Pharmacological Activities of Compounds of Leaves and Roots of Imperata Cylindrica with Its Antimicrobial and Structural Elucidation

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Abstract. The pharmaceutical constituents and structural elucidation of total phenols on the leaves and roots of imperata cylindrica plant were carried out to ascertain the active constituents that are responsible for its use as an astringent, febrifuge, diuretic, tonic and styptic action and its use for teething problems in infancy and others. The phytochemical analysis showed the presence of alkaloids, flavonoid, tannins, saponnins, steroids, terpenes and glycosides on the plant. The quantitative analysis of the phytochemicals present in the 4g of crude drug was determined using Van-Burden & Robinson 1981 method for Tannins, Obadoni & Ochuko 2001 method for Saponin, Harbone 1993 method for Alkaloids, Bohm &Kocipal Abyazan 1994 method for Flavonoids and Salkowske method for Terpenoids. Result showed that the crude drug contained Tannin (5.7%), Flavonoids (10.3%), Alkaloids (5.4%), Saponins (2.25%) and Terpenoids (43.3%). The antibacterial and antifungal activity screening showed strong inhibitory effect against test organism. E. coli, staphylococcus epidermis, Kleibsiella Pneumonia, pseudomonas aeruginosa, Streptococcus pyogenes, Asperigillus niger, Asperigellus flavus, Candida albican, Fusarium Oxysparum , Candida parapsilosis. Preparative thin layer chromatography and thin layer chromatography techniques were used to separate compounds from the extract which was subjected to structural elucidation using spectroscopic instruments such as GC-MS, UV-VIS AND FTIR. The spectral analyses suggested the presence of 1,6-anhydro-Heptanoic acid, 1,6-dichloro-cyclohexaneacetic acid, 1,6-anhydro-5-Hexenoic acid, 5-Hexenoic acid and cyclohexaneacetic acid. Fatty acid ethyl ester is known for its antibacterial, antifungal and also anti-inflammatory properties which would be effective in the management of bacterial and fungal infections. These results confirm the ethnomedicinal use of imperata cylindrica.

Keywords: Imperata cylindrica, Pharmacological Activities, Antimicrobial, Isolation & Structural Elucidation

1. INTRODUCTION

Man depended on plants as medicine [1] from time immemorial. In history perspective, it is evident that the fascination for plants is as old as mankind itself. The plant kingdom represent a rich store house of organic compounds, many of which have been used for medicinal purposes and could serve as lead for the development of novel agents having good efficacy in various pathological disorders in the coming years. Plants are the richest source of drugs for traditional medicines, modern medicines, nutriceutical food supplements, folk medicine, pharmaceutical intermediates and chemical entities for synthetic drugs [2]. The use of plant product as medicines could be traced as far back as the beginning of human civilization.

The active principle isolated, have provided leads in the development of several life saving drugs, which are in use today[3].

The isolated active compounds of the plants are secondary metabolites that occur naturally in plant with no nutritional value to human life[4]

Through the ages, majority of people in Africa use plant based traditional medicine for their cure medically. Example in Nigeria, the leaves and root of *imperata cylindrica* is being used for teething dumping in infancy. The leaves and the roots are used to stop a cut from bleeding. It is used to make the skin feel less oily. It is used to increase the flow of urine in human being. It is also used for the treatment of liver injury [5],[10].

Imperata cylindrica is widely distributed in topical regions. The korean folk medicine has described the rhizomes as diuretic, anti-flammatory , antipyretic, neuro-protective chromones, several arborane compounds such as arundoin, cylindrin and fernenol, in addition to cylindron, cylindrene, graminones, and imperarene . This study is a phytochemical and biological evaluation of imperata cylindrica. We describe the isolation and structural elucidation of several compounds: four methoxylated flavonoids 1-4,tetradecanoyl ester of sitosterol glucoside 5, and an aldehyde 6.These compounds are reported for the first time from genus imperata. In addition to daucosterol, B-sitostrol and a-amyrin .Moreover, the total methanolic extract showed potent hepato-protective activity and the compounds 1-5 showed cytotoxic activity elicited by the brine shrimp lethality assay [11].

The future of herbal medicine in Nigeria and the world at large is very bright owning to the fact that the field of herbal medicine is getting increasingly attractive, since there is increase in the awareness of the efficacy of healing herbs all over the world. Researchers are being carried out on the medicinal plants in order to see the increasing impact in the future especially when it is combined with the recent discoveries and development like gene alteration or gene splicing in plants and animals. The main trusts of these new medicinal plants are that they are genetically engineered to separate gene from disease causing microbes and then stop the disease [6].

Hence, modernized herbs would replace most of the traditional prescription of drugs that are currently in use. These herbs compliment the western medicine in terms of disease treatment provided that they are studied in terms of their biochemical contents and collective effects of the herbs as a whole [7].

In Africa, the demand for traditional medicine and pressure on medicinal plant resources will become greater if the principle bioactive medicinal constituents are been identified and isolated.

It is on these that studies on the chemical composition and structural elucidation of root and leaves of *imperata cylindrica* is being carried out [8].

Imperata cylindrica grass (leaves and root) are known to have economic value such as medicinal, nutritional and pesticides values. These have not been clearly investigated. This research and experiment is therefore centred on investigating, analyzing and justifying the medicinal, nutritional and pesticides values of these grasses. To know the chemical composition responsible for the medicinal value of these grass.

Imperata cylindrica (spear grass) are mainly seen as evasive grass in eastern part of Nigeria, Its pharmaceutical uses are scarcely known by general public, though it sometimes secretly used by some traditional medicine practitioners , Hence, this study will create more awareness of its phytochemical composition and medicinal values.

From literature, the root and leaves of *imperata cylindrica* are medicinal.

To justify the claims made on these grass for its medicinal and economic values, *imperata cylindrica* is responsible for teething dumping in infancy, stopping bleeding from a cut, making skin to feel less oil, increasing the flow of urine , antimicrobial and treatment of liver injury.

In addition, it has both theoretical and practical justification.

Table 1: The Taxonomy of Imperata cylindrica

Kingdom	**Plantae**
Division	Magndiophyta
Class	Liliopsida
Order	poales
Family	poaceae
Genus	Imperata
Species	I. Cylindrica
English name	Spear grass
Local name	Ata in Igbo

It is a perennial rhizomatous grass. It grows from 0.6 -3m (2-10 feet) tall. The leaves are about 2cm wide near the base of the plant and narrow to a sharp point at the top; the margins are finely toothed and are embedded with sharp silica crystals, The main vein is a lighter colour than the rest of the leaf and tends to be nearer to one side of the leaf.
The upper surface is hairy near the base of the plant, while the underside is usually hairless. Roots are up to 1.2 meters deep, but 0.4m is typical in sandy soil.

It has invaded every continent except Antarctica and is listed as an invasive weed in many areas. In the US, it survives best in the south east, but has been reported to exist as far north as West Virginia and Oregon. Worldwide, it has been observed from 45 N to 45 S. It grows on wet lands, areas of high salinity, organic soils, clay soils and sandy soils of pH from 4.0 and 7.5. It prefers full sun, but will tolerate some shade.

It is an aggressive plant which is spread both through small seeds, which are easily carried by the wind, and rhizomes, which can be transported by tilling equipment and in soil transport. The rhizomes create dense mats on the ground surface reducing the chances of the seeds of other plants reaching the soil and germinating successfully.

Silica crystals in its leaves deter all but mostly animals from eating its low food value leaves. The crystals cut the mouth(and do same to the legs of unwary humans who run through the fields of the stuff in shorts) and probably wear down teeth. It forms a very dense mat which prevents much competition from other plants. It may release an allelopatic phenoic chemical that may kill other plants or prevent seeds from germinating. The strong sharp tipped rhizomes penetrate the roots of other plants, perhaps to weaken them or leave them open to infection. It is because of this reason that the overall fodder value is very much reduced, and because of its ability to survive, it is considered to be an invasive pest in warmer climates.

The various investigation carried out by traditional researches from the early practitioners of herbal medicine revealed that the leaves and root of Ata (spear grass) are used for the treatment of liver injury, relief of teething problems and stomach ache in infancy ,stopping bleed in a cut, etc [5][9].

2. MATERIALS AND METHODS

2.1 Sample collection and Identification

The leaves and roots of the plant material were collected from Umuenechi village Nibo in Awka South Local Government Area of Anambra state. It was identified and classified by a plant kingdom scientific analyst (Consultant) Prof. J.C.Okafor of No 7 Dona Street Independence Layout Enugu, and Prof .V.I.Ajiwe of Department of Industrial Chemistry Nnamdi Azikiwe University Awka, as *Imperata cylindrica* plant . The fresh leaves and root of *Imperata cylindrica* were washed thoroughly with clean water and dried under room

temperature for a period of 7 days. The dried leaves and roots were pulverized with electric grinder and stored in an air tight container for further analysis.

2.2 .Extraction And Isolation Of Leaves And Root Of Imperata Cylindrica

10g of the pulverized leaves and roots of *imperata cylindrica* was homogenised for five minutes in methanol and water in the ratio of 4:1 (10 x volume) and was then filtered with a filter paper. The filtrate was evaporated to one ten the less the volume (1/10 < vol) at 40oc.The remaining mixture was acidified with 2ml H2SO4 after which it was extracted with chloroform (x3) in a separatory funnel.

There were two layers, the chloroform layer and the aqueous acid layer. The chloroform layer was dried and evaporated to obtain a moderately polar extract, which is likely to be terpernoids and phenolics. For the aqueous acid layer. It was basified to PH 10 with ammonium hydroxide and was extracted with chloroform and methanol in the ratio of 3:1 twice, followed by extraction with 20ml chloroform using a separatory funnel. This extraction gave two layers, the chloroform – layer and aqueous basic layer. The aqueous basic layer were evaporated and extracted with methanol. This yielded a white crystal alkaloid . 8g of ethyl acetate crude extract from plant was subjected to silica gel column chromatography (70 x 3.5cm) containing a layer of silica gel as adsorbent (mesh size 60 -120). The elution system was composed of neat hexane, hexane:ethyl acetate mixtures in other of increasing polarities (100:0, 90:10, 85:15, v/v). A total, of 56 fractions was collected which were mostly impure compounds, the fractions obtained again were pooled based on their TLC profiling. 7 combined fractions were obtained (IMP1 -7). The fractions were further purified by column chromatography using three solvent system. Three pure isolate were obtained IMP1, IMP2 and IMP3.

2.3. Structural Elucidation of the purified extract

The pure compounds were subjected to spectroscopic analysis using FTIR, UV-VISIBLE AND GC-MS spectroscopy.

3. RESULTS

The results and the spectrum of the analysis conducted on the compounds of Imperata Cylindrica Plant are presented below in tables and figures.

Table 2: Result Of Phytochemical Tests Of Imperata Cylindrica Plant

Class of Phytocompounds	Observation	Inferences
ALKALOIDS		
Wagner's reagent	White ppt	++
Mayer's reagent	Reddish brown ppt	++
SAPONNINS		
Frothing test	Persistent frothing	++
Emulsion test	Stable emulsion	++
Fehlings solution test	Light reddish ppt	+
FLAVONOID		
Ammonium test	Two layers of green and light green was formed.	++
NaoH/Acetic test	Two layers of green and light green was formed	++
TANNINS		
Ferric Chloride test	Greenish black precipitate	++

Lead acetate test	Cream precipitate	++
STEROIDS AND TERPERNOIDS		
Sample + ethanol +Chloroform + H_2SO_4	Reddish brown Interface layer	++
GLOCOSIDES		
Fehling test	Opaque brick red precipitate	++

Table 2: Results Of Quantitative Analysis Of Phytochemical Present In Imperata Cylindrica Plant

Parameters (4g)	Weight of sample(mg)	% weight
Tannin	0.057 ±	5.7
Flavonoids	0.103±	10.3
Alkaloids	0.054±	5.4
Saponins	0.023±	2.25
Terpenoids	0.433±	43.3

Table 3: Result of Antibacterial Inhibitory Activities of Aqueous Methanol (80%), Water and Ethyl Acetate Extracts of Imperata Cylindrica (Spear Grass)
Test organisms and zone of inhibition (diameter) in millimeters

S/NO	Test organism	80% methanol	Water	Ethyle acetate	+ve control	-ve control	Mic	MBC
1	Pseudomonas aeruginosa	11.88	5.64	9.14	26.00	NA	0.4	0.2
2	Bacillus leveus	6.89	7.00	0.82	11.11	NA	0.6	0.3
3	Staphylococcus Epidermis	13.24	10.69	8.86	21.23	NA	0.4	0.2
4	Kleibsiella pneumonia	3.05	3.12	4.00	12.36	NA	0.2	0.1
5	Escharichia coli	20.00	12.3	7.37	3.00	NA	0.8	0.3

MIC=Minimum inhibitory concentration
MBC = Minimal Bacteriacidal concentration

Table 4: Result Of Antifungal Inhibitory Activities Of Aqueous Methanol (80%), Water And Ethyl Acetate Extracts Of Imperata Cylindrica (Spear grass)
Test organism and zone of inhibition (diameter) in millimeters

S/NO	Test organism	80% Methanol	water	Ethyl acetate	+ve control	-ve control	Mic	MFC
1	Candida Albican	7.33	7.00	5.44	22.00	NA	0.8	0.3
2	Candida parapsilosis	2.87	2.00	2.00	8.77	NA	1.0	0.4
3	Aspergillus niveus	4.22	2.81	2.29	12.85	NA	1.0	0.3
4	Aspergillus parasiticus	10.01	7.36	8.23	18.91	NA	0.6	0.2
5	Microsporum gypseum	0.97	1.08	1.00	5.34	NA	1.0	0.4

MIC =Minimum inhibition concentration
MFC= Minimal fungal concentration

Table 5: FTIR spectral for IMP1 Ethyl acetate extract

FREQUENCY CM^{-1}	DESCRIPTION
1081.243	C-H,C-O deformation bonds for alkyl group and ketones
1185.495	C-O deformation bonds for ketones and acids group
1516.973	C=C stretch for alkenes

1629.901	C=O stretch for ketones, acids and amides groups
1929.567	C=O stretch for ketones, acids and amides groups
2226.756	CN stretch for nitriles
2402.066	CN stretch for nitriles
2551.897	CN stretch for nitriles
2660.344	C-H stretch for alkenes and aromatics
2786.765	C-H stretch for alkenes and aromatics
3125.234	N-H and OH stretch for amines ,alcohols and phenols
3344.173	N-H and OH stretch for amines ,alcohols and phenols
3702.685	OH unbound for alcohols

Table 6: UV-Visible spectroscopic Analysis Result for IMP1 Ethyl acetate

Extract	λMax (nm)	Chromophores/Description
Ethyl acetate	664.00	C=O n* transition. prominent
IMP1	532.00	C=C n* transition, not prominent
	439.00	C=C * transition, not prominent
	368.00	C=O * Transition, not prominent

Table 7: FTIR spectral for IMP2 Methanol extract

Frequency cm^{-1}	**Description**
690.1861	C-O,C-H deformation bonds for alkyl and methyl groups
746.006	C-O,C-H deformation bonds for alkyl and methyl groups
850.6318	C-O,C-H deformation bonds for alkyl and methyl groups
1157.5587	C-O deformation bonds for ketones and acids
1251.731	C-O stretch for ketones and alcohols
1389.406	C-O stretch for ketones and alcohols
1612.681	C=O stretch for ketones, acids and amides
1886.83	C=O stretch for ketones, acids and amides
2022.158	CN stretch for nitriles
2148.564	CN stretch for nitriles
2529.491	CN stretch for nitriles
2611.842	O-H stretch for acids
2724.26	C-H stretch for alkanes
2955.131	C-H stretch for aromatics
3093.978	C-H stretch alkenes
3249.372	O-H stretch for alcohols, acids and amides
3521.795	O-H stretch for alcohols, amides and acids
3663.988	O-H unbound for alcohols
3799.375	O-H unbound
3922.256	O-H unbound

Table 8: UV-Visible spectroscopic Analysis Result for methanol extract IMP2

Extract	λMax (nm)	Chromophores/Description
Methanol IMP2 extract	652.00	C=O n* transition not prominent
	413.00	C=O * not prominent
	400.00	C=C n* prominent
	396.00	C=O n* prominent
	393.00	C=O n* prominent
	386.00	C=O n* prominent

Table 9: FTIR spectra of the water IMP3 extract

Frequency cm^{-1}	**Description**
3784.126	O-H alcohols unbound
3572.74	O-H stretch
3341.766	for alcohols, Acids, and
3161.099	N-H stretch amides
2986.746	O-H stretch
2902.733	C-H stretch for acids, alkane, aromatics and alkene
2771.68	
2559.618	CN
2290.083	N-H stretch for Nitriles,amines and amides
2132.929	
2011.448	
1861.206	C=O stretch for ketones, acids and amides
1617.194	
1410.185	C=C stretch for alkenes and aromatics
1320.337	C-O stretch for ketones, acids and amides
1043.513	C-O, C-H deformation bond for alkyl and methyl
787.408	

Table 10: UV-Visible spectroscopic Analysis Result for water imp3

Extract	Max(nm)	Chromophores/description
Water imp3	662.00	C=O n* transition not prominent
	515.00	C=C n* transition prominent

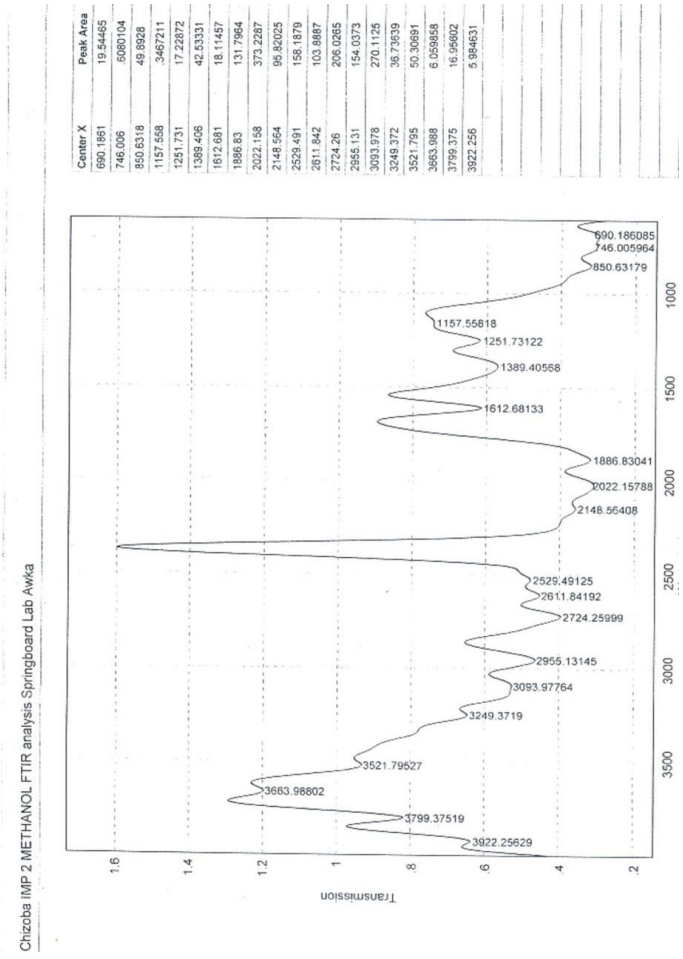

Figure 1: FTIR spectral for IMP2 Methanol extract

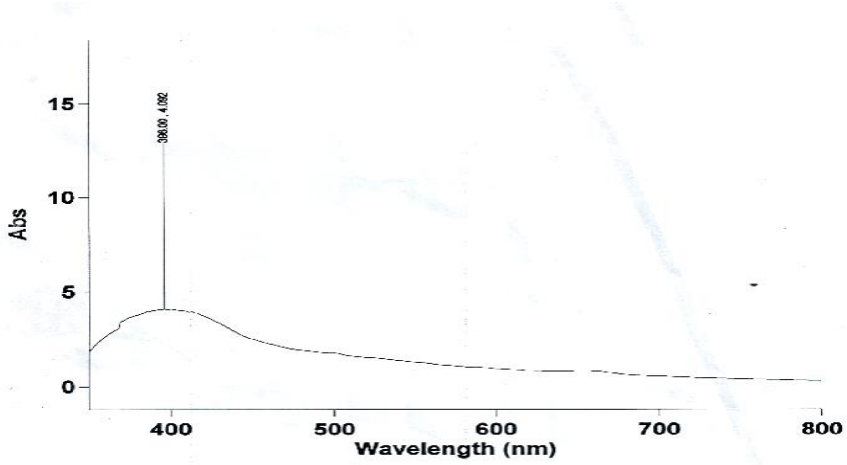

Figure 2 : UV-Visible spectroscopic Analysis Result IMP2 extract

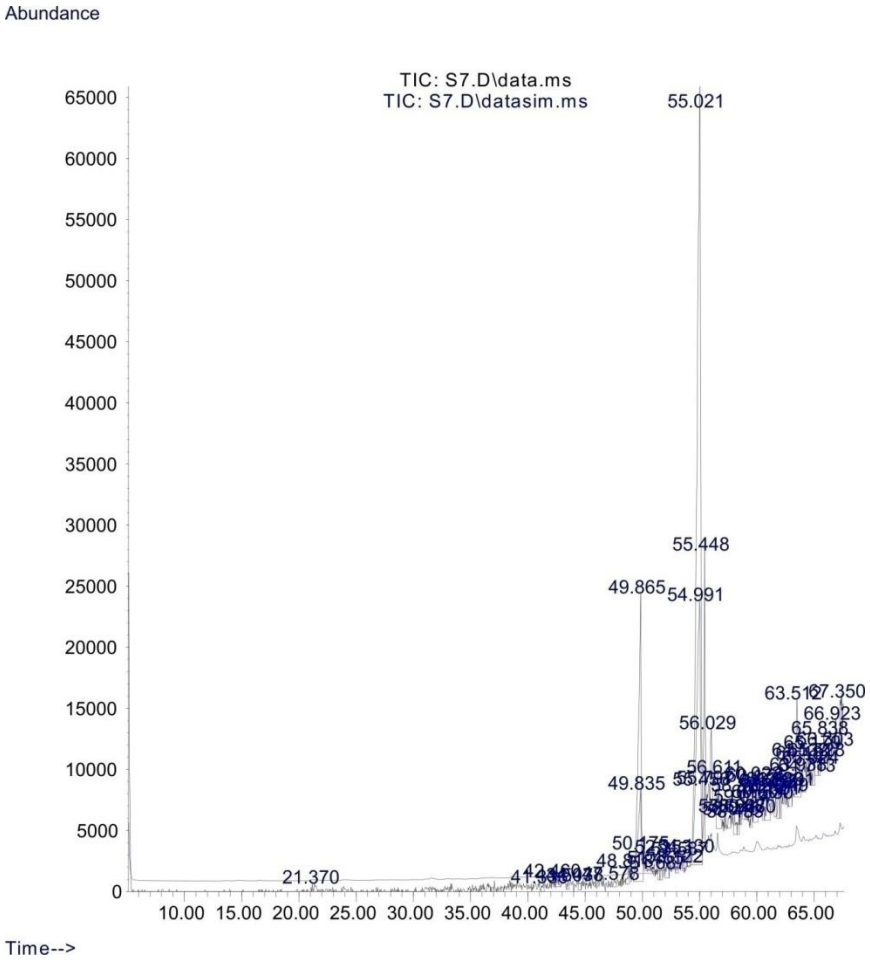

Figure 3: G C – MS IMP3 extract of Imperata Cylindrica Plant

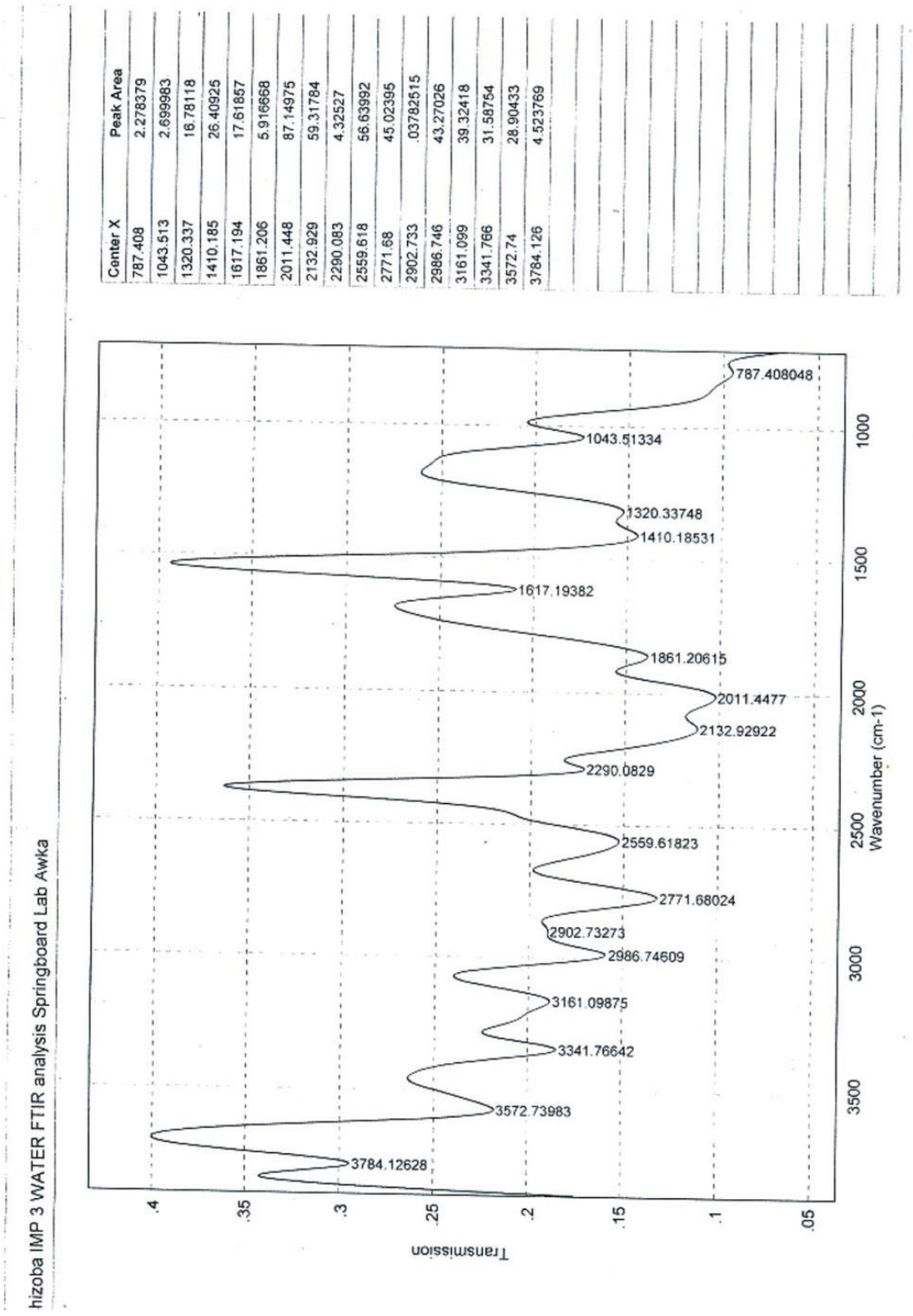
hizoba IMP 3 WATER FTIR analysis Springboard Lab Awka
Center X Peak Area
787.408 2.278379
1043.513 2.699983
1320.337 16.78118
1410.185 26.40925
1617.194 17.61857
1861.206 5.916668
2011.448 87.14975
2132.929 59.31784
2290.083 4.32527
2559.618 56.63992
2771.68 45.02395
2902.733 .03782515
2986.746 43.27026
3161.099 39.32418
3341.766 31.58754
3572.74 28.90433
3784.126 4.523769
787.408048
1043.51334
1320.33748
1410.18531
1617.19382
1861.20615
2011.4477
2132.92922
2290.0829
2559.61823
2771.68024
2902.73273
2986.74609
3161.09875
3341.76642
3572.73983
3784.12628
Transmission
.4
.35
.3
.25
.2
.15
.1
.05
1000
1500
2000
2500
3000
3500
Wavenumber (cm-1)

Figure 4: FTIR spectra of the water IMP3 extract

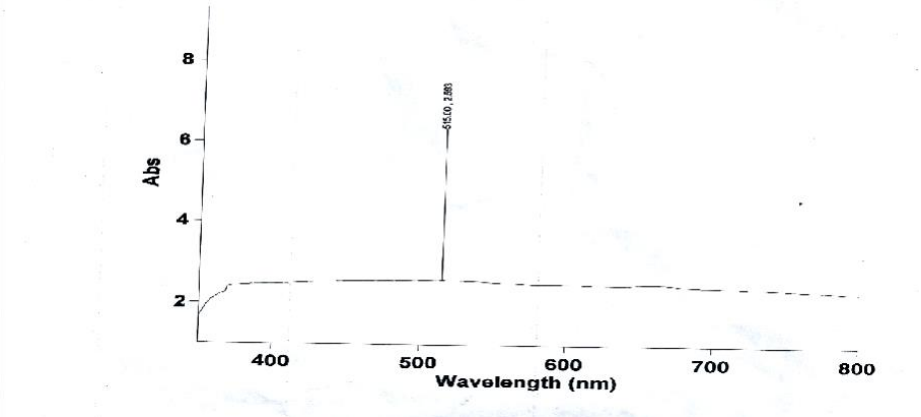

Figure 5: UV-Visible spectroscopic Analysis Result for water IMP3

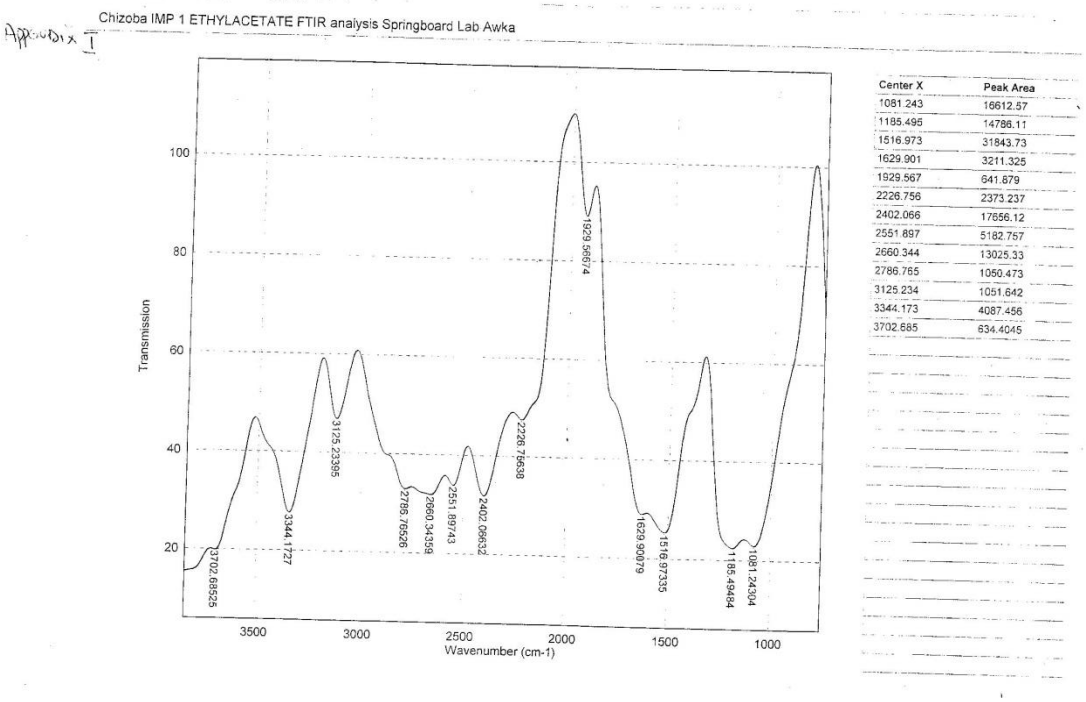

Figure 6: FTIR of Ethyl acetate IMP1 of Imperata Cylindrica Plant

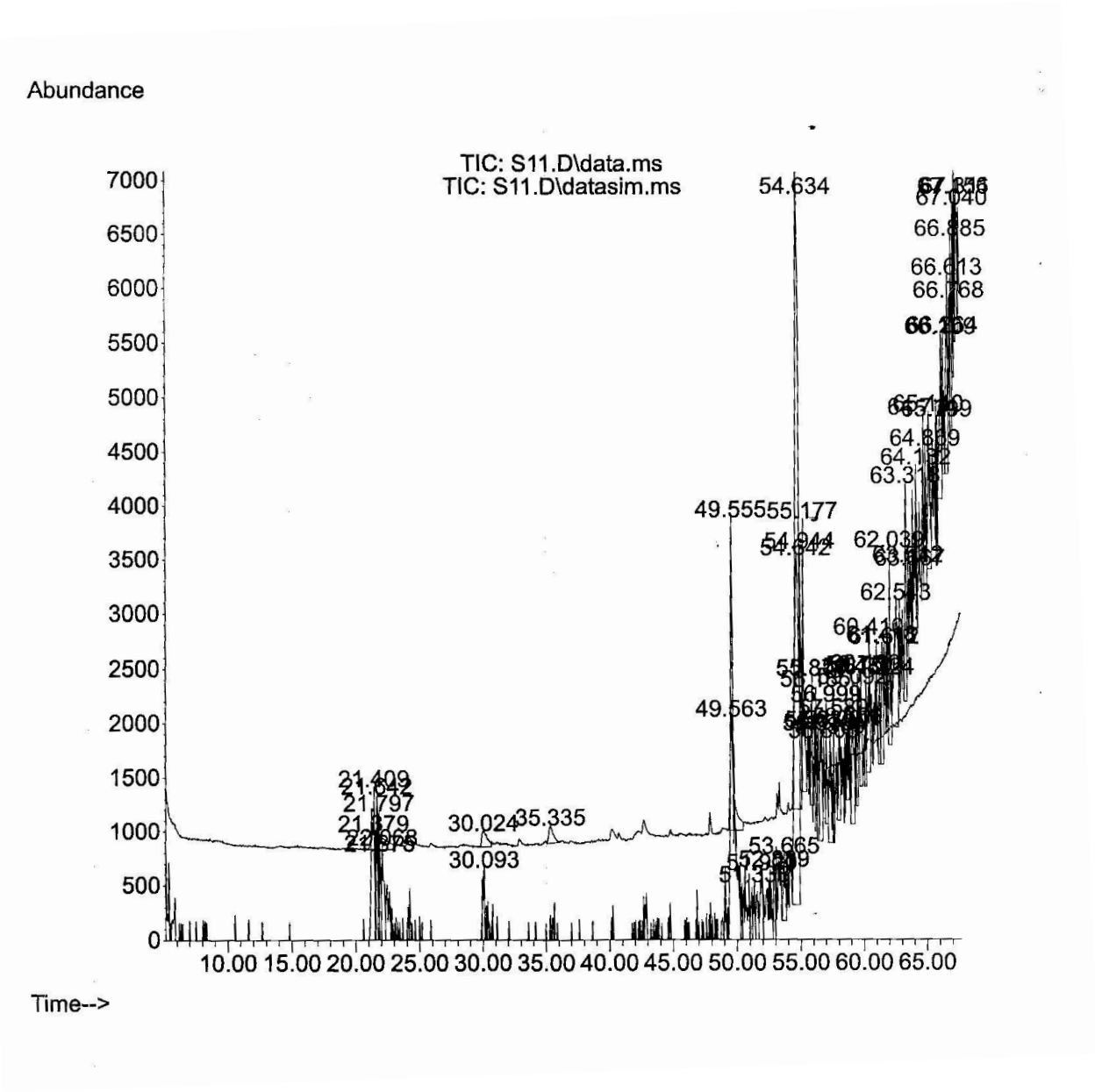

Figure 7: G C – MS spectra of IMP2 Methanol extract of IMperata Cylindrica Plant

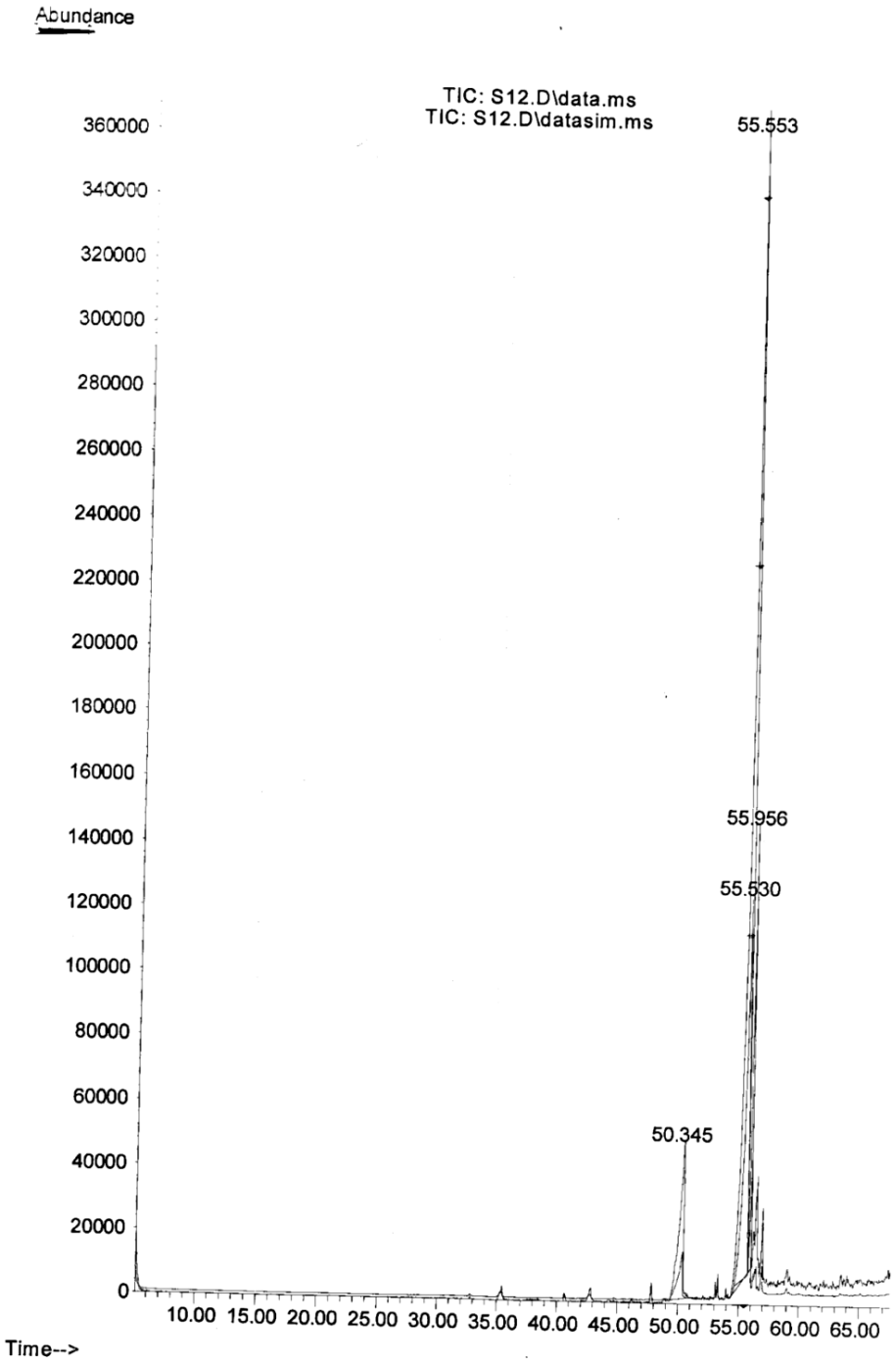

Figure 8: G C – MS Spectra of IMP1 ethyl acetrate of Imperata Cylindrica Plant.

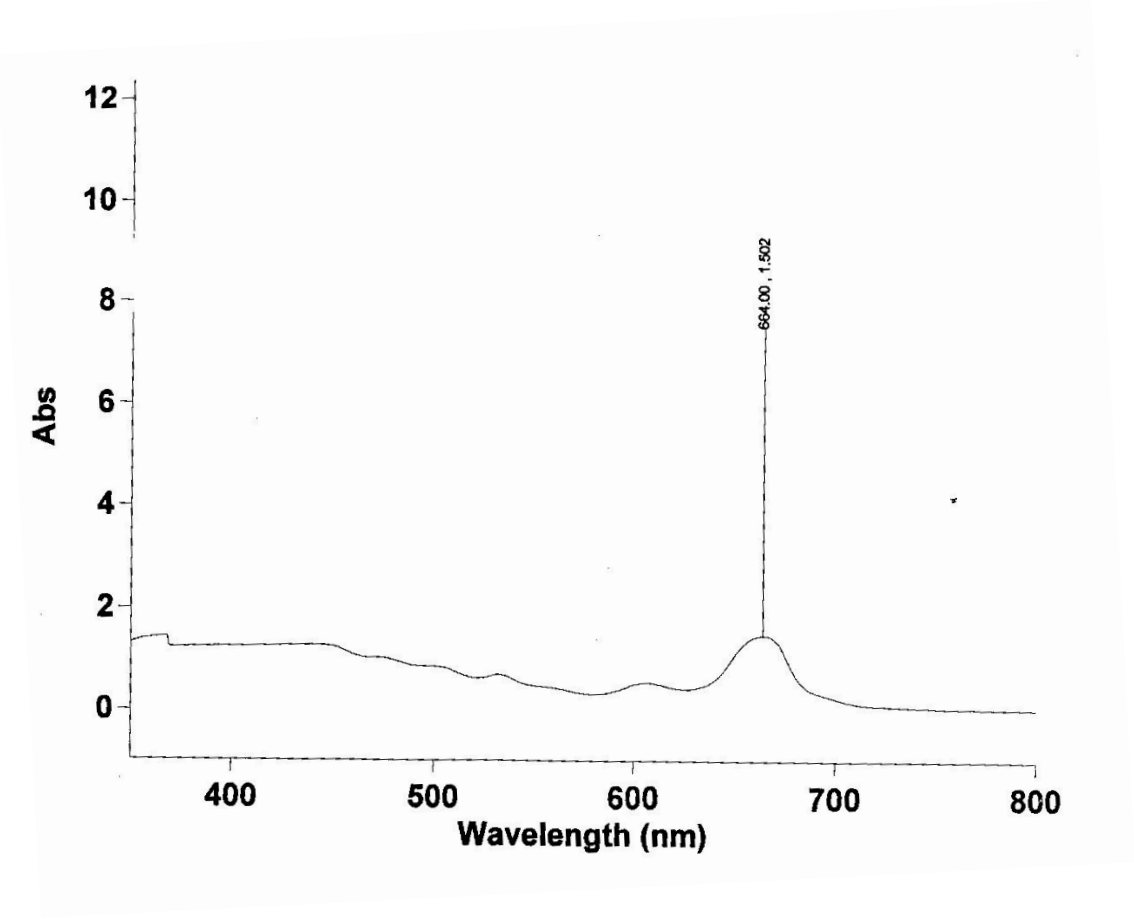

Figure 9: U V Spectra of IMP1 ethyl acetate extract of Imperata Cylindrica Plant.

4. DISCUSSION

Isolation and purification of Ethyl acetate, 80% methanol and water extracts have evaluated the phytochemical constituents of *imperata cylindrica* plant used as a herb in tradition medicine for the treatment of *Pseudomonas aeruginosa, Bacillus leveus, Staphylococcus Epidermis, Kleibsiella pneumonia, Escharichia coli, candida Albican, Candida parapsilosis, Aspergillus niveus, Aspergillus parasiticus, Microsporum gypseum and stomach disorderness* during teething in infancy. Its medicinal properties includes astringent, febrifuge, diuretic, tonic and styptic action. The results of the phytochemical analysis showed the presence of Alkaloid, Flavonoids , Tannins, Saponnins, Glucosides, steroids and terpernoids which are responsible for the treatment of these ailments in ethno medicine ascribed to plant. The phytochemical screening of the plant confirmed the presence of alkaloids- ascribing anti microbial activity within the plant, flavonoids ascribing pro-oxidants and affects the inner cell membranes. They are well known for their anti –inflammatory, anti-allergic and vaso-protective properties.

Test were carried out quantitatively on the leaves and roots of *imperata cylindrica* to determine the quantity of some phytochemicals in the grass like Tannins, Alkaloids, Flavonoids, Saponnins and terpenoids.

The results showed that the quantity of Tannins are 0.0575.7%, flavonoids 0.10310.3%, Alkaloids 0.054 5.4% , Saponinns 0.023 2.25% and terpenoids 0.433 43.3% .

The extract was further tested for their potency through anti bacterial and anti fungal activity against test organisms like *Pseudomonas aeruginosa, Bacillus leveus, Staphylococcus Epidermis, Kleibsiella pneumonia, Escharichia coli, candida Albican, Candida parapsilosis, Aspergillus niveus, Aspergillus parasiticus , Microsporum gypseum* . The anti-microbial screening showed that water extract, 80% methanol and ethyl acetate had higher activity against bacterial and fungal specie. The anti bacterial and anti fungal activity showed strong zones of inhibition zone effect against *E .Coli, staphylococcus Epidermis, albican candida and Aspergillus parasiticus*, both polar and non polar extracts of the plant showed high activity against the test organism at lower concentration.

The result of elucidation of the structure of IMP1, IMP2 and IMP 3 extracts are depicted in Tables 4.7 -4.9.1 and Appendixes 1 -9. The suggested compounds are 1,6-anhydro-Heptanoic acid, 1,6-dichloro-cyclohexaneacetic acid,1,6-anhydro-5-Hexenoic acid , 5-Hexenoic acid and cyclohexaneacetic acid. The suggested compounds from the analysis are possible antimicrobial because fatty acids are anti microbial.

Hence all these pharmacological screening on the *imperata cylindrica* plant confirmed its use in cure of several ailment ascribed to the plant traditionally.

5. CONCLUSION

The presence of useful bioactive class of phyto compounds like alkaloids, saponins, glycosides, flavonoids, terponoids in plant was an indication of the plant ethno pharmacological value. The phytochemical analysis of the *Imperata cylindrica* plant showed that the phyto compounds it contained, are the main basis for the plant medicinal properties.
Antimicrobial activity of both polar and non polar fractions of crude drug confirmed the high efficacy of the plant in the cure of diseases caused by *E.Coli, Staphyloccus Epidermis, Pseudomanas aeruginosa, Bacillus leveus, Kleibsiella Pneumonia,candida albican, Asperigellus flavus, Asperigellus niger,fusarium oxysparum and candida parapsilosis.*
Structural elucidation through combined techniques (UV-VIS, FTIR AND GC-MS) suggested these bioactive compounds: (1) 1,6-anhydro-Heptanoic acid, (2) 1,6-dichloro-cyclo hexaneacetic acid, (3) 1,6-anhydro-5-Hexenoic acid, 5-Hexenoic acid and cyclohexaneacetic acid.
In this study, the plant extract had been found to contain fatty acid ethyl ester and hence would be expected to exercise potent antibacterial, antifungal, anti –inflammatory and anti-oxidant properties.
From the present study, it have been recommended that the extract of *imperata cylindrica* should be formulated into tablets , injections, suspension and syrup for teething in infant and for use in treatment of diseases caused by *Pseudomonas aeruginosa, Bacillus leveus, Staphylococcus Epidermis, Kleibsiella Pneumonia and Escherichia Coli.*
Also it is recommended that the dosage toxicity and concentration at which the crude drugs should be used should be investigated. General priority for the following measures. Inventing and documenting various medicinal plant and herbs that are used to treat common diseases in each country. Setting of testing laboratories with adequate facilities for the assessment of the efficacy of *imperata cylindrica* plant.
Finally, further research should be done on the plant *imperata cylindrica* to know other properties of the plant metabolites.

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