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Research Article

Agriculture for Better Food

Ethno pharmacological Efficiency of *Andrographis Paniculata* against Tuber Rot Disease of *Manihot Esculenta* (Cassava)

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Abstract: Cassava is a chief and energy rich carbohydrates food, which might be the reason many parasitic fungi adhere within the plant that causes huge damage to the crop during the post harvesting stage or storage period. In the present study, the ethno-based possible eradication of parasitic fungi was highlighted. *Andrographis paniculata* Burm. f. Nees and *Stachytarpheta indica* were chosen for its popular medicinal values and the phytochemical contents were characterized. Its potency showed an inhibitory effects against tuber rot disease causing fungal pathogens *viz*., *Rhizopus* sp, *Mucor* sp., *Aspergillus niger*, *Aspergillus flavus* and *Fusarium oxysporum* in Cassava by disc – diffusion methods. The diameter of the zone of inhibition at various concentrations (2.5, 5, 7.5 and 10 w/v) of leaf extracts was observed with the maximum in *A. paniculata* (20mm with 10 w/v conc.) against *Fusarium oxysporum* followed by *A. niger* (17mm at 10w/v conc.). The preliminary phytochemical screening of *Andrographis paniculata* revealed the presence of phytoconstituents like alkaloids, flavonoids, phenols, and tannins. In GCMS analysis, Benzene,1,2,3-trimethoxy-5-(2-propenyl)-, Cyclopenta [c] pyran-4-carboxylic acid, 7-methyl-, methyl ester and 9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)- covered the high areas, that might be responsible or can possess the antifungal activity against Tuber root rot causing fungal pathogens. Furthermore, extensive studies are recommended to characterize each compound in the crude extracts of *A. paniculata* to enhance or to acclaim remedy for various diseases in plants. This study reveals the acknowledgement of the medicinal plant *A. paniculata* for its remarkable organic or eco-friendly fungicide potential against cassava tuber rot diseases.

Keywords: Antifungal activity, *Andrographis paniculata*, *Manihot esculenta* (Cassava), Tuber rot disease, GC-MS

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1. INTRODUCTION

Manihot esculenta Crantz is a dicotyledonous perennial woody shrub with an edible starch root, belongs to the family Euphorbiaceae which is commonly known as "Cassava". It is the most important staple food which is ready and cheap carbohydrate rich food and feed for livestock. The crop is concentrated within the southern states of Kerala, Tamil Nadu and Andhra Pradesh due to the favourable Climate and efficient utilization. Tamil Nadu has an area of 95,000 ha - 40% of the total area under Cassava in India and 60% utilized industrially to produce starch, sago and other value-added products. Also, for the production of bioethanol. Hence, it is an important engine for economic growth and development in many cassava producing countries of the world[1]. Despite the importance of cassava in the world, its production potentials are still undetermined by the activities of various disease agents which constitute serious production challenges that greatly reduce yield in many developing countries[2-3]. Different control measures have been suggested to control cassava tuber rot diseases especially, curing, use of resistant variety, and use of chemicals. Various types of synthetic fungicides have been used to control but major disadvantages are the development of resistance to many of the currently used chemicals in circulation, pollution of soil, ground and surface water [4-5]. Biological control, based on use of plant products as well as use of antagonists is the popular alternative of synthetic fungicide[6]. Some plants are known to synthesize phytochemical compounds with antimicrobial potential used successfully in the control of diseases in crops such as cassava, tomato, cowpea, rice, etc.[7-9]. The advantages of using these natural plant products are enormous and include little or no toxicity, local availability, biodegradability [10-11]. *A. paniculata* is an erect annual herbaceous plant which is extremely bitter in taste in all parts of the plant body native to India and Sri Lanka. It is widely cultivated in Southern and South eastern Asia, where it has been traditionally used to treat infections and some diseases [12-13]. 3-O-β-D-glucosyl-14-deoxyandrographolide, 14-deoxy andrographolide and 14-deoxy-11, 12-didehydro andrographolide are some phytoconstituents possess antifungal potential reported in *A. paniculata*[14]. Flavonoids present in plants showed potent inhibition of collagen, arachidonic acid, thrombin and platelet activation factor induced platelet aggregation. Furthermore, a diterpenoid compounds from leaves and roots possess the moderate vasorelaxing effect in rat thoracic aorta[15]. Moreover, ethanolic extract of plant inhibits adherence of *Streptococcus mutans* ATCC 25175 and *S. mutans* TPF-1 *in vitro* at the effective concentrations (0.5%). Whereas, antimicrobial and antifungal activity of aqueous extract of plant were reported in the sensitivity of the keratinophilic fungi on dry-weight method [16-18.] *Stachytarpheta indica*, commonly known as snake weed locally known for its Abortifacient, and in the management of Asthma, Headache, Alopecia, Bronchitis, Bruise, Chest Cold, Constipation, Itch, Diarrhea, Skin Sore, Vermifuge, Dysentery, Dysmenorrhea, Erysipelas, Fever, Inflammation, Liver Disease, Poisoning, Tumor, Venereal Disease, Cataract, Sedative, Anti-Fertility, Rheumatism [19]. In northern Nigeria, decoction of the leaves with natron is given for dysentery in humans and for similar conditions in horses[20]. Ethnobotanical, methanolic extracts of stems bark of *Stachytarpheta indica* exhibited antibacterial potential against *S. aureus, P. aeruginosa, E. coli, S. typhi* and *Shigella* spp [21]. Hence, the present study was taken up with the preliminary idea that the plant extracts of *Andrographis paniculata* and *Stachytarpheta indica* suggested for the management of the disease will be a preferred option since they are readily available with less complex preparation and application procedures. The objective focused on profiling phytoconstituents of potent plant based on its antifungal activity against the fungal species causing tuber rot disease of cassava.

2. MATERIALS AND METHODS

2.1 Collection of plant material

The leaves of *A. paniculata* and *S. indica* were collected from Kolli Hills, Namakkal district, South India (Lat:11.2485°N; Long:78.3387°E). The plant was identified by the Botanical Survey of India (Southern Circle), Regional Office, Coimbatore, Tamil Nadu, India. The voucher specimens (BOT – AAGAC – 005 - *A. paniculata* & BOT – AAGAC – 028 - *S. indica*) were deposited in the Department of Botany, Arignar Anna Government Arts College, Namakkal, Tamil Nadu, India. Local Tamil names of the plants – Nilavaembu or Siriyanangai (*A. paniculata*), Seemai Nayuruvu (*S. indica*). The fresh fully-grown plant leaves were selected. Collected plant leaves were cleaned to remove mud and other adhering weed plants. Fresh leaves were dried at shade dried for 2-3 days. The dried leaves samples were mechanically powdered, sieved using 80 meshes and stored in an airtight container. These powdered materials were used for further analysis[22].

2.2 Preparation of extract

The shade dried leaves were subjected for pulverization in order to get fine coarse powder. A known quantity of each grounded plant material was soaked separately in methanol for 48hrs and filtered through 4-fold muslin cloth followed by Whatman filter paper. These extracts were concentrated to dryness in a flash evaporator under reduced pressure and controlled temperature (40-50 C) to get crude extract.

2.3 Isolation of pathogenic fungi

Isolation of rotted tubers of cassava was made directly from the fields which were washed and surfaced sterilized with 1% mercuric chloride solution. Pieces of the rotted cassava tissue (5 mm) were directly plated on solidified potato dextrose agar in petri dishes using a flame-sterilized scalpel. The inoculated plates were incubated at room temperature (27 °C for 5-7 days) and observed daily for emergence of colonies. Sub-culturing was obtained by hyphal tip technique and maintained in agar slants. The isolates were identified by microscopic observation using Lactophenol cotton blue staining method. A four-day-old culture of the fungus was prepared for the stock culture and used throughout the duration of the experiment.

2.4 Antifungal assay

Agar diffusion method was carried out for the assessment of the methanolic extract of A. *paniculata* and *S. indica* against the test pathogenic fungi. One hundred microlitres of inoculums (10^6 CFU/ml) of each test fungus were spread evenly using a sterile glass spreader onto Sabouraud dextrose agar plates[23]. The plates have been kept to dry and a sterile cork borer (5 mm in diameter) was then used to punch wells in the agar medium at different sites on the plates. Subsequently, wells were filled with various

concentrations of leaf extracts (2.5, 5.0, 7.5 and 10 mg) and allowed to diffuse at room temperature for 2h. DMSO solvent was used for negative control whereas Ketoconazole as positive control. The inoculated plates were incubated at 25 °C for 3 days. The minimum inhibitory concentration (MIC) should be recorded as the lowest concentration of the plant extract that inhibits the growth of the test organisms. The diameter of inhibition zones was recorded. Triplicate was maintained for each assay.

2.5 Gas Chromatography-Mass Spectroscopy (GC-MS)

Methanolic plant extracts (*A. paniculata*) were subjected to GC-MS (GC-MS-5975C (AGILENT)) analysis, to find out the active compounds. Chromatography was performed on a DB-Wax capillary column (30 m×0.25 mm ID and 0.25 µm film thickness). The carrier gas was helium at a flow rate of 1.0 ml/min and 1 µl of sample was injected. The injector and detector temperatures were 230 °C and 200 °C, respectively. The column oven was programmed as follows: initial temperature 60 °C, initial time 2.0 min, program rate 10 °C/min; final temperature 250 °C; final time 10 min. The samples were then dissolved in CH_2Cl_2 and a split injection technique was recommended. The identification of the compounds was based on comparison of their retention indexes (RI), obtained using n-alkanes (C_{11}-C_{31}) and retention time and also confirmed by comparison of their mass spectra with the NIST/NBS - Wiley library data. Relative percentage amounts were calculated from TIC (Total Ion Chromatogram) by the computer[24].

3. RESULTS AND DISCUSSION

3.1 Isolation of pathogenic fungi

In the present investigation, *A. flavus, A. niger, Mucor* sp., *Rhizopus* sp. and *Fusarium oxysporum* were isolated from the infected Cassava (Figure 1). Based on the morphology of the fungal colony, mycelia as well as the characteristic of the conidia, the fungus was identified. Many fungal diseases of cassava have been reported in some countries of Africa such as the Republic of Congo, Tanzania, Togo, Nigeria, Uganda and other world parts. *Phytophthora drechsleri* [25], *Sclerotium rolfsii* [26] and *Rosellinia nectarix* [27]. The present study supported the isolated fungal strain of *Fusarium oxysporum*, *Botryodiplodia theobromae, Aspergillus niger*, *Aspergillus flavus, Fusarium solani* and *Macrophomina phaseolina*[28]. while, pathogens like *F. oxysporum, F. moniliforme, A. flavus, A. niger, Rhizopus sp, A. ochraceus, P. purpurogenum* and *Pestalotia* sp. were also reported in tuber rot in cassava, Nigeria[29]

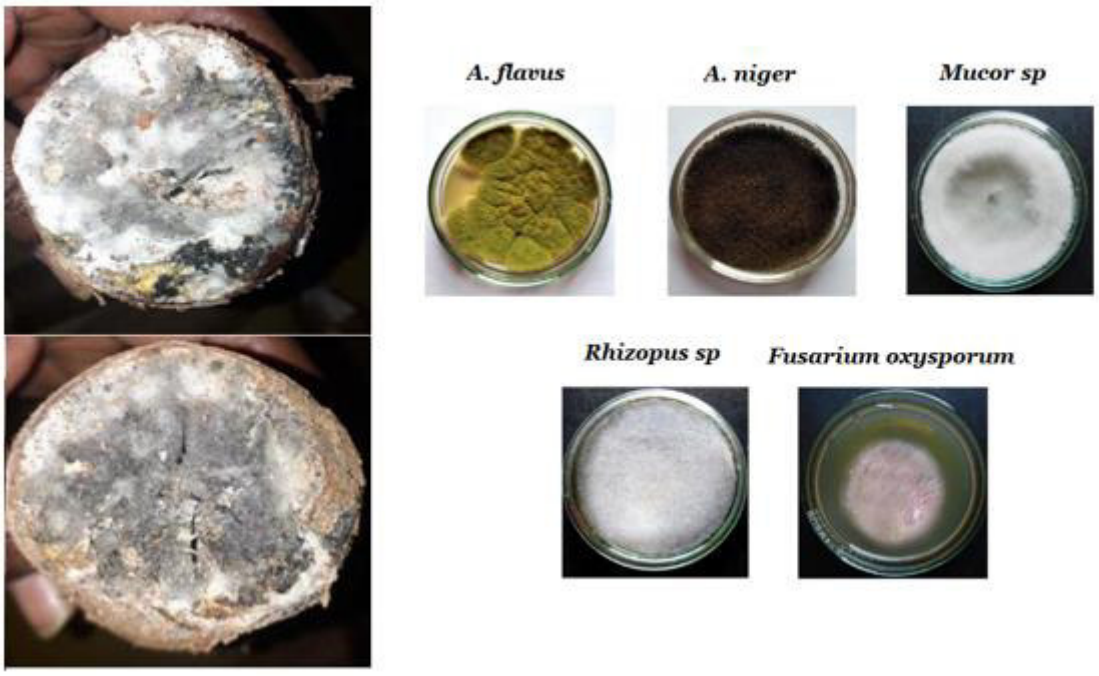

Fig: 1 Tuber rot disease in cassava and colony morphology of pathogenic fungi

3.2 Antifungal assay

In this study, methanolic extracts of *A. paniculata* showed high antifungal activity against *Fusarium oxysporum* (20mm), *A. niger* (17mm), *A. flavus* (16mm) and 13mm range was observed in *Rhizopus* and *Mucor* sp. with the concentration of 10mg (Table 1). *S. indica* also exhibited its activity against *Fusarium oxysporum* (14mm), *A. niger* (12mm) and *A. flavus* (12mm) respectively. Comparatively, *A. paniculata* possesses maximum inhibitory effects compared to *S. indica* (Figure 2). Methanolic extract of *A. paniculata* showed lowest minimum fungicidal concentration (MFC) value (250 µg/mL) against *A. niger*. MEOH extract afforded 3-O-β-d-glucosyl-14-deoxy andrographolide, 14-deoxyandrographolide, and 14-deoxy-11,12 didehydro andrographolide as good antifungal compounds[30]. Also, Methanolic extracts of *A. paniculata* exhibited spore germination inhibition of *F. solani* and *A. solani*[31]. *A. paniculata* acts against *Mucor* sp., *Aspergillus flavus, Rhizopus* sp., *Aspergillus niger* and *Fusarium* sp. were also tested and similar findings were reported[32]. *Fusarium* species isolated from rotted cassava in Africa include *F. moniliforme sensu lato*, *F. semitectum, F. oxysporum*, and *F. solani*[33]. *F. solani* and *F. oxysporum* species complex also have been reported as pathogens of cassava in Colombia[34]. In a survey, conducted in Nigeria during 1998 and 1999, the most frequently isolated pathogen was *B. theobromae* at 75% frequency, while *F. solani*

and *F. oxysporum* together were isolated at a frequency of 45%[35]. In another survey, conducted during the dry season in 1996, *B. theobromae* was isolated at a frequency of 28% in Nigeria and at 7.7% in Benin, while *Fusarium* species were isolated at a frequency of 13 and 12% in Nigeria and Benin, respectively. In a subsequent survey in Benin during the rainy season in 1997, the isolation frequency of *Fusarium* species was comparatively higher (23 to 32%) than reported before[36]. Comparatively, *A. niger* shows more virulence, causing the highest percentage of root rot disease in cassava compared to *Trichoderma viride*[37]. It has also been recorded that *A. niger* promotes a leading cause of postharvest fungal root rot of cassava especially in South-East Nigeria[38-39] inciting storage root rot. It may be due to the highly adoptable nature of *A. niger* can sustain in many factors, thus may enter *via* wounded holes in the tubers than any other pathogens and utilize the nutrients of the stored tubers as substrates for growth and development. In the present study, *Fusarium oxysporum* and *A. niger* was reported and the antifungal potency of *A. paniculata* extract revealed with maximum inhibitory effects against these tuber root rot causing pathogens.

Table 1 Zone of inhibitory effects of the plant extracts

S. No	Isolates	Different concentrations of extract (mg)											
		Andrographis paniculata ZoI (mm)						*Stachytarpheta indica* ZoI (mm)					
		2.5	5	7.5	10	DMSO	+ve	2.5	5	7.5	10	DMSO	+ve
1	*Rhizopus sp.*	-	-	-	13±1.632	-	-	-	-	-	-	-	-
2	*Mucor sp.*	-	-	10.33±1.247	13±2.247	-	-	-	-	-	-	-	-
3	*A. niger*	11±0.816	12±1.632	15±1.632	17.33±2.054	-	15±1.632	-	-	-	12.66±2.054	-	15±1.632
4	*Fusarium oxysporum*	12±1.632	13±1.632	13.66±1.247	20±0.816	-	-	-	8.33±1.247	10.33±1.247	14±1.632	-	-
5	*A. flavus*	-	12±1.632	13.66±1.247	16±1.632	-	13±1.632	-	-	-	12±1.632	-	13±1.632

P< 0.05 when compared to control; ± value – Standard Deviation; ZoI – Zone of Inhibition (mm) represents* mean *zones of inhibition measured in millimetres

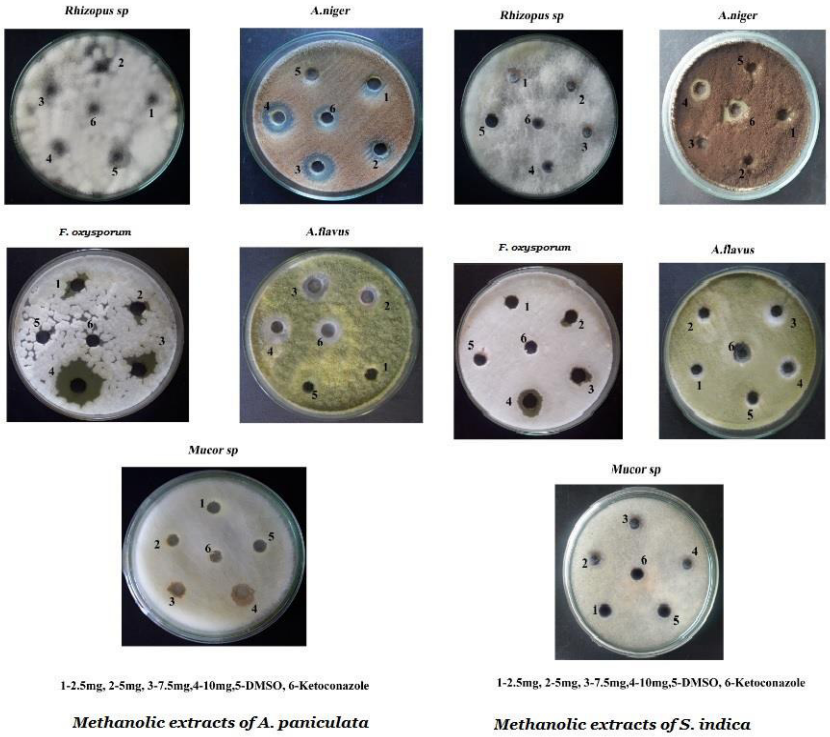

Fig: 2 Antifungal activity of the plant extracts

3.3 *Gas Chromatography-Mass Spectroscopy (GC-MS)*

The GC chromatogram of the methanolic extract of *A. paniculata* revealed the presence of 20 phytochemicals with the retention time, Molecular weight and the relative percentages of the compounds present in the leaves of *A. paniculata* were recorded (Figure 3). Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Techniques (NIST08s), WILEY8 and FAME having more patterns. The name, molecular weight, molecular formula and structure of the test material were determined (Table 2).Benzene,1,2,3-trimethoxy-5-(2-propenyl)-, Cyclopenta [c] pyran-4-carboxylic acid, 7-methyl-, methyl ester and 9,12,15-

Octadecatrienoic acid, methyl ester, (Z, Z, Z)- covers the maximum percentage area in the extracts. These phytochemicals have a wide range of medicinal properties like antifungal, antibacterial and antiallergic properties [40-42]. Other antioxidants are Hexadecanoic acid, vitamin – E, 9,12-Octadecadienoic acid, methyl ester, Hexadecanoic acid, methyl ester was also recorded. The therapeutically important active principle andrographolide was reported in aerial parts of *A. paniculata* [43]. Three xanthones, 1,8-dihydroxy-3,7-dimethoxy-xanthone, 4,8-dihydroxy-2, 7-dimethoxy-xanthone, 3,7,8-trimethoxy-1-hydroxyxanthone are reported from the root's[44]. 2-Vinyl-9-[beta-d-ribofuranosyl] hypoxanthine, one of the phytochemicals possess the antibacterial activity[45] with the similar compounds recorded in this study.

Table 2 Phytochemical components of *A. paniculata* methanolic extracts as identified by GC-MS analysis

No.	RT	Area %	Phytochemical Constituents [ab]	MF	MW
1	8.809	0.93	2-Pyrrolidinone, 1-methyl-	C_5H_9NO	99.13
2	9.198	2.38	Benzofuran, 2-methyl-	C_9H_8O	132.15
3	9.287	2.80	2-Cyclopenten-1-one, 3-methyl-	C_6H_8O	96.127
4	11.686	0.83	3-Amino-5-cyclopropyl pyrazole	$C_6H_9N_3$	123.16
5	12.831	1.56	2-Vinyl-9-[beta-d-ribofuranosyl] hypoxanthine	$C_{12}H_{14}N_4O_5$	294.26
6	14.208	0.78	2-Amino Phenanthrene	$C_{14}H_{11}N$	193.24
7	14.519	1.05	3-(4-Isopropyl-phenyl)-acrylic acid	$C_{12}H_{14}O_2$	190.24
8	14.653	5.95	Indole-1-carboxylic acid, 4-amino-, methyl ester	$C_{10}H_{10}N_2O_2$	190.2
9	14.730	**17.37***	Cyclopenta [c]pyran-4-carboxylic acid, 7-methyl-, methyl ester	$C_{11}H_{10}O_3$	190.062
10	15.264	**18.61***	Benzene, 1,2,3-trimethoxy-5-(2-propenyl)- **(Synonym – Elemicin)**	$C_{12}H_{15}NO_5$	253.25
11	15.564	0.86	3-Hydroxy-7,8-dihydro-. beta. -ionol	$C_{13}H_{20}O_2$	208.29
12	16.752	0.79	Ethanone, 1-[5-(2-furanylmethyl)-2-furanyl]-	$C_{11}H_{10}O_3$	190.19
13	17.352	1.92	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-	$C_{10}H_{18}$	138.25
14	18.252	9.54	Hexadecanoic acid, methyl ester (Synonym – Methyl palmitate)	$C_{17}H_{34}O_4$	270.45
15	18.585	7.94	n-Hexadecanoic acid (Synonym – Palmitic acid)	$C_{16}H_{32}O_2$	256.4
16	19.874	2.09	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294.47
17	19.941	**15.84***	9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)- **(Synonym – Methyl linolenate)**	$C_{19}H_{32}O_2$	292.45
18	20.030	6.62	Phytol (Synthetic form – Vitamin – E & K_1)	$C_{20}H_{40}O$	296.53
19	20.174	1.27	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298.50
20	26.073	0.87	9-Fluorenone-4-carbonyl chloride	$C_{14}H_7ClO_2$	242.65
Total		100			

[a] Compounds listed in order of elution from DB 35-MS Capillary Standard non-polar column.
[b] Compounds identified based on computer matching of the mass peaks with WILEY and NIST Library.

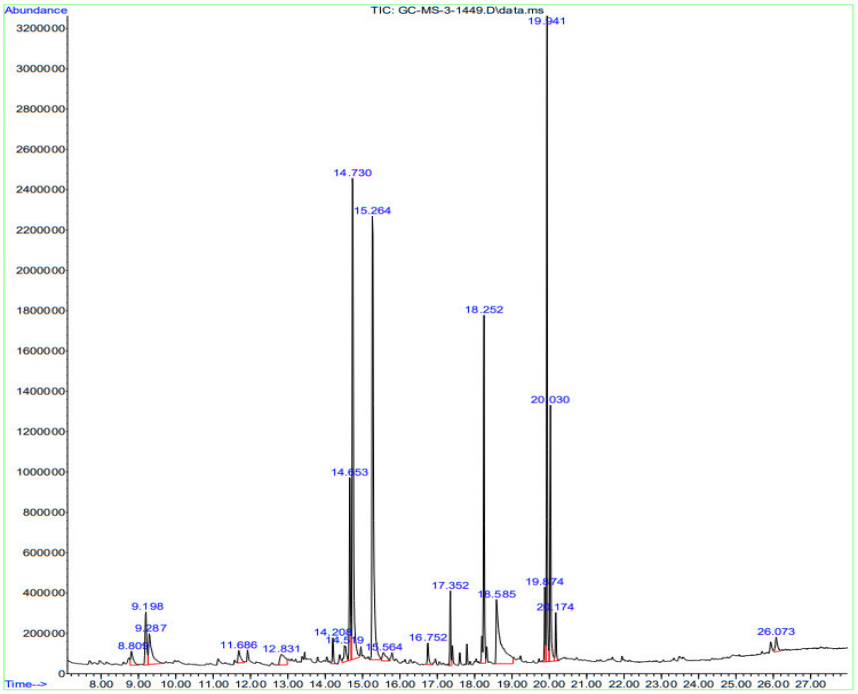

Fig: 3 GC-MS chromatogram of *A. paniculata*

4. CONCLUSION

This observation recommends that methanolic leaf extracts of *A. paniculata* exhibited the highest zone of inhibition against the tested fungal pathogens causing tuber root rot diseases in cassava. Hence, the findings supports the use of *A. paniculata* extracts as ethnopharmacological information in developing phyto-antimyco formulations to control this tuber root rot disease in *Manihot esculenta*. Further studies are recommended for the characterization studies in order to elucidate the structures of active compounds from *A. paniculata*. Since these natural products and their analogues are remarkably considered not only for the management of fungal pathogens but also an important source of eco-friendly organic fungicide in replacements of synthetic toxic chemicals.

5. AUTHORS CONTRIBUTION STATEMENT

Ms. Palani Ruba and Dr. Michael Helan Soundra Rani collected the samples from the Kolli hills, Namakkal district and sketched the concepts in processing & extraction procedures. Albert Hannah Selvakumari designed and performed the antifungal activity and GCMS characterization study. Dr. Edward Gnanaraj Wesely and Dr. Michael Helan Soundra Rani analysed the data with regards to Methodology & Validate the original draft writing with suggestions and inputs from all authors.

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8. CONFLICT OF INTEREST

Conflict of interest declared none.

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