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GCMS Based Characterization of Compounds Present in the Latex of Manilkara zapota, Plumeria acuminate, Tabernaemontana coronaria and its Evaluation for Antimicrobial Activity and Allelopathic Effect

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Abstract*— About 10% of all flowering plant species (angiosperms) exude latex upon tissue damage and this latex has no known function in primary metabolism. It is more phytochemically diverse than resins, mucilages, and gums, and often contains complex mixtures of secondary metabolites. In the previous study it was found that latex of Manilkara zapota, Plumeria acuminate and Tabernaemontana coronaria has potential bioactives. Present study investigated the volatile components of latex in Manilkara zapota, Plumeria acuminate and Tabernaemontana coronaria using GCMS analysis. The latex extracts were further evaluated for their antimicrobial action and allelopathic effect on seed germination. GC-MS analysis revealed that latex extracts of all three plant species under study contain a variety of chemical compounds. These components changed when extraction was carried out at variable temperatures. A detrimental response in seed germination study was observed in the test species. The test organisms hinted at an antimicrobial effect of these latex extracts. Thus latex exuded from these plant species and the extracted compounds may have a potential to be used as natural herbicides or disinfectants.

Index Terms: GC-MS, latex, allelopathic effect, antimicrobial activity.

I. INTRODUCTION

Latex is a milky white fluid secreted by various plant species. Latex is well known for its sticky properties, which have been used to produce rubber (from *Heveabrasiliensis Euphorbiaceae* and other species), chicle from *Manilkara spp. (Sapotaceae)* used in chewing gum. Latex from various plant species contains bioactive compounds including alkaloids such as morphine in Papaver spp. (*Papaveraceae)*; cardiac glycosides in Asclepias spp. (*Apocynaceae*); terpenes such as the sesquiterpene lactone, lactucin, from lettuce (Lactuca spp. Asteraceae); and digestive cysteine proteases in *Carica papaya* (Caricaceae) and Ficus spp.[1] Pharmaceuticals used in modern medicine aspirin, atropine, ephedrine, digoxin, morphine, quinine, reserpine and tubocurarine serve as examples of drug discovered through observation of indigenous medical practices. Polyphenols are the most numerous and widely distributed class of phytochemicals many polyphenols, particulary the flavonoids, had been found to possess relatively potent antioxidant, antiatheroscleortic, antitumour, antiinflammatory, antimutagenic and antiviral activities.[2]. While many synthetic drugs and natural bioactives are discovered, it is also known in general that bacteria have the genetic ability to transfer and gain resistance to drugs used as therapeutic agents. The only way to prevent antibiotic resistance is by using new compounds which have not based on the existing synthetic anti-microbial agents [3] On the other hand, allelopathy concerns the effects of one plant on another due to chemical released by them or the breakdown of their metabolites. It is expected to be an important mechanism in the plant invasion process because the lack of co-evolved tolerance of resident vegetation to new chemical produced by the invader could allow these newly arrived species to dominant natural plant communities. [4] Thus study of plant bioactive components could address some of these emerging issues.

II. REVIEW OF LITERATURE

Papaya latex is effectively useful for therapeutic purpose against dyspepsia and is externally functional to burns and scalds. This milky white substance is antagonistic to fungal growth, especially for *Candida albicans* and act as folk medicine to treat skin eczema caused by this fungus. A leaves and young fruit extract are inimical to intestinal worms and successfully use for the treatment of boils [5] Studies have shown that Asclepias latex, the protoplasmic content derived from the laticiferous cell which characterizes this genus, contains specialized substances including the poison, asclepione, a proteolytic enzyme, cardiac glycosides toxic to

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Vol 11, Issue 8, August 2024

birds, and other substances poisonous to livestock. These plants and their latex were extensively used as folk medicines, including the treatment of various forms of cancer. [6] The aqueous and ethanol extract of Calotropis procera leaf and latex were investigated for antimicrobial activity against pathogenic organisms (Eschericia coli, Salmonella typhi, Bacillius subtilis, Candida albicans, Aspergillus niger) using the Agar well diffusion method. The ethanolic latex extract showed significant activity against all the test organisms and demonstrated strong and better inhibitory activity on the test organisms than the leaf extract. [7] Studies on the allelopathic potentiality of Heveabrssiliensis leaf extract, on germination and seedling growth of four common legumes (Cicerarietinum, Vignamungo, Lensculinaris and Vigna radiate) have shown species specific effect. Both the aqueous (0.62% -2.5%) and methanol (0.1 mg/ml – 0.7mg/ml) extract of leaf were strongly toxic and hinder the seed germination of three test crops, Cicerarietinum, Lensculinaris and Vignaradiata, in very low concentration. [8]

III. MATERIALS & METHODS

A. Extraction of components

Extraction of latex was carried out using methanol as solvent and the extract was subjected to agitated hot maceration at 45˚ Celsius and 60˚ Celsius in a rotary shaker cum incubator for 24 hours at 100rpm. Extracts were filtered using 0.22um syringe filters and used for GCMS analysis. Aqueous extracts were also prepared at 45˚ celsius using the same procedure and used in study of allelopathy and antimicrobial activity.

B. GC-MS analysis

GC-MS analysis of methanolic extracts was performed on a Shimadzu GC-2010 Gas Chromatography with Shimadzu GCMS-QP2010 Ultra mass spectrometer system equipped with a Rtx-5MS capillary column (30 m x 0.25 mm id, film thickness 0.25 µm). The oven temperature was programmed from 60-280°C at the rate of 10°C/min. The ion source was set at 220 °C and electron ionization at 70 eV. Helium was used as the carrier gas at a flow rate of 1.32 mL/min. Scanning range was 55 to 600 m/z. The mass spectrum of the compounds present in the latex extracts was compared with the spectrum of the known components stored in the NIST and NIST 11 library.

C. Study of Antimicrobial activity

Minimum Inhibitory Concentration test of the aqueous latex extracts was performed on the strains of *Escherichia coli* (ATC 1133D) and *Staphylococcus aureus* (ATC 6538P). Half strength nutrient broth was used as media. Negative controls were setup to check the microbial load of extracts and positive control was setup to evaluate the viability and growth of the selected test organisms. All the control tubes and test tubes were incubated in a bacteriological incubator at 37 ˚ celsius for over 48 hours. Test readings were taken on colorimeter at 520 nm over 48 hours. However, the viability of the organisms cannot be predicted by optical density of the MIC tubes. Hence, the spread plate method was used further to check the viability and assess efficacy of the aqueous extracts against the microbes. The plates were incubated and observed.

D. Study of Allelopathic effect

Vigna radiata (Mung) seeds were selected as test seeds from the crop family. The experiment was set up in triplicates of 10 seeds each in sterile petri plates lined with Whatmann filter paper No.41. HPLC grade water (3 ml in each plate) was used to moisten the plates and provide the seeds with a suitable growth environment. No treatment was given to the seeds in control plates. 30 seeds each were dipped in the crude latex aqueous extracts of *M.zapota, P.acuminate* and *T.coronaria* for 30 minutes. These seeds were then arranged in sterile petri plates in sets of 10 and studied for next 7 days. Seed germination and root or shoot length if any was checked on all days for every seed.

IV. RESULTS AND DISCUSSION

A. GCMS analysis of Methanolic latex extracts

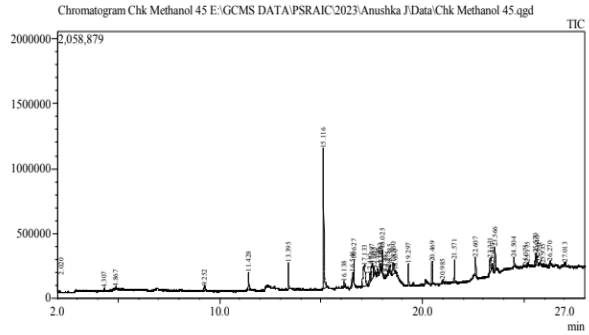

Fig. 1. a) GCMS spectrum of M.zapota extracted at 45 ˚ Celsius.

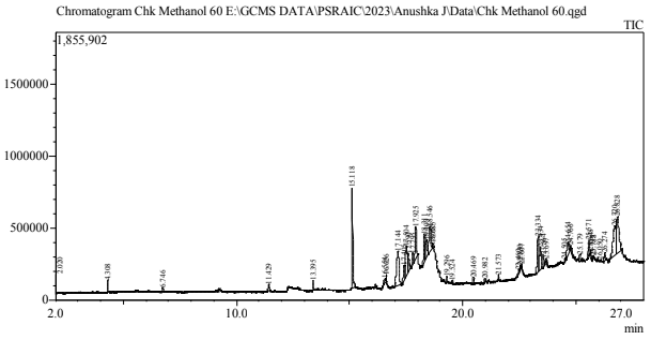

Fig. 1. b) GCMS spectrum of M.zapota extracted at 60 ˚ Celsius.

ISSN (Online) 2456 -1304

International Journal of Science, Engineering and Management (IJSEM)

Vol 11, Issue 8, August 2024

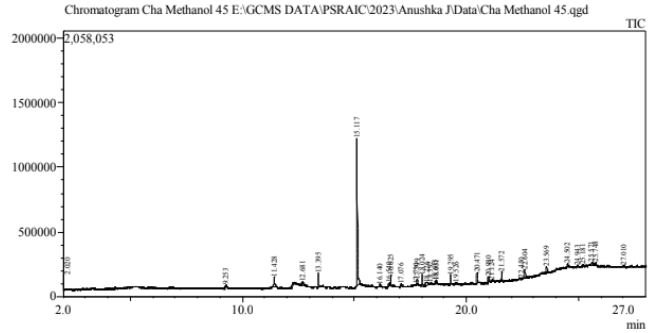

Fig. 1. c) GCMS spectrum of P.acuminate extracted at 45 ˚ Celsius.

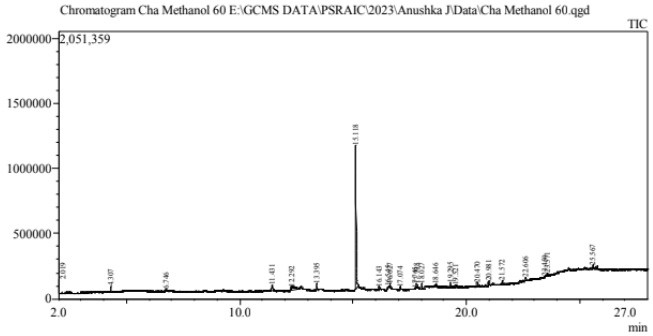

Fig. 1. d) GCMS spectrum of P.acuminate extracted at 60˚ Celsius.

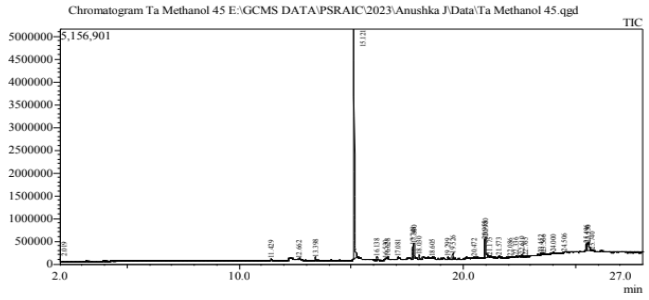

Fig. 1. e) GCMS spectrum of T.coronaria extracted at 45 ˚ Celsius.

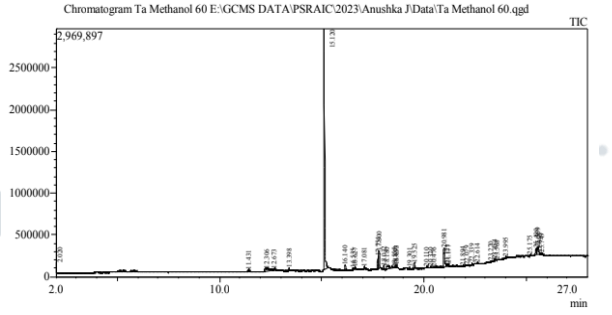

Fig. 1. f) GCMS spectrum of T.coronaria extracted at 60 ˚ Celsius.

Table I: GCMS compounds identified from Manilkara zapota methanolic extract.

***Manilkara zapota* methanolic extract**		
Extraction at 45^0 celsius		
Retention time	Compound	Mol.wt
11.428	Cycloheptasiloxane, tetradecamethyl- $$ 2,2,4,4,6,6,8,8,10,10,12,12,14,14-Tetradecamethylcycloheptasiloxane # $$ Tetradecamethylcycloheptasiloxane	518
13.395	Cyclooctasiloxane, hexadecamethyl- $$ Hexadecamethyl-cyclooctasioxane $$ Hexadecamethylcyclooctasiloxane $$ 2,2,4,4,6,6,8,8,10,10,12,12	592
16.546	Phthalic acid	278
16.627	Cyclodecasiloxane, eicosamethyl-	740
17.133	l-Valine, n-pentafluoropropionyl-, isobutyl ester	319
17.798	beta.-Alanine, n-pentafluoropropionyl-, butyl ester	291
18.025	Cyclooctasiloxane, hexadecamethyl-	592
18.560	Eseroline, tertbutylcarbamate(ester)	317
21.571	Cyclononasiloxane, octadecamethyl-	666
23.331	Pyrazine, 2,5-bis(1,1-dimethylethyl)-, 1,4-dioxide	224
24.504	Silicic acid, diethyl bis(trimethylsilyl) ester	296
25.570	7-Hydroxy-7,8,9,10-tetramethyl-7,8-dihydrocyclohept	252
Extraction at 60 ^{0} celsius		
Retention time	Compound	Mol.wt
2.020	5H-Benzo[b]pyran-8-ol, 2,3,5,5,8a-pentamethyl-6,7,8,8a	222
4.308	Cyclotetrasiloxane, octamethyl-	296
11.429	Cycloheptasiloxane, tetradecamethyl	518

ISSN (Online) 2456 -1304

International Journal of Science, Engineering and Management (IJSEM)

Vol 11, Issue 8, August 2024

***Manilkara zapota* methanolic extract**		
13.395	Cyclooctasiloxane, hexadecamethyl-	592
16.554	Urs-12-ene, 3-methoxy-, (3.beta.)-	440
17.144	Urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+)-	468
18.403	Lup-20(29)-en-3-ol, acetate, (3.beta.)-	468
18.546	Betulin	442
19.296	Tetracosamethyl-cyclododecasiloxane	888
19.524	Stannane, diethenyldimethyl	204
20.469	Cyclodecasiloxane, eicosamethyl-	740
23.334	Lup-20(29)-en-28-oic acid, 3-hydroxy-, methyl ester	470
23.564	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl	578
23.697	Taraxasterol	426
26.828	.alpha.-Amyrin	440

Table II: GCMS compounds identified from *Plumeria acuminate* methanolic extract.

***Plumeria acuminate* methanolic extract**		
Extraction at 45 ^{0} Celsius		
Retention time	Compound	Mol.wt
11.428	Cycloheptasiloxane, tetradecamethyl-	518
12.681	L-Valine, N-(trifluoroacetyl)-, 1-methylpropyl ester	269
13.395	Cyclooctasiloxane, hexadecamethyl-	592
17.076	Isoamyl nitrite	117
18.024	1,3,5,7,9-Pentaethyl-1,9-dibutoxypentasiloxane	500
19.295	Cyclononasiloxane, octadecamethyl-	666
19.526	Oxirane, (butoxymethyl)-	130
21.124	1,1,3,3,5,5-Hexamethyl-1,5-bis(2-methylpropoxy)trisiloxane	352
22.450	3,3-Dibutoxy-1,1,1,5,5,5-hexamethyltrisiloxane	352
25.571	Silicic acid, diethyl bis(trimethylsilyl) ester	296
Extraction at 60 ^{0} Celsius		
Retention time	Compound	Mol.wt
4.307	Cyclotetrasiloxane, octamethyl-	296
11.431	Cycloheptasiloxane, tetradecamethyl-	518
12.292	3-Cyano-5,5-dimethoxycarbonyl-N-methylisoxazolidine	228
13.395	1,3,5,7,9-Pentaethyl-1-butoxycyclopentasiloxane	442
18.027	1,3,5,7,9-Pentaethyl-1,9-dibutoxypentasiloxane	500
19.521	Oxirane, (butoxymethyl)-	130
22.606	3,3-Dibutoxy-1,1,1,5,5,5-hexamethyltrisiloxane	352
25.567	Silicic acid, diethyl bis(trimethylsilyl) ester	296

Table III: GCMS compounds identified from *Tabernaemontana coronaria* methanolic extract.

***Tabernaemontana coronaria* methanolic extract**		
Extraction at 45 ^{0} Celsius		
Retention time	Compound	Mol.wt
2.019	Stannane, diethyldimethyl-	208
11.429	Cycloheptasiloxane, tetradecamethyl-	518
12.662	Phthalic acid, 3-bromobenzyl ethyl ester	362
13.398	Cyclooctasiloxane, hexadecamethyl-	592
16.138	Tricyclo[4.2.2.0(2,5)]dec-7-ene, 7-(5-hexynyl)-	214

ISSN (Online) 2456 -1304

International Journal of Science, Engineering and Management (IJSEM)

Vol 11, Issue 8, August 2024

***Tabernaemontana coronaria* methanolic extract**		
16.530	Stannane, chlorotrimethyl-	200
16.628	Cyclodecasiloxane, eicosamethyl-	740
17.081	Stannane, triethenylmethyl-	216
17.748	2-Amino-3-cyano-4,5-decamethylenethiophene	262
18.030	Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost	430
18.605	2-Trimethylstannyl-5-trichloromethyl-6-chlorobicyclo[2.2.1]hepta ne	410
19.299	Tetracosamethyl-cyclododecasiloxane	888
20.955	5,10-Pentadecadien-1-ol, (Z,Z)-	224
20.980	9-[4-Hydroxybutyl]hypoxanthine	208
21.175	Astaxanthin	596
22.086	5H-Benzo[b]pyran-8-ol, 2,3,5,5,8a-pentamethyl-6,7,8,8a-tetrahydro-	222
22.336	1,2-Dimethoxy-4-(1-methoxy-1-propenyl)benzene	208
22.610	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl	578
24.000	Silicic acid, diethyl bis(trimethylsilyl) ester	296
Extraction at 60 ^{0} Celsius		
Retention time	Compound	Mol.wt
12.306	3-Cyano-5,5-dimethoxycarbonyl-N-methylisoxazolidin	228
12.673	L-Valine, N-(trifluoroacetyl)-, 1-methylpropyl ester	269
13.398	1,3,5,7,9-Pentaethyl-1,9-dibutoxypentasiloxane	500
17.800	Oxalic acid, butyl cyclobutyl ester	200
21.894	1,1,3,3,5,5-Hexamethyl-1,5-bis(2-methylpropoxy)trisiloxa	352
22.339	2,6-Lutidine 3,5-dichloro-4-dodecylthio-	375
22.614	3,3-Dibutoxy-1,1,1,5,5,5-hexamethyltrisiloxane	352
23.270	Tris(tert-butyldimethylsilyloxy)arsane	468
23.995	Silicic acid, diethyl bis(trimethylsilyl) ester	296
25.490	7-Hydroxy-7,8,9,10-tetramethyl-7,8-dihydrocyclohep	252

GCMS analysis of all three plant species under study showcased presence of wide variety of chemical compounds. The effect of extraction temperatures is also observed as most of the compounds extracted are different at different extraction temperatures. Thus the developed GCMS method is found to be suitable for simultaneous identification of compounds from the latex of all three plant species.

B. Study of antimicrobial activity of latex extracts each of 250ppm, 500ppm and 1000ppm from all three plant species on E.coli and S.aureus

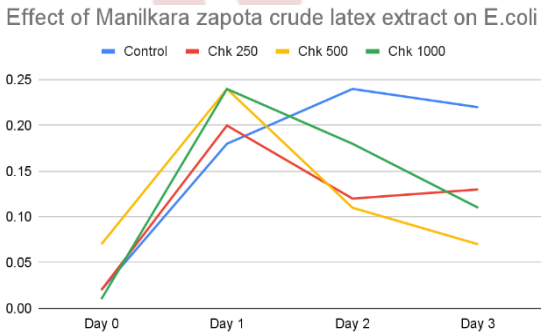

Fig. 2. a) Effect of M.zapota aqueous latex extracts on optical density of E.coli

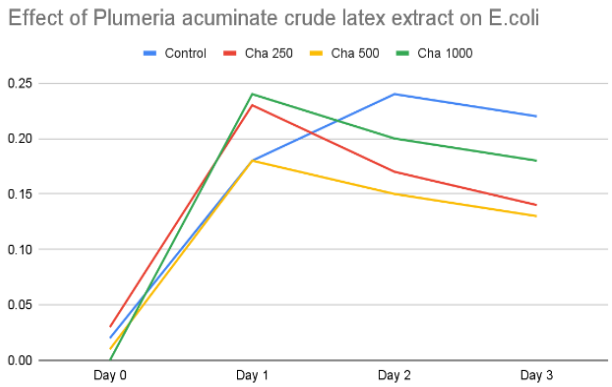

Fig. 2. b) Effect of P.acuminate aqueous latex extracts on optical density of E.coli

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International Journal of Science, Engineering and Management (IJSEM)

Vol 11, Issue 8, August 2024

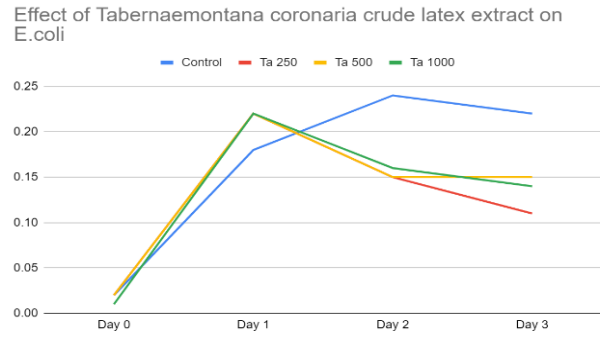

Fig. 2 c) Effect of T.coronaria aqueous latex extracts on optical density of E.coli

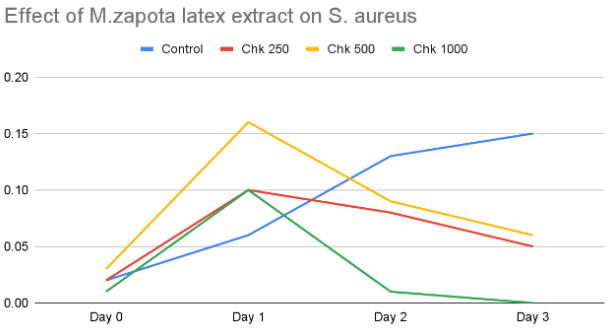

Fig. 2. d) Effect of M.zapota aqueous latex extracts on optical density of S.aureus

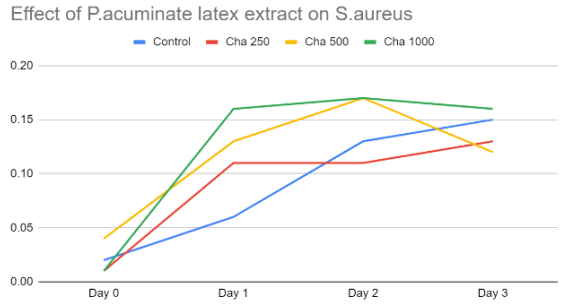

Fig. 2. e) Effect of P.acuminate aqueous latex extracts on optical density of S.aureus

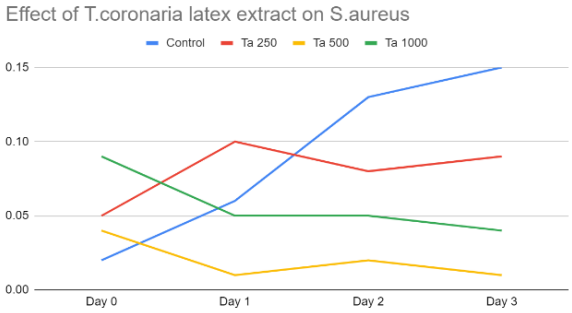

Fig. 2. f) Effect of T.coronaria aqueous latex extracts on optical density of S.aureus

After comparing the effect of latex extracts of three plant species it is observed that 500 ppm and 1000 ppm concentrations of all three plant latex extracts are inhibiting the growth of *E.coli.* It is also observed that *M. zapota* latex extract and *T. coronaria* latex extract are showing inhibitory effect on growth of S.aureus whereas *P.acuminate* latex extract is not showing any significant inhibition on the growth of *S.aureus.*

C. Allelopathic effect on seed germination of Vigna radiate (mung) seeds

Table IV: Average root length response recorded for all the three latex treated plates in the roots of V.radiata.

	Average root length (in cm)			
	Control	**M. zapota aqueous latex extract treated plate**	**P. acuminate aqueous latex extract treated plate**	**T. coronaria aqueous latex extract treated plate**
Day 0	0	0	0	0
Day 1	0.713	0.163	0.137	0.43
Day 2	2.34	1.043	0.68	1.2434
Day 3	3.03	1.433	1.0634	1.61
Day 4	3.41	1.433	1.1134	1.767
Day 5	3.58	1.433	1.1134	1.88
Day 6	3.58	1.433	1.1134	1.88
Day 7	3.58	1.433	1.1134	1.88

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Vol 11, Issue 8, August 2024

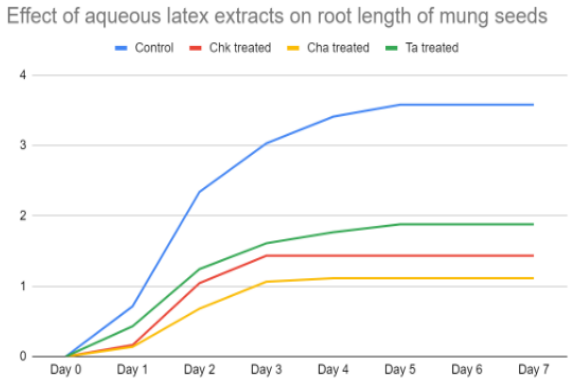

Fig. 3. Effect of aqueous latex extracts on average root length of V.radiata(mung) seeds.

Control plate was subjected to distilled water. The roots show an increase in their length with each passing day when recorded for seven days in the control plates. The *M.zapota*, *P.acuminate* and *T.coronaria* extract treated plates show a stunted growth in roots. Thus presence of compounds acting as allelopathic agents is suspected in all three aqueous latex extracts. Thus the extracts can be tested and optimized for a natural herbicide effect in other plant and weed speices.

V. CONCLUSION

Gas chromatography mass spectrometry was used as a tool to investigate the presence of bioactive compounds present in the latex of *M.zapota*, *P.acuminate* and *T.coronaria*. The method developed was found to be effective for simultaneous identification of compounds present in all three samples and thus can be used as a qualitative method. The results showed majority of different bioactive compounds in the latex of all three species and they were found to be different at different extraction temperatures. Thus the stability of these compounds can also be evaluated in future as stability can influence their activity. All three latex extracts were found to contain active antimicrobial agents which acted selectively on the test species *E.coli* and *S.aureus*. The aqueous latex extracts also showed inhibition against seed germination response in *V.radiata* seeds and thus can be used as potent natural herbicides in lower concentrations.

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