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Qualitative Phyto Chemical Screening and GC-MS Analysis of Ethanolic Extract of Acalypha Indica Linn

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ABSTRACT

Medicinal plants have been used for centuries before the advent of orthodox medicine. The plant kingdom represents an enormous reservoir of biologically active compounds with various chemical strctures and protective disease and preventive properties. The qualitative phytochemical screening of aerial part of Acalypha indica extracts analysed in petroleum ether, chloroform, ethanol and aqueous extracts. The phyto chemical extract revals the presence of alkaloids, saponins, phenols, tannins, flavonoids and glycosides. The results showed that the ethanol was more efficient than other extracts. Acalypha indica aerial part of ethanolic extract using Gas Chromatography – Mass Spectrometry method. In the present study plant, 13 phytochemical constituents were identified by GC-MS Chromatogram. The phytochemical compounds which could be exploited for the development of plant based drugs.

KEYWORDS: Ethanolic extract, GC-MS, phytochemical screening, Acalypha indica, phyto compounds, chromatogram.

I. INTRODUCTION

Phytochemicals are responsible for medicinal activity of plants these are non – nutritive chemicals that have protected from various diseases[1,2]. The major constituent consists of alkaloid, flavonoids, saponins, phenolic compounds, phytosterols, proteins and aminoacids, gums and mucilage and lignin[3]. Phytochemical constituents are the basic source for the establishment of several pharmaceutical industries the constituents are playing a significant role in the identification of these plants lies in some chemicals substances that produces a define physiological action on the human body. The most important property of these bioactive constituents of plants is that they are more effective with little or no side effects when compared to the commonly used synthetic chemotherapeutic agents. The plants are widely used in the world's pharmaceuticals. The well – studied bioactive constituents of plant kingdom include alkaloid, tannins, steroids, terpenoids, flavonoids, carotenoid and glycosides[4].

Phenolic compounds such as quercetin, rutin, narigin, catechin, caffeic acid, gallic acid and chlorogenic acid are very important plant constituents[5]. Flavonoids are the large group of naturally occurring phenolic compounds, which occurs in different plant parts in free state and as glycosides[6]. They are found to have many biological activities including anti microbial, anti ulcer, anti arthritic, anti cancer, anti angiogenic, anti inflammatory, protein kinase inhibition etc. The flavonoid and flavonols are most widely distributed of all the phenolics[7]. Flavonoids are particularly beneficial, acting as anti oxidants and giving protection against cardio vascular disease, certain forms of cancer and age related degeneration of cell components. Their poly phenolic nature enables them to scavenge injurious free radicals such as super oxide and hydroxyl radicals[8]. A variety of dietary plant flavonoids inhibits tumor development in experimental models[9].

Gas Chromatography – Mass Spectrometry (GC-MS) is a systematic thechnique that have been used in the identification of bioactive molecules in plants. GC-MS has two advantages over other spectroscopic techniques. One, the capillary column has good separation precision and can produce a high – quality chemical finger print that can be used to interpret bio active molecules. Secondly, the special data base on the information of the chemical composition of the plant extract which would be important in the structural elucidation and in the synthesis of novel drug candidate molecules and nutraceutical products[10]. The indigenous knowledge on the use of plant is one of the

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determining factor that helps in defining the cultural identities and provides link with the previous knowledge of their use[11].

II. MATERIALS AND METHODS

Qualitative Phyto Chemical Screening of Acalypha Indica

Test for Carbohydrates

Fehling's Test

5 ml of Fehling's solution was added to 2 ml of extract and boiled in a water bath. The formation of yellow or red precipitate indicates the presence of reducing sugars[12].

Test for Proteins and Aminoacids

Dragendorff's Test

To 1 ml of the extract, 1 ml of Dragendorff's reagent was added. The appearance of orange-red precipitate indicates the presence of alkaloids[13].

Test for Flavonoids

Shinoda's Test

To 1 ml of the extract, magnesium turnings and 1-2 drops of concentrated hydrochloric acid were added. Formation of pink colour indicates the presence of flavonoids[14].

Test for Glyeosides

Legal's Test

The extract was dissolved in pyridine and freshly prepared sodium nitroprusside solution was added. The formation of pink or red colour indicates the presence of glycosides[15, 16].

Test for Tannins and Phenolic Compounds

a. To 1 ml of the extract, 2 ml of 5% neutral ferric chloride solution was added. The development of a dark bluish-black colour indicates the presence of tannins.
b. To 1 ml of the extract, 2 ml of gelatine solution was added. The formation of white precipitate indicates the presence of tannins and phenolic compounds[17].

Test for Saponin

Foam Test

About 1 ml of the extract was diluted with 20 ml of distilled water and was shaken in a graduated cylinder for 15 min. The formation of 1 cm layer of foam indicates the presence of saponins[18].

Test for Steroids and Sterols

Salkowski's Test

The extract was dissolved in 2 ml of chloroform and equal volume of concentrated sulphuric acid was added along the sides of the test tube. The upper layer turns red and lower layer turns yellow with green fluorescence, indicating the presence of steroids and sterol compounds in the extract[19].

Test for Terpenoids

Libermann-Burchard's Test

The extract was dissolved in 2 ml of chloroform and added 10 drops of acetic anhydride and 5 drops of concentrated sulphuric acid. Appearance of red to violet colour indicates the presence of terpenoids[14].

Test for Volatile Oil

2 ml of the extract was evaporated on a porcelain crucible. If the residue has an aromatic smell, it indicates the presence of volatile oil[20].

GC-MS Analysis of Components of Acalypha Indica

Preparation of the Extract

2 µl of ethanolic extract of Acalypha indica was employed for GC-MS analysis.

Instruments and Chromatographic Conditions

GC-MS analysis was carried out on a GC Clarus 500 Perkin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions. Column Elite-1 fused silica capaillary column (30 × 0.25 mm ID × IEM df, composed of 100% Dimethy l poly siloxane), operating in electron impact mode at 70 eV; helium gas (99.999%) was used as a carrier gas at a constant flow of 1 ml / min with an injection temperature of 250^0C, an injection volume of 0.5 EI employed with a split ratio of 10 : 1, and ion-source temperature of 280^0C. The oven temperature was programmed from 110^0C (isothermal for 2 min), with an increase of 10^0C/min to 200^0C/min, then 5^0C/min to 280^0C/min, ending with an isolthermal for 9 min at 280^0C. Mass spectra were taken at 70eV; a scan interval of 0.5 s and fragments from 40 to 550 Da[21].

III. RESULTS AND DISCUSSION

The preliminary phyto chemical screening presented in Table 1 revealed the presence of

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alkaloids, glycosides, flavonoids, saponins, phenols and tannins in ethanol extract. The results showed that the ethanol extract was more efficient than other extracts. Hence the ethanolic extract of plant sample was selected and used for further study.

The phyto chemical components identified by GC-MS analysis of ethanolic extract of Acalypha indica are shown in Table 2 and Fig 1 with their retention time, peak area percentage, molecular formula and probability. In the present study plant, 13 phyto chemical constituents were identified by GC-MS Chromatogram. Out of which Five major phyto chemical constituents namely mome inositol, C(E) -1- (tert – butyldimethyl silyl) -4, 4-dimethyl – 2 – penten – 1 – one, 2, 3 – Dihydro – 3, 5 – dihydroxy – 6 – methyl – 4 H – pyran – 4 – one, cytidine (CAS) and 3 – phenyl – 1, 2 – pyrazole were found in abundance with peak area % ranged between 2.83 and 47.29. These phyto constituents were found to show the retention time between 7.01 and 29.12.

Medicinal plant based drugs have shown the added advantage of being simple effective, free from side effects and offera broad spectrum of activity with great emphasis on preventive action of chronic and degenerative diseases. Medicinal plants are the richest bio-resource of drugs of traditional medicines, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. The medicinal plants have chemical substances called phyto chemicals that produce various physiological action on the human body.

Phyto chemical screening is an essential step towards the discovery of new drugs as it provides the information regarding the presence of particular primary and secondary metabolites in the plant extract of clinical significance. Phyto chemicals derived from the plant sources are used for prevention and treatment of diabetes mellitus. Cancer, heart diseases and high blood pressure. The therapeutic effect of several medicinal plants have been attributed to the presence of phenolic compounds such as flavonoids, phenolic acid, prosanthocyanidins, diterpenes and tannins.

Medicinal plants have been used for centuries as remedies for human illness. They are the richest bio – resource of drugs traditional systems of medicine, modern medicine, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as tannins, phenols, alkaloids, flavonoids, steroids, terpenoids, glycosides, saponins etc. A large number of medicinal plants are now widely used all over the world for production of both traditional and modern drugs and development of new drugs. These medicinal herbs constitute indispensable components of the traditional medicine practiced world wide due to the low cost, easy access and less side effects. Plants represent a reservoir of effective bio active compounds and play a dominant role in the maintenance of human health and these are non phyto toxic, more systemic and easily bio degradable. Acalypha indica can provide many benefits for several treatments when consumed orally including abortion, diarrhoea, emetic, anti inflammatory, anti bacterial, anti fungal, anti cancer, anti ulcer, constipation, insect bites and diuretic.

Table 1
QUALITATIVE PHYTOCHEMICAL SCREENING OF AERIAL PARTS OF ACALYPHA INDICA

Plant constituents	Petroleum ether extract	Chloroform Extract	Ethanol Extract	Aqueous extract
Alkaloids	-	+	+	-
Glycosides	-	+	+	-
Carbohydrates	-	-	-	+
Phytosterols	+	-	-	-
Steroids	-	-	-	-
Flavonoids	-	+	+	-
Saponins	-	+	+	-
Phenols	-	-	+	-
Tannins	-	-	+	+
Proteins and amino acids	-	-	-	+
Terpenoids	+	-	-	-
Fixed oils and fats	-	-	-	-

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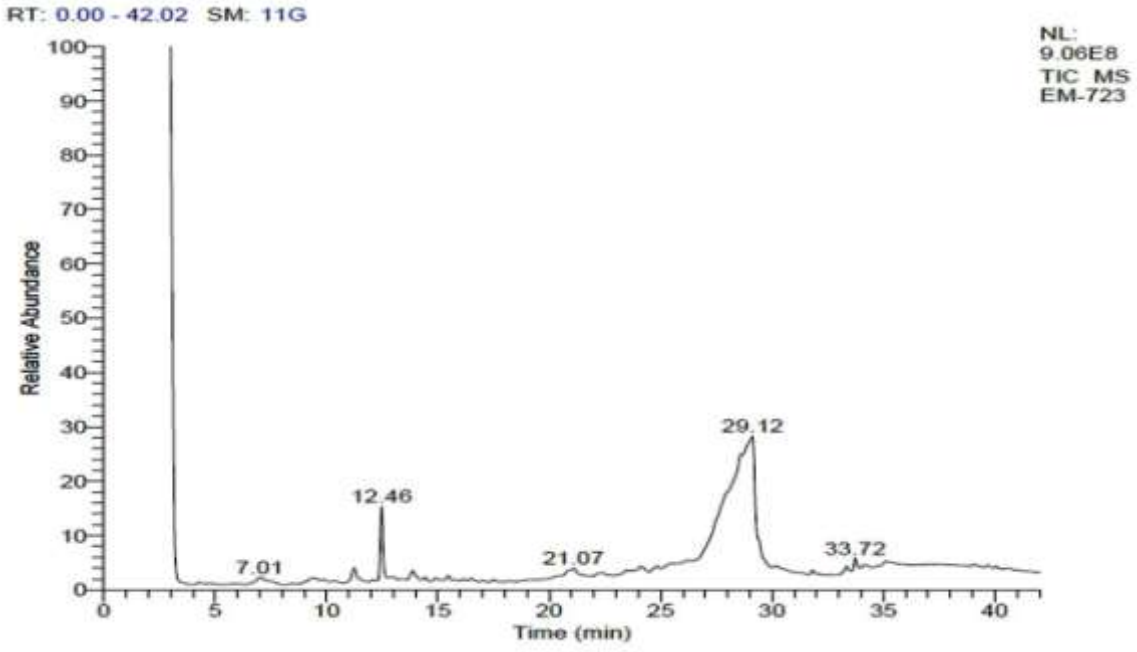
\+ = Indicates the presence of the constituents.
\- = Indicates the absence of the constituents.

Table 2
GC-MS ANALYSIS OF ETHANOLIC EXTRACT OF ACALYPHA INDICA

S. No.	Compounds	Retention Time (Min.)	Peak Area %	Molecular Formula	Probability
1.	Phenyl Methylhydrazino N-sulfamoylisosemicarbazide	4.27	0.49	$C_8H_{12}N_4O_3S$	11.51
2.	3-Phenyl-1,2-pyrazole	7.01	2.83	$C_9H_8N_2$	44.17
3.	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	11.12	3.51	$C_6H_8O_4$	92.77
4.	(E)-1-(tert-butyldimethylsilyl)-4,4-dimethyl-2-penten-1-one	12.46	11.57	$C_{13}H_{26}OSi$	7.79
5.	Thiophene, 2-propyl- (CAS)	13.86	2.71	$C_7H_{10}S$	36.97
6.	dimethyl citraconate	15.45	0.80	$C_7H_{10}O_4$	63.32
7.	Cytidine (CAS)	21.07	3.36	$C_9H_{13}N_3O_5$	12.47
8.	3-Oxo-20-methyl-11-à-hydroxyconanine-1,4-diene	24.11	1.84	$C_{22}H_{31}NO_2$	18.74
9.	Mome inositol	29.12	47.29	$C_7H_{14}O_6$	87.19
10.	Hexadecanoic acid, 2,3-dihydroxypropyl ester CAS)	33.33	1.52	$C_{19}H_{38}O_4$	40.44
11.	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	33.72	1.82	$C_{18}H_{30}O_2$	44.43
12.	4-cyanomethylquinoline	35.11	1.15	$C_{11}H_8N_2$	41.75
13.	Di-(2-ethylhexyl)phthalate	40.07	0.51	$C_{24}H_{38}O_4$	51.57

Fig 1
GC-MS CHROMATOGRAM OF ETHANOLIC EXTRACT OF ACALYPHA INDICA



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