.95	22.78	4.85	46.11
	Acetone	7.5	16.67	7.5	16.67
	Chloroform	7.5	16.67	6.15	31.67
Root	Methanol	7.05	21.67	4.75	47.22
	Ethyl acetate	6.55	27.22	5.85	35
	Petroleum ether	7.5	16.67	7.4	17.78
	Acetone	7.25	19.44	4.25	52.78
	Chloroform	7	22.22	5.6	37.78
Callus	Methanol	6.4	28.89	5.25	41.67
	Ethyl acetate	7.5	16.67	6.45	28.33
	Petroleum ether	7.5	16.67	7.5	16.67
	Acetone	7.3	18.89	7.15	20.56
	Chloroform	7.5	16.67	6.35	29.44
Control		9	—	9	—
LSD(P≤0.05) solvent (S)		0.27	—	0.55	—
LSD(P≤0.05) Type (T)		N.S	—	0.39	—
LSD(P≤0.05) S x T		0.46	—	0..95	—

[a] Mean of three replicates [b] Inhibition % = (Control-Treatment)/ control)*100)

used compared to shoot and root extracts with the exception of methanol extract.

Effect of *A. officinalis* L. extracts on the linear growth of *B. cinerea* on the PDA medium given in table 10 showed that different extracts caused a significant inhibition on the linear growth compared to the control. Petroleum ether and acetone extracts in the case of different fresh biomass types caused non-significant inhibition of each other on the linear growth at different concentrations used with the exception of callus at high concentration caused a significant inhibition of each other on the linear growth. Root ethyl acetate extract showed the highest inhibition percent (91.67 and 100%) at the concentration of 400 and 600 µg ml^{-1}, respectively, in comparison with the same solvent and the other fresh biomass types. Different callus extracts showed the highest inhibition percent at high used concentration in comparison with shoot and root extracts except for ethyl acetate and chloroform extracts. Study on *A. officinalis* showed that these bioactivities differ between *in vitro* and *in vivo* grown plants. Khorasani *et al.*, 2010 studied the antimicrobial activity of different ethanol extracts of A. officinalis (in vivo plant, in vitro and callus tissues) against some gram-positive and gram negative. Showed that only antimicrobial activity was obtained from in callus tissue against *B. cereus* (+ve). Flavonoids are phenolic compounds that possess a wide range of biological activities, including antifungal activity obtained from "triguero" asparagus by-products against *F. oxysporum* (Rosado- Álvarez *et al.*, 2014).

Antibacterial activity

Inhibition effect of different dry biomass of *A. officinalis* L. on growth of two bacteria (+ve and -ve) on PDA medium by using different solvents

In this study, the different dry shoot and root extracts of *A. officinalis* L. were tested for their antibacterial activity against Gram-negative and positive bacteria. Strains of *R. solanacearum* and *B. subtilis* were used. Dry shoot and root methanol extracts showed antibacterial activity (positive and negative Gram (+ve & -ve)) similar to the control tables 11 and 12. Dry shoot ethyl acetate

Table 9: Effect of different fresh biomass types extracts of (*A. officinalis* L.) plant on the linear growth of *A. tenuissima* on the PDA medium by using different extraction solvents.

Bio-mass type	Solvent	Concentration µg ml^{-1}			
		400		600	
		Linear growth (cm)	Inhibition[b](%)	Linear growth (cm)	Inhibition[b](%)
Shoot	Methanol	5.95	33.89	5.65	37.22
	Ethyl acetate	6.25	30.56	4.15	53.89
	Petroleum ether	3.75	58.33	3.6	60
	Acetone	6.05	32.78	3.8	57.78
	Chloroform	4.55	49.44	0.9	90
Root	Methanol	5.55	38.33	4.6	48.89
	Ethyl acetate	5.6	37.78	5.1	43.33
	Petroleum ether	6.7	25.56	5.75	36.11
	Acetone	6.25	30.56	5.7	36.67
	Chloroform	5.4	40	4.1	54.44
Callus	Methanol	6.4	28.89	5.55	38.33
	Ethyl acetate	5.25	41.67	1.45	83.89
	Petroleum ether	6.5	27.78	1.75	80.56
	Acetone	5.45	39.44	3.3	63.33
	Chloroform	2.75	69.44	0	100
Control		9	—	9	—
LSD(P≤0.05) solvent (S)		0.44	—	0.71	—
LSD(P≤0.05) Type (T)		0.31	—	0.5	—
LSD(P≤0.05) S x T		0.77	—	1.2	—

[a] Mean of three replicates [b] Inhibition % = (Control-Treatment)/ control)*100)

Table 10: Effect of different fresh biomass types extracts of (*A. officinalis* L.) plant on the linear growth of *B. cinerea* on the PDA medium by using different extraction solvents.

Bio-mass type	Solvent	Concentration µg ml^{-1}			
		400		600	
		Linear growth (cm)	Inhibition[b](%)	Linear growth (cm)	Inhibition[b](%)
Shoot	Methanol	7.5	16.67	5.45	39.44
	Ethyl acetate	6	33.33	0.5	94.44
	Petroleum ether	7.5	16.67	7.5	16.67
	Acetone	7.5	16.67	7.5	16.67
	Chloroform	7.5	16.67	1	88.89
Root	Methanol	1.6	82.22	1	88.89
	Ethyl acetate	0.75	91.67	0	100
	Petroleum ether	7.5	16.67	7.5	16.67
	Acetone	7.5	16.67	7.5	16.67
	Chloroform	6.55	27.22	4.55	49.44
Callus	Methanol	1.5	83.33	0.95	89.44
	Ethyl acetate	7.3	18.89	6.05	32.78
	Petroleum ether	7.5	16.67	6.2	31.11
	Acetone	7.45	17.22	2.5	72.22
	Chloroform	7.5	16.67	7.5	16.67
Control		9	—	9	—
LSD(P≤0.05) solvent (S)		0.65	—	N.S	—
LSD(P≤0.05) Type (T)		0.46	—	0.65	—
LSD(P≤0.05) S x T		1.12	—	1.59	—

[a] Mean of three replicates [b] Inhibition % = (Control-Treatment)/ control)*100)

Table 11: Antibacterial activity of different types of dry biomass extracts of (*A. officinalis* L.) plant against selected strains of bacteria.

Biomass type	Solvent	Conc.	Strains of bacteria	
			R. solana-cearum(-ve)	*B. subti-lis*(+ve)
Shoot	Methanol	Low	+	+
		High	+	+
	Ethyl acetate	Low	-	+
		High	-	+
	Petroleum ether	Low	-	-
		High	+	+
	Acetone	Low	-	+
		High	-	+
	Chloroform	Low	+	-
		High	+	+
Root	Methanol	Low	+	+
		High	+	+
	Ethyl acetate	Low	+	+
		High	+	+
	Petroleum ether	Low	-	-
		High	-	-
	Acetone	Low	+	+
		High	+	+
	Chloroform	Low	-	-
		High	-	+

Table 12: Inhibition zones of different concentrations of dry biomass extracts of (*A. officinalis* L.) plant against selected strains of bacteria.

Biomass type	Solvent	Conc.	Strains of bacteria	
			R. solana-cearum(-ve)	*B. subti-lis*(+ve)
Shoot	Methanol	Low	0.4	1.1
		High	1.2	1.6
	Ethyl acetate	Low	0	0.67
		High	0	0.77
	Petroleum ether	Low	0	0
		High	1.2	1.1
	Acetone	Low	0	1.2
		High	0	1.9
	Chloroform	Low	0.9	0
		High	1.13	1
Root	Methanol	Low	1.1	1.07
		High	1.63	1.23
	Ethyl acetate	Low	0.43	1.1
		High	1.23	1.67
	Petroleum ether	Low	0	0
		High	0	0
	Acetone	Low	0.83	1.03
		High	1.6	1.27
	Chloroform	Low	0	0
		High	0	0.9

extract caused inhibition against Gram- positive bacteria (+ve) but dry root ethyl acetate extract caused inhibition against Gram- positive and negative bacteria (+ve & –ve). Dry shoot and root petroleum ether extracts were not active against Gram-positive and negative bacteria (+ve &–ve), except shoot petroleum ether extract against Gram- negative and positive bacteria (-ve & +ve) at high concentration. Dry shoot acetone extract inhibited only Gram-positive bacteria (+ve) but dry root acetone extract inhibited both types of bacteria. Dry shoot chloroform extract was active against Gram-positive and negative bacteria (+ve and –ve) at high and low concentrations, except dry shoot chloroform extract against Gram-positive bacteria (+ve) at low concentration. Dry root chloroform extract was only active against Gram-positive bacteria (+ve) at high concentration.

Inhibition effect of different fresh biomass of *A. officinalis*L. on growth of two bacteria (+ve and -ve) on PDA medium by using different solvents

Tables 13 and 14 show the effect of fresh shoot, root and callus extracts against the two Gram-positive and negative bacterial strains mentioned before. Fresh shoot and root methanol extracts at low and high concentrations did not affect the two bacterial strains (+ve & -ve). While, in callus, it gave inhibitory effect at both concentrations against Gram-positive bacteria (+ve) as well as at high concentration against Gram-negative bacteria (-ve). Callus chloroform extract gave inhibition effect against two bacterial strains (+ve & -ve) at both low and high concentrations, while in fresh root chloroform extract, this effect exhibited only in Gram-positive bacteria (+ve). On the other hand, fresh shoot chloroform extract did not affect the bacterial activity. In the case of fresh shoot acetone extract, the effect was only valid against Gram-negative bacteria at high concentration but had no effect against Gram-positive bacteria. Fresh root and callus acetone extracts had no effect against both bacterial strains at low and high concentrations. Fresh shoot and callus ethyl acetate extracts gave inhibitory effect against both strains (+ve & -ve) at low and high concentrations but root ethyl acetate extract affected only gram-positive bacteria (+ve) at both concentrations. Fresh root petroleum ether extract did not record any inhibitory effect against both bacterial strains (+ve & -ve). While shoot and callus extracts at high concentration were valid against gram-negative and positive bacteria (-ve & +ve), respectively.

Nair and Chanda (2006) mentioned that the aqueous and ethanol extracts of *A. racemosus* showed the least antibacterial activity compared with medicinal various plants against seven gram-negative and five gram-positive

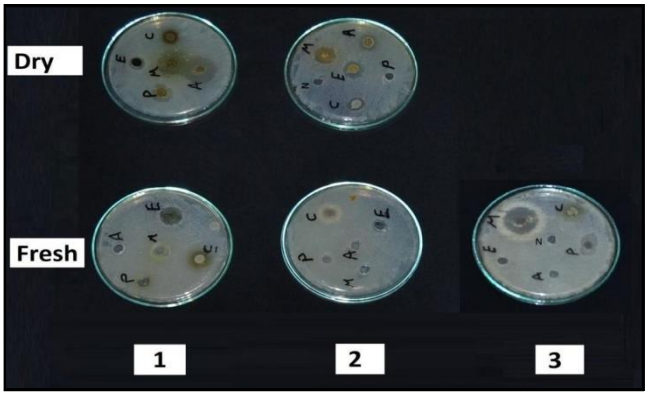
Table 13: Antibacterial activity of different types of fresh biomass extracts of (*A. officinalis* L.) plant against selected strains of bacteria.

Biomass type	Solvent	Conc.	Strains of bacteria	
			R. solana-cearum(-ve)	*B. subti-lis*(+ve)
Shoot	Methanol	Low	-	-
		High	-	-
	Ethyl acetate	Low	+	+
		High	+	+
	Petroleum ether	Low	-	-
		High	+	-
	Acetone	Low	-	-
		High	+	-
	Chloroform	Low	-	-
		High	-	-
Root	Methanol	Low	-	-
		High	-	-
	Ethyl acetate	Low	-	+
		High	-	+
	Petroleum ether	Low	-	-
		High	-	-
	Acetone	Low	-	-
		High	-	-
	Chloroform	Low	-	+
		High	-	+
Callus	Methanol	Low	-	+
		High	+	+
	Ethyl acetate	Low	+	+
		High	+	+
	Petroleum ether	Low	-	-
		High	-	+
	Acetone	Low	-	-
		High	-	-
	Chloroform	Low	+	+
		High	+	+

Table 14: Inhibition zones of different concentrations of fresh biomass extracts of (*A. officinalis* L.) plant against selected strains of bacteria.

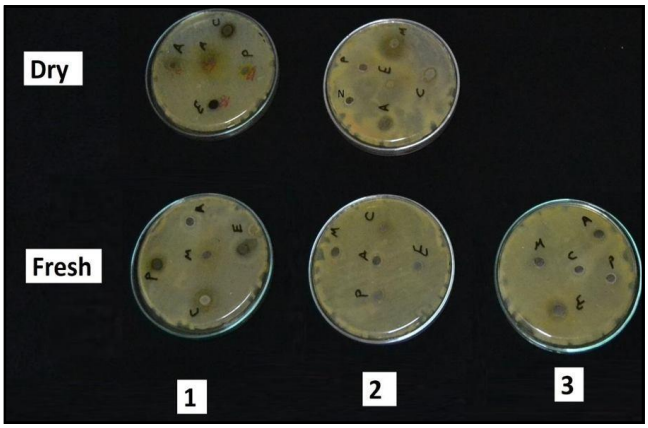
Biomass type	Solvent	Conc.	Strains of bacteria	
			R. solana-cearum(-ve)	*B. subti-lis*(+ve)
Shoot	Methanol	Low	0	0
		High	0	0
	Ethyl acetate	Low	0.63	0.73
		High	1	1.03
	Petroleum ether	Low	0	0
		High	1.6	0
	Acetone	Low	0	0
		High	1.2	0
	Chloroform	Low	0	0
		High	0	0
Root	Methanol	Low	0	0
		High	0	0
	Ethyl acetate	Low	0	0.7
		High	0	1.07
	Petroleum ether	Low	0	0
		High	0	0
	Acetone	Low	0	0
		High	0	0
	Chloroform	Low	0	0.6
		High	0	1.6
Callus	Methanol	Low	0	0.67
		High	0.3	1.5
	Ethyl acetate	Low	0.6	0.7
		High	1.2	1.37
	Petroleum ether	Low	0	0
		High	0	0.9
	Acetone	Low	0	0
		High	0	0
	Chloroform	Low	0.6	0.3
		High	0.9	0.67

bacteria. Aqueous extracts and ethanol extracts of *A. racemosus* showed no effect against different used bacteria (+ve and –ve) except *Bacillus cereus* (+ve). Mishra *et al*., (2014) used roots methanol extract of *(A. racemosus*) against gram-positive and negative bacteria which showed significant *in vitro* antibacterial efficacy against gram-negative bacteria (*Escherichia coli*, three types of Shigella bacteria (*Shigella dysenteriae*, *S. sonnei*, *S. flexneri*), *Vibrio cholerae*, two types of salmonella bacteria (*Salmonella typhi*, *S. typhimurium*) and *Pseudomonas putida*) and gram-positive bacteria (*B. subtilis and Staphylococcus aureus*). Sinha and Biswas (2011) mentioned that bactericidal activity of crude extracts from *A. racemosus* roots was screened against eight pathogenic strains belonging to gram-positive bacteria (*B. subtilis*, *Micrococcus luteus* and *S. aureus*) and gram-negative bacteria (*E. coli*, *Pseudomonas aeruginosa*, two types of Shigella bacteria (*S. dysenteriae* and *S. flexneri*) and *Vibrio cholerae*). Patel and Patel (2013) used that A. racemosus leaves extracts with different solvents like acetone, chloroform, ethyl acetate, methanol, petroleum ether and water. Recorded that different extract of *A. racemosus* leaves have antimicrobial activity against Gram positive as well as Gram negative bacteria. Shevale *et al.*, (2015) demonstrated *in vitro* antibacterial activity of crude ethanol and acetone extract of *A. racemosus* roots against gram-positive (*S. aureus* and *B. subtilis*) and gram-negative (*E. coli*, *Klebsiella pneumoniae* and *S. typhi*). Kishor *et al*., (2019) also found that *in vitro*



1: Shoot 2: Root 3: Callus N: Control M: Methanol A: Acetone P: Petroleum C: Chloroform E: Ethyl acetate

Fig 6: Inhibition zones of different concentrations of fresh and dry biomass extracts of (*A. officinalis* L.) plant against B. *subtilis* (+ve) at high concentration.



1: Shoot 2: Root 3: Callus N: Control M: Methanol A: Acetone P: Petroleum C: Chloroform E: Ethyl acetate

Fig. 7: Inhibition zones of different concentrations of fresh and dry biomass extracts of (*A. officinalis* L.) plant against *R. solanacearum* (-ve) at high concentration.

antibacterial activity of *A. racemosus* roots against different gram positive bacteria *(S. aureus* and *B. cereus*) and gram-negative (*E. coli* and *S. Typhimurium*).

Conclusion

It could be concluded from the previously mentioned results that the biomass type plays an important role in inhibiting microbial activity. Root extract is more effective than shoot extract at most extracts. The concentration of the extract has a positive relationship with inhibition activity against fungal growth. Also, the type of fungus is considered one of the important factors. Using different types of *A. officinalis* L. biomass extracts as an antifungal led to positive results. In the case of using the fresh shoot and dry root methanol extract, the obtained inhibition percent was more than 91% against *F. oxysporum*. As well as ethyl acetate extract against *R. solani* and *B. cinerea*when dry shoot and root, fresh shoot and root were used. Also, the callus ethyl acetate extract gave the same result against *R. solani*. Acetone extract gave positive results against *A. tenuissima*, *R. solani* and *B. cinerea* when dry root was used. When petroleum ether extract was used, the result was more than 91% against *R. solani* and *B. cinerea* when dry shoot and dry root were used, respectively. In the case of chloroform extract, the realized result was more than or equal 90% against *A. tenuissima* when callus, fresh shoot, dry shoot and dry root were used at high concentration. *M. phaseolina* gave the least inhibition percent.

The concentration of the extract has a positive relationship with inhibiting activity against bacterial growth for example; fresh shoot petroleum ether extract against gram negative bacteria (–ve). Also, the bacterial type is considered one of the important factors for example; fresh root ethyl acetate extract has inhibitory effect against gram positive bacterial (+ve). Using different types of *A. officinalis* L. biomass extracts as an antibacterial led to positive results. In the case of using dry root ethyl acetate, acetone ether or chloroform extract, they had an inhibitory effect against Gram- negative bacteria (-ve) compared to dry shoot of the same solvents extracts. Also, methanol extract for dry shoot and root had an inhibitory effect on bacterial strains (+ve & -ve).

Further studies are needed using isolates from other fungi and bacteria. Other *in vivo* experiments are also needed to confirm the effect of plant extracts.

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