Anti-microbial Activity of *Alpinia Galanga Rhizome* Extracts Against Some Plant Phathogens

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Abstract: Ethanol, petroleum ether and Acetonitrile extracts of *Alpinia galanga* have been evaluated against bacteria viz. *Pectobacterium carotovorum* S.S *carotovorum* NRRL B- 4072, *Pseudomones syringae* SS. *Syringae*, DSM 50302, MP50, *Rhizobium radiobacter*, DSM 30204, and *Corynebacterium glutamieum* 1220 T. using agar well diffusion method and four fungal species: *Aspergillus Flavus*, *Aspergillus niger*, *Drechslera hawaiiensis* and *Humicola Fuscoatra* by food poison technique. All the extracts showed significant antibacterial and antifungal activities. The highest inhibition zone (29.33mm) was recorded for ethanolic extract against *C. glutamieum* bacteria and in *A. Flavus* fungi the EC_{50} recorded 3237 ppm. Ethanol extracts have shown excellent activity towards all the pathogens. The inhibition zone for bacteria and Ec_{50} for fungi values ranged from 25.25-29.3mm and 3237.0-9033.7ppm, repetitively. GC- MS analysis of Ethanal extracts Benzofuran was the predominant compound (84.32%), followed by barbituric acid 3.81%, beta farnesene 3.29%, 4 Ethoxychroman 1.59%, trans – Isocroweacin 1.56% and 1.8 cineole 1.19%, which could be responsible for its broad spectrum activity. So, *Alpinia galanga* can be quite resourceful for the development of new generation antimicrobe.

Key words: *Alpinia glanga*- Antimicrobial activity- GC- MS analysis- pathogens- plant extracts.

1. Introduction

Control of plant bacterial diseases remains difficult due to the limited availability of bactericides. Only few chemical products are available, and their use is hampered by limited efficacy in the field beside for their negative potential effects either in the environment or on human and animal health. Moreover the sue of antibiotics in plant protection is limited because of the possibility to select pathogen population resistant to bactericides and the potential transfer of resistant genes to animal and human pathogenic bacteria **(McManus *et al.*, 2002)**.

Fungal phytopathogens are the cause of many plant diseases and much loss of crop yields, especially in subtropical and tropical regions **(Brimner and Boland 2003)**. The requirement is to find effective source to cheek pathogenicity. Chemical fungicides are extensively used in contemporary agriculture. However, these products may cause problems such as environmental pollution and negative affect against human health. Further problems include the development of resistance in the pathogens and to a decrease in the diversity of non- target organisms. To reduce the worsening problems in fungicide usage, new methods for plant protection, which are less dependent on chemicals and are more friendly to environment should be discovered and developed.

The use of chemical pesticides is very popular practice to control various plant diseases management as compare to natural one which are prepared from plants or plant parts. But, consumer now demands less use of synthetic fungicides due to the non-biodegradability, pollutive nature and residual toxicities of chemical pesticides. Several studies have revealed that the plant extracts could be reasonable source of natural pesticides that need excellent efforts to recognise new natural control agents **(Arokiyaraj *et al.*, 2008; Gangadevi *et al.*, 2008; and Brindha *et la.*, 2009)**.

Galangal (*Alpinia galanga*), is belonging to members of the zingiberaceae family. Its rhizomes have been extensively used as condiment for flavoring and local medicines. It is known to contain antimicrobial agents **(Deepa and Prabakaran 2012 and Anupama *et al.*, 2015)**.

The objective of the present study is to determine the antimicrobial bioactivity of *Alpinia 1galanga* rhizome extracts with different solvent systems like Ethanol, Acetonitrile and petroleum ether and identify the main component of the galangal extracts using GC/MS.

2.Materials and Methods

2.1.1 Plant Material: The Rhizomes of the *A. galanga* are existed in the local market. Identities of plants species were authenticated by referring standard literature. Rhizomes then cut to small pieces and powdered using electrical blender for further use.

2.1.2 Extract preparation:

Fifty grams of shaded dried powdered materials of rhizome were soaked in 300ml of each of the solvent Viz ethanal, petroleum ether (40-60°C) and acetonitrile for 72 hour at room temperature until complete exhaustion of the material. Each extract was stirred once every 24 hours using sterile glass rod. At the end of 72 hour each extract was fitrated through watmann No 1 filter paper then filtrate act concentrated by using vaccum rotary evaporator until final volume was 50ml. The extracts were stored in dark bottle and kept in refrigerator at 4°C **(Kochuthressia et al., 2012)**.

2.2 Microbial strains and culture media:

2.2.1 Bactrerial strains

Stock cultures of bacteria, used throughout this study were kindly provided by the laboratory of microbiology resources center (Cairo Mircen). Egypt microbial culture collection (EMCC). Faculty of agriculture, Ein Shams University. *Pectobacterium Carotovorum* SS. *Carotovorum* NRRL B- 4072, *Pseudomonas Syringae* SS. *Syringae*. DSM 50302, MP50, *Rhizobium radiobacter*, DSM30204 and *Corynebacterium glutamieum* 1220 T. They were

maintained on Nutrient agar slants and stored at 5°C for further studies.

2.2.2 Fungal species:

Phytopathogenic fungi that were used in this study are *Aspergillus Flavus, Aspergillus niger, Drechslera* hawaiiensis and *Humicola Fuscoatra*. They were provided from Fungicides, Bactericides and Nematicidis Department, Central Agricultural pesticides laboratory (CAPL). Each fungus were maintained on potato dextrose agar (PDA) and stored at 5°C for further studies.

2.3 Experimental procedure:

2.3.1 Antibacterial assay:

The plant extract were tested for their antitnicrobial activity by the well diffusion method **(Chung et al., 1990)**. This method depends on the diffusion of the various extracts from a cavity through the solidified agar layer of petri dish to an extract in which the growth of the added microorganism is prevented entirely in circular area or zone around the cavity containing the extracts **(Cote and Chemal 1994)**. Using micropipette 0.5ml of each of the media broth containing 10^5- 10^6 cfu/ml. The tested organisms were incubated on the three plates of solidified agar and were spread uniformly with a glass spreader. Then four well were cut out in the agar layer of each plate with an aluminum bore of 5mm diameter to contain 50µl extract and standard solvent. All the work was carried out in freeze for one day. After the addition of the extract it was allowed to diffuse the solution in the medium and then incubated for 37°C for 24 hours for antibacterial activity. After the incubation period the mean diameter of the inhibition zone was measured in mm.

2.3.2 Antifungal activities:

Antifungal activity was measured by using food poison technique elucidated by **Mohanty et al., (2012)**. All concentrations from this extract were added to 100ml of PDA media. The plates were left for 15mints to solidify. Then 5mm diameter of fungal colony punched with borer was placed onto plates containing media with extract in aseptic condition. Plates were incubated for 3.5 days at 28°C. The medium without any treatment served as control. The test fungi were inoculated and percent inhibition of mycelial growth was determined. After three days colony diameter was measured in centimeter. For each treatment three replicates were maintained. The fungi toxicity of the extracts in terms of percentage inhibition of mycelial was calculated by using formula:

$$\%I = \frac{C - T}{C} \times 100$$

Where; % I = percentage inhibition, C= Colony diameter in control, T= Colony diameter in treatment.

2.4 Analysis by GC- MS

For GC- MS analysis, the samples were injected into Agilent 6890 gas chromatograph equipped with an Agilent mass spectrometric detector, with a direct capillary interface and fused silica capillary column PAS- 5ms (30mmX0.25µm film thickness), Helium was used as carrier gas at approximately 1ml/ min_1., Pulsed splitless mode. The solvent delay was 3min. and the injection size was 1.0 µl. The mass spectrophotometeric detector was operated in electron impact ionization mode in joning energy of 70 ev. scanning from m/z 50 to 500. The ion source temperature was 230°C and the quadrupole temperature was 150°C, and the electron multiplier voltage (EM voltage) was maintained 1250v above auto tune. The instrument was manually tuned using perfluorotributyl amine (PFTBA). The GC temperature program was started at 80°C for 3 min then elevated to 280°C at the rate of 8°C/ min, and 10min. hold at 280°C the injector temperature was set at 280°C. Wiley and Nist 05 mass spectral data bas was used in the identification of the separated peaks.

Compound identification was obtained by comparing the retention times with those of authentic compounds and the spectral data obtained from Library data of corresponding compounds (CAPL).

2.6 Statistical Analysis

The data were analyzed statistically using SPSS analysis of variance technique and least significant difference test was assessed at 5% probability level to compare treatment means.

The concentration of the extracts that inhibiting the fungi mycelium growth by 50% (EC_{50}) values were determined by the linear regression (LPD) line computer program of the probit of the tested fungs percentage in hibition VS- Log the concentrations (ppm) of the tested extracts.

3. Results and Discussion

3.1 Antibacterial activities of extracts.

Three different solvent systems ethanol, petroleum ether and acetonitrile were used for antimicrobial extraction. The phyto-constituents of rhizome were extracted separately. Four different bacterial genus were used to evaluate antibacterial activity of *Alpinia galanga*. The activity of plant extracts diminished with decrease in solvent polarity., i.e ethanol > acetonitrile > petroleumenter. The results in Table 1 clearly indicated that among the three solvents used for the study, the activity of ethanol extracts were prevailed the two others. The potentials were assessed by the presence or absence of inhibition zones, (IZ). The inhibition zones for ethanol extracts ranged from 29.33mm (*C.glutamieum*) to 25.25mm (P.carotovorum S.S *Carotovorun*), 28.58mm for (*P. syringae*. S.S. *syringae*) and 25.83mm (*R.* vadiobacter).

This observation is in agreement with **Sathish Kumar et al., 2008 and Bualeea et al., 2007**, who reported that the ethanol extract produced strong inhibitory activities against some microorganisms. The acetonitrile extract showed reasonable activity against bacteria such as *P. carotovorum S.S carotovorum, R.* radiobaeter, *P. syringe S.S syringae* and *C.glutamieum* IZ values ranging 22.75mm, 18.92, 18.50 and 18.42, respectively. The petroleum ether rhizome extract showed higher level of inhibition zone in *P. carotovrum s.s carotovorum* (18.33mm) and *R. radiobacter* (17.0mm). The results are also in substantial agreement with many studies **(Burt, 2004; and Maillard 2002)**. The *P. carotovorum s.s carotovorum* was found to be the most sensitive to the

extracts. The varying degrees of sensitivity of the bacterial test organisms may be due to both the intrinsic tolerance of microorganisms and the nature and combinations of phytocompounds present in the extract (**Krittika Norajit *et al.*, 2007)**.

At present, however, the mode of action of extraction on microorganism is not fully understood. Nevertheless, in the view of their hydrophobicty, it is generally considered that they are involved in such mechanism as cytoplasmic membrane, coagulation of cell contents and disruption of the proton motive force **Burt (2004)**.

Table 1: Bactericidal activity of different solvent extracts of *A. galanga* rhizome express as zone inhibition.

Test bacteria	Diameter of inhibition zone (mm)			
	Solvent extract			
	Ethanol	Petroleum ether	Acetonitrile	
P. carotovorum S.S carotovorum	25.25^{B}	18.33^{DE}	22.75^{C}	16.58^{A}
C. glutamieum	29.33^{A}	15.17^{G}	18.42^{DE}	15.73^{AB}
P. syringae S.S syringae	28.58^{A}	15.42^{FG}	18.50^{DE}	15.63^{B}
R. radiobacter	25.83^{B}	17.00^{EF}	18.92^{D}	15.44^{B}
Mean	**27.25^{A}**	**16.48^{B}**	**19.65^{C}**	
Cont	0.0^{H}	0.0^{H}	0.0^{H}	

A,B,C….. means with the same letters are not significant difference.

3.2 Antifungal activity:

Rhizome ethanol extract showed maximum inhibition in A. Flavus (Ec_{50} 3237.0 ppm) while it was 4951.41ppm and 7337.0 in acetonitrile and petroleum ether respectively. Effectiveness of the tested extracts was regularly increased with an increase in the concentration. The lowest inhibitory effect was occurred to the radial growth of the tested fungi by petroleum ether, Ec_{50} ranged from 7337.0ppm for *A.vlavus* to 11033.7 to *H. fuscoatra*. All the extracts of *A. galanga* showed antifungal activity against, *A. flavus*, A. niger, *D. australiensis H. Fuscoatra* presented (Table 2, 3 and 4). Similarly activity against *C. albicaus*, *Trichophyton*, *colleotricum*, *Fusarium* and *Rhizopus* by Alpinia has also been previously described by other workers **(Trakranrungsieh and Khunkitti 2008., and Janssen and Scheffer, 1985)**.

Table 2: Antifungal activity of rhizome (*A. galanga*) extract by ethanol food poison technique.

Test fungi	% inhibition at different concentration (ppm)							Ec_{50}
	1000	2000	4000	6000	8000	10000	12000	
Aspergillus flavus	29.7	41.4	58.3	65.8	76.5	86.7	92.6	3237.0
Aspergillus niger	14.5	22.0	34.0	42.2	53.5	64.6	78.3	6449.0
Drechslera Hawaiiensis	14.7	22.0	31.0	41.3	52.3	63.8	73.3	6927.9
Humicola Fuscoatra	6.8	21.2	25.1	32.5	38.7	45.6	68.3	9033.7

Table 3: Antifungal activity of rhizome (*A. galanga*) extract by acetonerile food poison technique.

Test fungi	% inhibition at different concentration (ppm)							Ec_{50}
	1000	2000	4000	6000	8000	10000	12000	
Aspergillus flavus	23.4	35.6	44.1	62.3	71.2	81.5	85.8	4951.4
Aspergillus niger	14.3	21.7	30.5	43.0	51.1	60.9	69.7	7121.9
Drechslera Hawaiiensis	9.9	15.6	22.3	25.0	52.0	60.0	63.5	7737.7
Humicola Fuscoatra	9.2	14.4	19.8	21.2	33.7	54.7	55.6	9578.9

Table 4: Antifungal activity of rhizome (*A. galanga*) extract by petroleum-ether- food poison technique.

Test fungi	% inhibition at different concentration (ppm)							Ec_{50}
	1000	2000	4000	6000	8000	10000	12000	
Aspergillus flavus	12.3	16.7	30.4	42.9	58.3	65.8	86.7	7337.0
Aspergillus niger	9.0	14.0	22.1	34.0	42.0	53.5	75.6	8549.0
Drechslera Hawaiiensis	9.2	14.1	22.3	32.2	41.5	52.3	72.3	8927.9
Humicola Fuscoatra	9.7	21.4	26.6	32.0	38.7	45.8	62.0	11033.7

3.3 Identification of galangal extract compounds

The extracts of galangal obtained by three solvent, ethanol, acetonitrile and petroleum ether were analyzed using GC- MS. Through the use of the GC-MS, the main compounds of all extract were found to consist of 1,8- Cineal, trans- beta farnesene and farnesyl acetate, whereas α farnesene, B farnesene and caryophyllene were found in both of ethanol and petroleum ether and absence in acetonitrile. Each component has different retention time, the characterization of individual compounds were performed with the mass spectrometry (Fig1,2 and 3). 1,8 cineole is an oxygenated mono terpenes, while β Caryophyllene is a ssquiterpene. In addition, B-bisoabolene is Ethareterpenes. **Mallavarapu et al., (2002)** also reported similar main compounds in galangal, i.e., 1,8- cineole, α- fenchyl acetate and camphor. They reported the existence of 1,8- cineal, α-fenchyl acetate and camphor; in addition to 1,8- cineole as the main component of galangal, which is in agreement in this study.

The GC- MS analysis results of ethanol are presented in table 5 and Fig 1. Eighteen compounds were identified. The extract profile shows Benzofuran as the main compound (84.32%); other major compounds were barbituric acid, β farnesene 3.81 % and 3.29 respectively.

The profile of petroleum ether extract (Table 6) and (Fig 2) showed the most abundant compound, penta lene carboxlic acid (87.24%) as the major peak from 27 compounds. The other significant compounds were beta- farnesene, 1,4 dihyrophenan threne and coumarin (3.10%, 1.86% and 1.67) respectively.

Table 5: GC- MS results of Phizome *A. galanga* extracted with Ethanol.

Name of the compound	Rt (min)	Percentage %
1,8 Cineole	4.95	1.19
P- Mentha	10.61	0.15
Neryl acetates	11.31	0.19
Caryophyllene	11.96	0.09
Beta- Farnesene	12.47	3.29
4- Ethoxychroman	12.80	1.59
α- Farnesene	13.30	0.19
Eugenol acetate	13.67	0.69
Benzenamine	13.90	0.43
Pyrazolo	14.29	0.98
Benzofuran	15.64	84.32
2- Butanone	16.78	0.41
Barbituric Acid	17.49	3.81
Farnesyl Acetate	17.94	1.12
Trans – Isocroweacin	18.19	1.56
Ethyl Palmitate	19.76	0.22
Ethyl oleate	21.76	0.07
Ethyl stearate	22.04	0.08

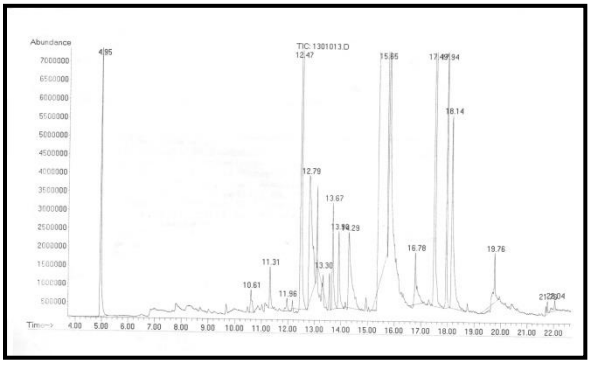

Fig. 1: Chromatogram of positive active compound from rhizome *A. galanga* extracted with ethanol.

Table 6: GC- MS results of rhizome *A. galanga* extracted with Petroleum-ether.

Name of the compound	Rt (min)	Percentage %
1,8 Cineole	4.95	0.92
2- Alpha-Dimethyl aminocholes	11.19	0.00
2,6 Octadien	12.32	0.04
Trans- caryophllne	12.42	0.10
α- Farnesene	12.64	0.08
Beta. Farnesene	12.93	3.10
Caryophyllene	13.35	0.05
Germacrene- D	13.47	0.10
Pentadecane	13.58	1.37
Beta- Bisabolene	13.90	0.21
Beta- sesquiphellandrene	14.15	0.15
Trans-Gama- Bisabolene	14.28	0.06
Ethyl eugenol	14.54	0.11
Fugenol acetate	14.58	0.13
4- chromanal	15.11	0.14
2-methyl-4- trifluoromethyl	15.11	0.25
Zingiberenol	15.63	0.09
3 methoxy-5methyl- benzaldehyde	15.74	0.04
Penta lenecar boxylic acid	16.26	87.24
Pentodecene	16.37	0.97
2,4 Dimethyl enzoic acid	16.50	0.17
Heptadecane	16.66	0.06
1,4- dihydrophenanthrene	18.29	1.86
Farnesyl acetate	18.76	0.84
Coumarin	19.13	1.67
Tetra carbonitril	19.60	0.16
Piperonal	19.74	0.08

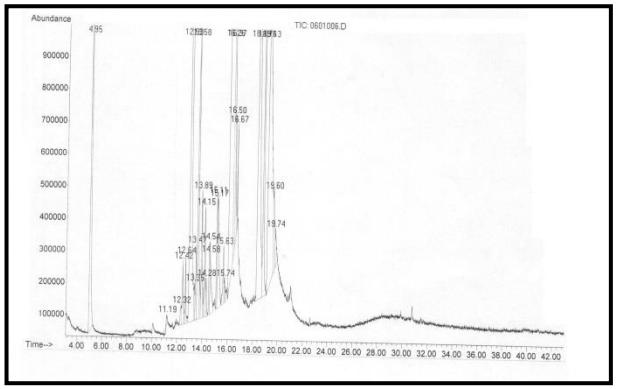

Fig. 2: Chromatogram of positive active compound from rhizome *A. galanga* extracted with petroleum ether.

The less compound obtained from acetonitrile extract in table 7 and Fig. 3. 2,5 dimethyle benzoate (90.20%) as the major compound from seven. The other significant compounds trans beta- farnesene, coumarin and barbituric acid were 2.99%, 2.54% and 2.43%, respectively. These results is different from that described by other authors **(Mallavarapu *et al.*, 2002; Oonmetta- aree *et al.*, 2006)** because the composition of any plant is influenced by several factors such as planting, climatic seasonal and experimental conditions **(Daferera *et al.*, 2000)**.

Table 7: GC- MS results of rhizome *A. galanga* extracted with acetonitrile

Name of the compound	Rt (min)	Percentage %
1,8 Cineole	4.98	0.49
trans- beta. farnesene	12.93	2.99
Pentadecane	13.58	0.51
2,5 dimethylebenzoate	16.35	90.20
Barbituric acid	18.27	2.43
Farnesyl Acetate	18.76	0.83
Coumarin	18.99	2.54

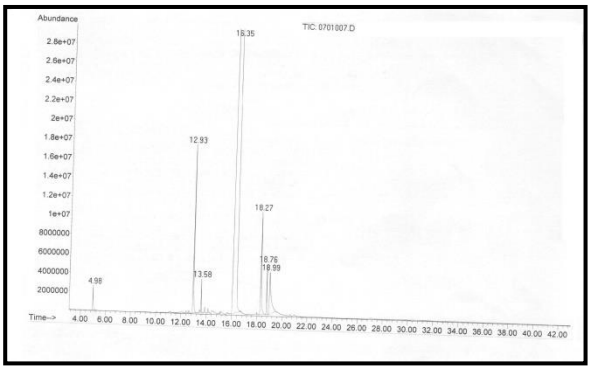

Fig. 3: Chromatogram of positive active compound from rhizome *A. galanga* extracted with Acetonitrile.

In summary, the major compounds in three solvents from rhizome *A. galanga* were (Benzofuran, Beta Farnesene, barbituric acid, penta lenecarboxylic acid, pentadecan, 1,4- dihydrophenathrene, coumarin, 2,5 dimethyle benzoate and barbituric acid). Generally it could be considered that their mechanism of action may be result as the influence on the mechanism such as cytoplasmic membrane, coagulation of cell contents and disruption of the proton motive force **(Burt, 2004)**. There fore a galangal can be used for preservation of food, as it possesses characteristic flavour as well as antimicrobial activity.

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