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Characterization of saponins from *Acalypha indica* L and Carica papaya L and its microbial activities

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ABSTRACT

The advent of science in the search for antibiotics principally depends on medicinal plants as raw materials. This in-vitro study corroborated the antimicrobial activity of the plants used mostly in folklore medicine. The extract of Carica papaya and acalypha indica were tested against bacterial strains, Pseudomonas fluorescens, E. coli, Staphylococcus aureusand Bacillus subtillisand fungal strains, Fussariumoxysporum, and Phytophtherainfestans. both extracts showed activity against those isolates. The qualitative and quantitative phytochemical analyses of the plant extracts were carried out via GC–MS for the essential phytocomponents and HPLC analysis was used to determine the saponins purity of both extracts. The methanol extracts of the plants were the most active based on the antifungal assay. The qualitative phytochemical screening revealed the presence of alkaloids, tannin, saponins, flavonoids, steroids, and terpenoids in the plant. The HPLC analysis revealed a quantitative phytochemical analysis of the most active extract in the plants, the saponins with the highest content of 43.17% and 23.67%. The GC–MS analyses of the plants revealed the presence of Thiophene,2,3-dihydro (47%), Pyridine-4-aldehyd,N-ethoxycarbonylhydrazon (15.5%) in acalifa indica and 2-Trifluoroacetoxydodecane (38.24%), 1,3,5-Triazine-2,4-diamine, N,N'-bis(1-methylethyl)-6-(methylsulfonyl)-(12.20%) in Carica papaya. The present study revealed that the Carica papaya and Acalypha indica extract were composed of a variety of metabolites and therapeutic active substances, in addition to novel substances. These substances can be isolated and evaluated experimentally to confirm their biological and medicinal activities as well as verify their mechanism of action.

Keywords: Carica papaya, Acalypha indica, GC–MS, HPLC, antibacterial activity and antifungal activity

1. INTRODUCTION

Traditional medicinal plants are a therapeutic resource used by the population of the African continent specifically for health care, which may also serve as starting materials for drugs [1], Iwu et al. [2] reported that infectious diseases account for one-half of all deaths in tropical countries. As a result, people of all continents have long applied poultices and imbibed infusions of indigenous plants dating back to prehistory for health purposes [3]. It comprises therapeutic practices in existence for hundreds of years before the development of modern scientific medicine and is still in use today without any documented evidence of adverse effects.

According to the World Health Organization [4] "a medicinal plant" is any plant that in one or more of its organs contains substances that can be used for therapeutic purposes, or which are precursors for the synthesis of useful drugs. This definition distinguishes plants whose therapeutic properties and constituents have been established scientifically and plants that are regarded as medicinal but have not yet been thoroughly investigated. The term "herbal drug" determines the part/parts of a plant used for preparing medicines (for example leaves, flowers, seeds, roots, barks, stems, etc) [5] (Anon, 2007a). Furthermore [6], defines medicinal plants as herbal preparations produced by subjecting plant materials to extraction, fractionation, purification, concentration, or other physical or biological processes which may be produced for immediate consumption or as a basis for herbal products.

Medicinal and aromatic plants contain biologically active chemical substances such as saponins, tannins, essential oils, flavonoids, alkaloids, and other chemical compounds [7,8], which have curative properties. These complex chemical substances of different compositions are found as secondary plant metabolites in one or more of these plants. Tyler [9], has reported that plants also contain certain other compounds that moderate the effects of the active ingredients.

Medicinal and aromatic plants have demonstrated their contribution to the treatment of diseases such as HIV/AIDS, malaria, diabetes, sickle-cell anemia, mental disorders[10, 11], and microbial infections [2,12]. According to the World Health Organisation [6], 80% of the population of the world uses medicinal plants for the treatment of diseases and in African

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countries, this rate is much higher.

Iwu et al., [2], reported that the primary benefits of using plant derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment. The use of medicinal plants in developing countries as a normative basis for the maintenance of good health has been widely observed [13]. Furthermore, the increasing reliance on the use of medicinal plants in industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural remedies [14].

Moreover, in these societies, herbal remedies have become more popular in the treatment of minor ailments and also on accountt of the increasing costs of personal health maintenance. The survey conducted by the WHO Roll back in malariaprogram in1998, showed that in Ghana, Mali, Nigeria and Zambia, more than 60% of the children with high fever were treated at home with herbal medicines [15]. Saponins are glycosides of triterpenes and steroids characterized by their bitter or astringent taste, foaming property, hemolytic effect on red blood cells and cholesterol binding properties [16]. Saponins have been shown to possess both beneficial (lowering cholesterol) and deleterious (cytotoxic and permeabilization of intestinal epithelium) properties andto exhibit structure dependent biological activity. In medicine,it is used to some extent as an expectorant and an emulsifying agent[7].

In recent years, research on medicinal plants has drawn a lot of attention globally for its versatile applications. Medicinal plants are the wealthiest bioresources of drugs of the traditional system of medicines, modern medicines, food supplements, nutraceuticals, folk medicines, pharmaceutical intermediates, and chemical activities for synthetic drugs. Scientific experiments on the antimicrobial properties of plants and their constituents have been documented in the late 19th century [17]. Large body of evidence has been gathered to demonstrate the promising potential of medicinal plants used in various traditional, complementary and alternate systems of treatment of human diseases.

Traditionally used medicinal plants produce different compounds of identified therapeutic properties. This revitalization of interest in plant derived drugs is mainly due tothe current extensive belief that, “green medicine is safe”.Many works have been done which aim at knowing thedifferent antimicrobial and phytochemical constituents of medicinal plants and using them for the treatment of microbial infections (both topical and systemicapplications) as possible alternatives to chemically synthesized drugs to which many infectious microorganisms have become resistant. During the last ten years the paceof development of new antimicrobial drugs has slowe down while the frequency of resistance (especiallymultiple) has increased astronomically [18].

For a long period of time, plants have been precious sources of natural remedy for maintaining human health, especially in thelast decade, with more intensive studies for natural therapies.Plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made immense contributions to human health and wellbeing. Plants contain numerous biologically active compounds, many of which have antimicrobial properties [3]. Numerous studies have beenperformed with the extracts of various plants, screening antimicrobial activity as well as for the discovery of newantimicrobial compounds [19,20-21]. Acalypha indica (family: Euphobiaceae) is a small annual herb grows up to 60cm along the roadsides having medicinal properties [22]. It is distributed in Asian countries such as Sri Lanka, India, Pakistan,Africa and South America [23,24]. It is commonly known as Indian Copper leaf (T- Kuppaimeni, S- Kuppameniya) [25].Leaves aree little triangular and ovate. Leaf stalks are longer than the 3-5 cm long blades. Flowers are borne on erect of axillary spikes which are stalkless. Male flowers are minute where the female flowers are scattered along the inflorescence axis[26,27&28].

The plant extract is used fortreating pneumonia, jaundice, piles, asthma, rheumatism, bedsores, wounds, skin infections and eczema. Has been stated to have wound healing activity, snake venom neutralizing properties, antibacterial activity, and antiurolithiatic activity [29,30,31,32,33&34].The whole plant is diuretic, expectorant, emetic, anthelmintic [35].

The previous findings of Suresh et al, revealed that A. indica showed considerable antibacterial activityagainst S. aureus and E. coli. The aqueous extracts of A. indica showed inhibition against E.coli and alcoholic extract show inhibition towards Staphylococcus aureus and Salmonella typhi [36].

The phytochemical content of dried Anting-anting plants has the benefit of treating therapeutic problems, one of which is anthelmintic [37], and also mentioned in the research of Syahiranet et al., [38], that the leaves of Anting-antings- Anting-antings can be consumed orally as anthelmintic and are more often consumed because the amount is abundant and easily separated from plant parts rather than stems roots and flowers. According to Nahrstedt et al.,[39], some chemical constituents have been isolated from Acalypha indica, including kaempferol glycosides, Mauritian, clitorine, nicotiflorin and biorobin, tannins, pyranoquinolinoneflindersin alkaloids. Its ethanol extract is known to contain polyphenols, flavonoids, monoterpenes,sesquiterpenes, steroids, triterpenoids, and quinones [40], also flavonoids, tannins, saponins and glycosides in ethanol extract Anting-antings and alkaloids in petroleum ether extract, acetone and methanol [41].

Carica papaya is commonly known as papaya and belongs to family Caricaceae. Active constituent papain induces teratogenic effects and antiovulatory activity in rats. papaya (Caricapapaya) is a special fruit crop having high nutritionalvalue and potential for both fresh and processed marketuses. Papaya exceeds apple, peach, and grapes invitamins, minerals, amino acid, and food energy values asa fruit source. The green fruit is evident to comprise ofprotein, fat, carbohydrate, fiber, ash, Ca, P, Fe, Na, K, beta carotene equivalent, thiamine, riboflavin, niacin,ascorbic acid, and Vitamin E. It has also been found tocontain sinigrin, the enzyme myrosin, and carpasemine[42] (Amosu AM etal., 2014).

Papaya has many biologically active compoundsincluding chymopapain and papain, which helps indigestion [43]. Papaya root, seed and leaf extract possess highly anti-tumor and pesticidal properties [44]. The green papaya is used for the cure of ulcer and impotence [45].The unripe fruits have uses as antiseptic, in cleansing the intestines of bacteria and enabling the intestine to absorb vitamins and minerals, especially vitamin B12 [46]. Secondary metabolites such as alkaloids, flavonoids, saponins and tannins in green papaya are serving as a potent free radical scavengers and are antimicrobial in action [47]. The leaves, fruits, and latex obtained from the papaya plant have medicinal uses. The fruit has been found to contain

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certain immune-stimulating and antioxidant agents.
Antimicrobials from natural products have inspired antibiotic discovery and have been used for microbial control. These include plant extracts, small antimicrobial peptides, essential oils, bacteriocins and various groups of compounds [48]. There areseveral reports showing the antimicrobial activity of free compounds isolated from natural sources. Natural products such as tannins aregood substances to control microbial growth by interact with bacterial proteins and precipitating them [49]. Chalconederivative from Croton anisodontus Mull. Arg. acts as acompetitive inhibitor Mep A efflux pump and potentiatesciprofloxacin's action against multidrug-resistant Staphylococcus aureus. Betulinic acid was reported to have stringinhibition against Candida albicans [50]. Nimbolidefrom Azadiracta indica A. Juss possesses significant bactericidal activity against Helicobacter pylori by killing free-living bacteria and cells within biofilm [51].
The objective of this study is to extract and estimate the saponins from Acalypha indica and Carica papaya, also to assessthe saponins antimicrobial activity and antifungal activity.

2. Materials and Methods

2.1 Collection of plant materials. The plants used in this study are Acalypha indica, Carica papaya. The different plants were simultaneously collected from forests, open fields,abandoned and cultivated farms in the Hyderabadarea of Telangana State,India. The plants were identified in their fresh states and collected in sterile polythene zip lock covers to transport the laboratory for analysis.

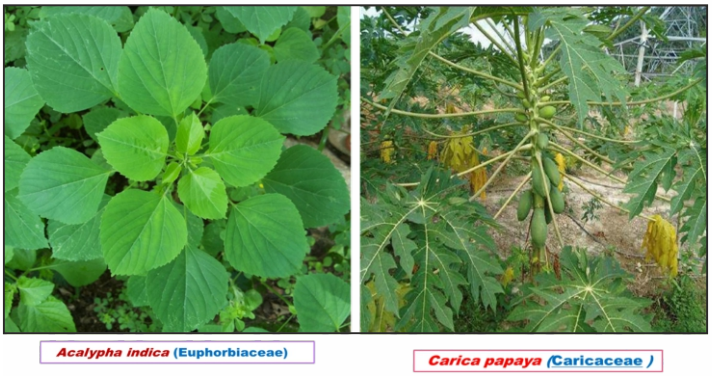

Figure 1: Pictures of medicinal plants Acalypta indica and Carica papaya.

2.2 Preparation of plant extracts
The collected plant parts were separated from undesirable materials and were washed with distilled water. They were sun-dried for one week and ground into fine powder with the help of a grinder. The powder was stored in an airtight container and kept in a cool, dark, and dry place until analysis commenced. The bioactive components were extracted according to the methods of Pandey et al., [52], with slight modification. The powered materials were dissolved in80% methanol (1:10); 1 g sample should be dissolved in 10 ml of solvent Pandey et al. [52]., Mixtures were kept in sterilized beakers wrapped with aluminium foil to avoid evaporation and exposure to light. The beakers were then kept in dark for 3 days at room temperature accompanying occasional shaking andstirring. After 3 days, mixtures were filtered through Whatman no. 1 filter paper. The filtrates obtained were concentrated using a water bath.

2.3 Test for saponins
The extract (50mg) was diluted with 20 ml of distilled water, and it was agitated in a graduated cylinder for 15 minutes. The formation of 1 cm layer of foam showed the presence of saponins.

2.4 Sample Preparation for HPLC analysis
Two grams of each plant sample powder was extracted in soxhlet apparatus with 150 mL of 70% ethanol for 7 hrs at 45°C. The extraction procedure was executed in triplicate for each plant sample. It was transferred into a flat bottom flask and concentrated with a rotary evaporator. The concentrate was then dissolved in 10mL of HPLC-grade methanol.

2.5 HPLC analysis
30µl of these extracts were passed through. 45µm syringe filter and that filtrate was used for HPLC analysis. The HPLC system (Shimadzu lab chromo 2010 HT HPLC, UV detector) was used. The software package used for analyzing results was Shimadzu lab chromo HPLC control and auto-sampling. Chromatographic analysis was carried out using a c-18 column at 35 ^{0}C temperature. Prior to analysis, the column was equilibrated with the corresponding. Running conditions included: injection volume 15µl; mobile phase: acetonitrile: water (40:60) running time 25 min., flow rate 1 ml/min; and detection at 203nm. The separation of filtered methanolic plant extracts, as well as a mixture of authentic standard samples of saponins, was done. The peak area of standards and samples was calculated to determinate concentration.

2.6 Gas Chromatography-Mass Spectroscopy Analysis
To determine the chemical composition of the samples, a Trace GC Ultra-ISQ mass spectrometer was used. The analysis was performed using a TG-5MS column with dimensions of 30m x 0.25mm x 0.25µm film thickness. The temperature of the oven was programmed to increase from 60°C to 150°C at a rate of 5°C/min, then increased to 280°C at a rate of 10°C/min, holding for 2 minutes at 150°C. Helium gas was used as a carrier at a constant flow rate of 1 ml/min, and the inlet and transfer line temperatures were kept at 250°C. A solvent delay of 3 minutes was used, and 1µl of diluted samples were automatically injected in split mode using Auto sample AS3000. Mass spectra were collected in full scan mode at 70 eV ionization voltages over the range of m/z 40-650 [53].

2.7 Anti-bacterial activity
Nutrient agar plates are prepared and pathogenic bacterial lab cultures that are Pseudomonas fluorescens: MTCC 9768, E.coli: MTCC 424, Staphylococcus aureus: MTCC 96, Klebsiella pneumoniae: MTCC 272, and Bacillus subtillis: MTCC 3053 were spread in the agar plates. Then activated samples are placed using the paper dip method and incubated for 24hrs. After 24hrs of incubation clear zone of bacterial inhibition was observed around the sample, which was measured, and the measurement of the zone was recorded. Samples which were showing the antibacterial activity are used for further study [54].

2.8 Anti-fungal activity
The anti-fungal activity was detected by the dual culture method. FussariumoxysporumNCIM1008 and Phytophtherainfestans MTCC 8707 were grown on PDA

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medium. An agar block (five mm dia) was cut from an actively growing (96 h old) fungal culture and placed on the surface of fresh agar medium at the centre of petri plate. A paper disc dipped in the respective sample was placed onto the plate at different locations of a 90 mm dia Petri plate and plates were incubated at 30 ±2 °C. Inhibition zone between two cultures was measured after 5 days of incubation.

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

Where,
I = Inhibition % of mycelia growth (growth reduction over control)
C = Radial growth of fungus in the control plate (mm)
T = Radial growth of fungus on the plate inoculated with bacteria (mm)

3. Results and Discussion:

3.1 GCMSanalysis of Acalifa indica

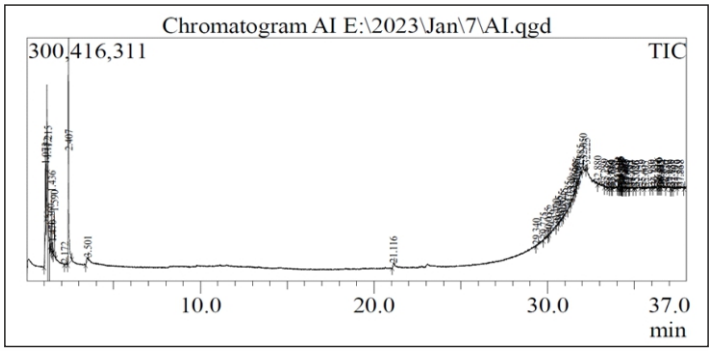

Figure 2: Chromatogram of Gcms analysis Acalifa Indica.

Table 1: Phytochemicals identified in methanolic plant extract of Acalifa indica by GC-MS.

Pea	R.Time	Area	Area%	Height	A/H	Basem/z	Name
1	1.073	169501472	6.07	74790793	2.27	48.70	Propanoicacid, 2-chloro-
2	1.172	103581708	3.71	20397489	5.08	59.70	Pentaborane(11)
3	1.215	1331644146	47.65	0	0.00	84.75	Thiophene,2,3-dihydro-
4	1.360	21079744	0.75	27899534	0.76	57.10	Propane,1-(ethenyloxy)-2-methyl-
5	1.436	97250416	3.48	73374675	1.33	84.95	Chloroform
6	1.470	11189036	0.40	13487273	0.83	56.05	Cyclopentane,methyl-
7	1.590	94439361	3.38	55119576	1.71	73.05	Propane,2,2-dimethoxy-
8	2.172	4755265	0.17	1588915	2.99	44.00	Pentanal
9	2.407	433310132	15.51	112846630	3.84	93.80	Pyridine-4-aldehyd,N-ethoxycarbonylhydrazon
10	3.501	27131282	0.97	3847012	7.05	43.05	2-Pentanone,4-hydroxy-4-methyl-
11	21.116	14421802	0.52	4010556	3.60	41.05	n-Hexadecanoicacid
12	29.340	6868000	0.25	1722118	3.99	207.00	(9-Oxo-9,10-dihydroacridin-4-yl)aceticacid
13	29.775	9590520	0.34	1930680	4.97	207.00	Benzene,2-[(tert-butyldimethylsilyl)oxy]-1-isopropyl-4-methyl-
14	30.035	4651958	0.17	2228614	2.09	207.00	Propiophenone,2'-(trimethylsiloxy)-
15	30.162	4979300	0.18	2357177	2.11	133.05	n-Propylamine,N-acetyl-3-[2-acetyl-3,4,5-trimethoxyphenyl]-
16	30.490	9390819	0.34	2325397	4.04	73.05	Silane,trimethyl[(3,7,11-trimethyl-2,6,10-dodecatrienyl)oxy]-
17	30.645	8429432	0.30	2387583	3.53	73.10	Silane,trimethyl[(3,7,11-trimethyl-2,6,10-dodecatrienyl)oxy]-
18	30.825	8002646	0.29	2042767	3.92	253.05	1-Trimethylsilyl-4-(1-methyl-1-silacyclobutyl)benzene
19	30.945	9555031	0.34	2281982	4.19	281.05	1H-Indole-2,3-dione,1-(tert-butyldimethylsilyl)-5-chloro-,3-(O-ethyloxime)
20	31.185	8659981	0.31	2524044	3.43	96.10	Pseduosarsasapogenin-5,20-dien methylether
21	31.330	4551350	0.16	1773465	2.57	73.05	Silane,[1,4-dioxane-2,3-diylbis(oxy)]bis[trimethyl-,cis-

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22	31.485	10450667	0.37	3832685	2.73	281.05	1,3,5,7-Tetraethyl-1,7-dibutoxytetrasiloxane
23	31.600	5559430	0.20	2535191	2.19	281.05	2-Methyl-7-nonadecene
24	31.655	10687457	0.38	3320104	3.22	281.10	Haloxazolam
25	31.696	4426073	0.16	3637583	1.22	209.00	Silane,(9,19-cyclo-9.beta.-lanost-24-en-3.beta.-yloxy)trimethyl-
26	31.885	23580593	0.84	5850074	4.03	67.05	2-(4-Hydroxybutyl)cyclohexanol
27	32.050	37206432	1.33	11388039	3.27	281.10	E-10,13,13-Trimethyl-11-tetradecen-1-olacetate
28	32.225	19344260	0.69	6792112	2.85	69.10	2,10-Dodecadien-1-ol,3,7,11-trimethyl-,(Z)-
29	32.880	5327769	0.19	2568993	2.07	208.00	Pentasiloxane,1,1,3,3,5,5,7,7,9,9-decamethyl-

Interpretation on mass spectrum GC-MS wasconducted using the database of National Institute
Standard and Technology (NIST) having more than62,000 patterns. The spectrum of the unknowncomponent was compared with the spectrum of theknown components stored in the NIST library. Thename, molecular weight and structure of thecomponents of the test materials were ascertained inTable 1 figure 2.MethanolicAcalifa indicaplant extractwas subjectedto GC-MS study for identification of medicinalproperties, according to the results, the total 29 Phytocomponents are screened, and most of themedicinal properties areThiophene,2,3-dihydro (47%), Pyridine-4-aldehyd,N-ethoxycarbonylhydrazon (15.5%), Propanoicacid, 2-chloro (6.07%), Pentaborane(11) (3.71%), Chloroform (3.48%), Propane,2,2-dimethoxy- (3.38%), E-10,13,13-Trimethyl-11-tetradecen-1-olacetate (1.33%) relative abundance respectively.

3.2 GCMS ANALYSIS OF CARICA PAPAYA

The analysis of extracts using GC MS technique also proved that there are effective compounds in papaya plants according to the solvent used as shown in Table (2) and Figure (3). Table (2) and figure (3) showed the presence of 12 phytocomponentsscreened in the methanolic papaya leaf extract. Through comparative examination, the main components present in the papaya plant extract of the local variety in terms of their relative abundance were 2-Trifluoroacetoxydodecane (38.24%), 1,3,5-Triazine-2,4-diamine, N,N'-bis(1-methylethyl)-6-(methylsulfonyl)-(12.20%), ,5-Heptadien-3-yne (5.71%), 1-Propanethiol (3.73%), Methylene Chloride (3.53%), Chloroform (2.80%) and Hexane (1.32%)relative abundance respectively.

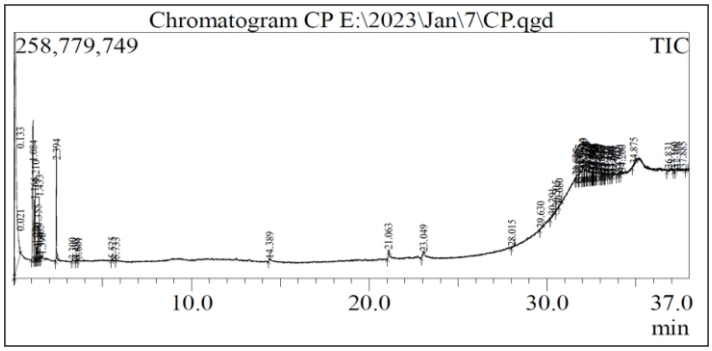

Figure 3 : Chromatogram ofPhytocomponents in the papaya plant extract.

Table 2:Phytochemicals identified in methanolicplant extract of Carica papaya by GC-MS

Peak	R.Time	Area	Area%	Height	A/H	Base m/z	Name
1	0.021	26644071	0.81	48981498	0.54	44.05	2-Heptanamine, 5-methyl-
2	0.133	1254601570	38.24	125255983	10.02	41.10	2-Trifluoroacetoxydodecane
3	1.084	400227497	12.20	102705694	3.90	62.85	1,3,5-Triazine-2,4-diamine, N,N'-bis(1-methylethyl)-6-(methylsulfonyl)-
4	1.166	122228164	3.73	63195535	1.93	46.10	1-Propanethiol
5	1.210	115777816	3.53	81287753	1.42	83.95	Methylene Chloride
6	1.270	22829949	0.70	15520228	1.47	43.05	Pentane, 2-methyl-
7	1.308	16905316	0.52	12578035	1.34	57.15	Pentane, 3-methyl-
8	1.355	43959187	1.34	34446590	1.28	57.15	Hexane
9	1.433	91906495	2.80	65324924	1.41	82.95	Chloroform
10	1.465	13117068	0.40	13106320	1.00	56.10	Cyclopentane, methyl-
11	1.590	10929492	0.33	3578291	3.05	73.10	Propane, 2,2-dimethoxy-
12	2.394	187335547	5.71	105599693	1.77	93.00	1,5-Heptadien-3-yne

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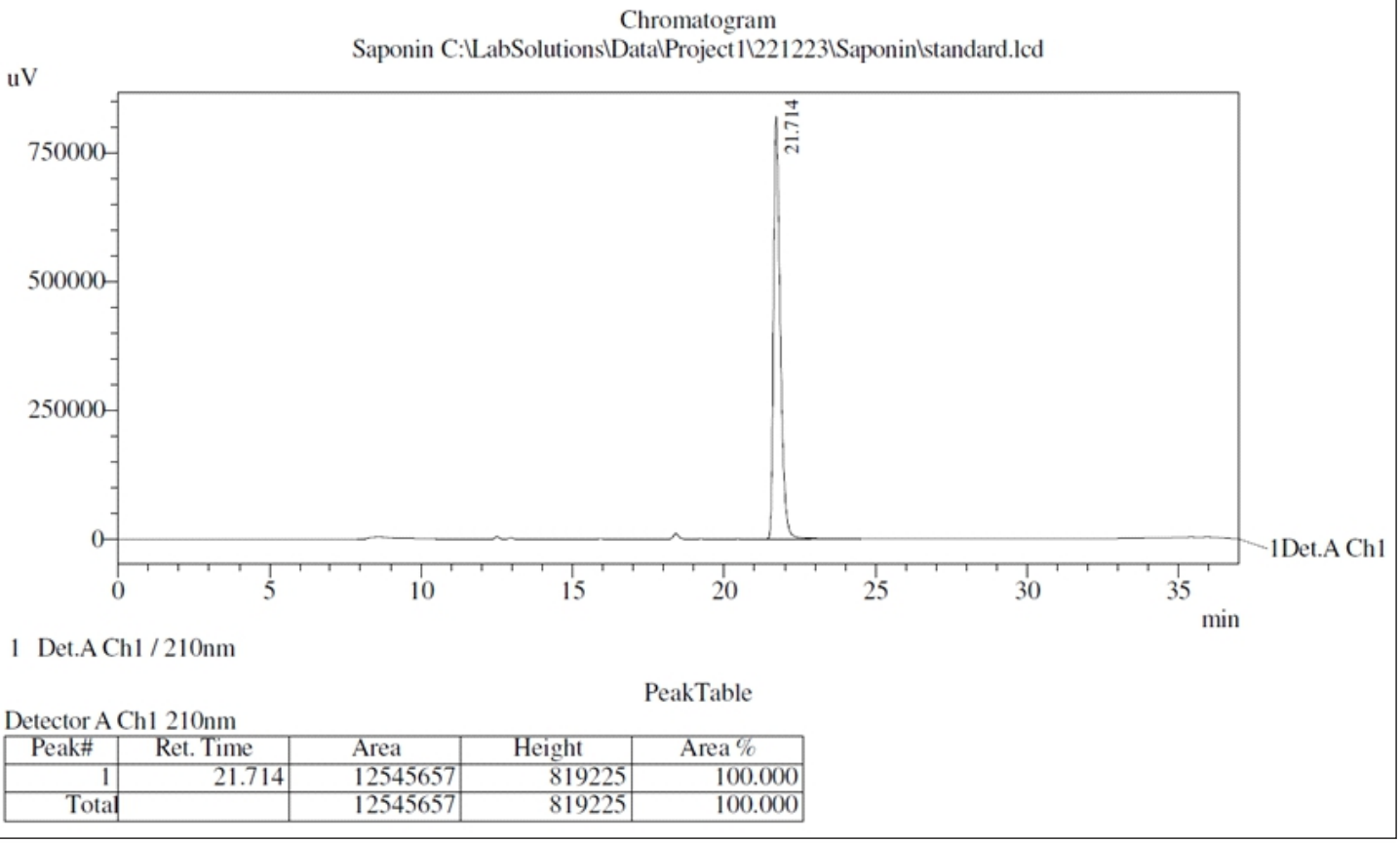
3.3 Standard saponin HPLC



PeakTable

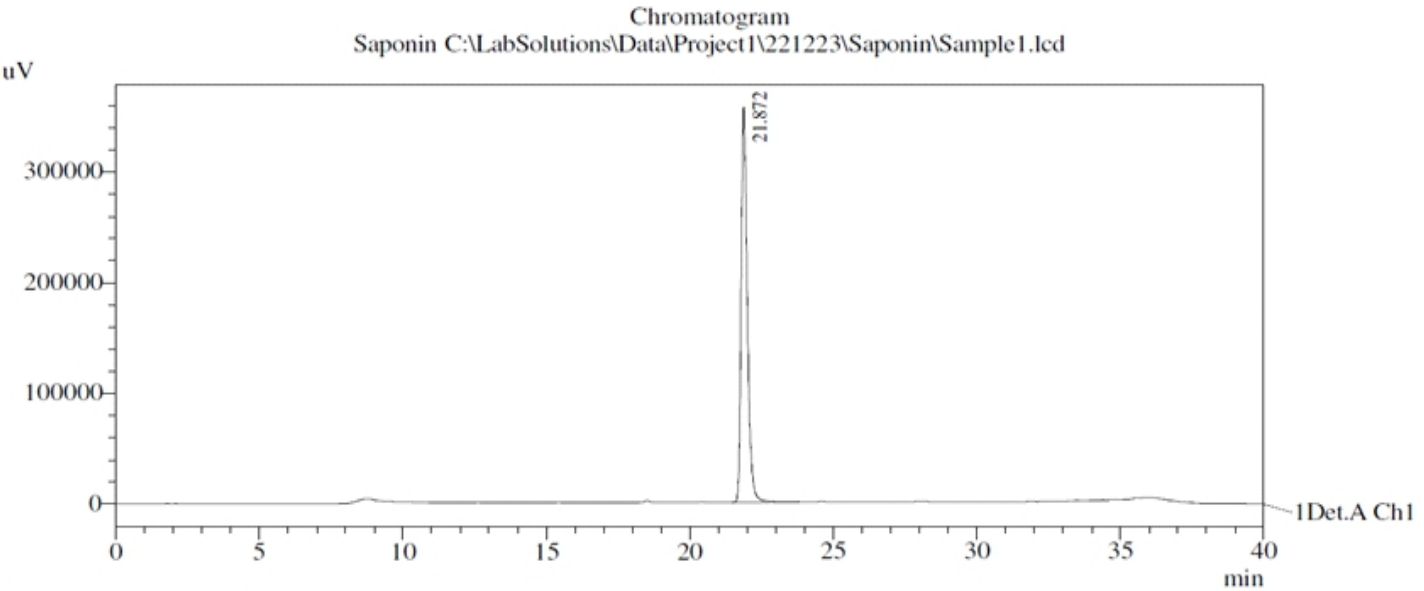
Detector A Ch1 210nm

Peak#	Ret. Time	Area	Height	Area %
1	21.714	12545657	819225	100.000
Total		12545657	819225	100.000

Figure 4: Standard saponin chromatogram by using HPLC.

HPLC was used to estimate saponin in plant extracts. NIST traceable standard saponin was procured from the local market. HPLC was run up to 35 minutes and the saponin was eluted at 21.7 minutes. The standard saponin chromatogram was compared with two plant extracts to know the saponin purity.

3.4 HPLC of Acalifa indica



PeakTable

Detector A Ch1 210nm

Peak#	Ret. Time	Area	Height	Area %
1	21.872	5356652	357263	100.000
Total		5356652	357263	100.000

Figure 5: Acalifa indica plant extract chromatogram by using HPLC.

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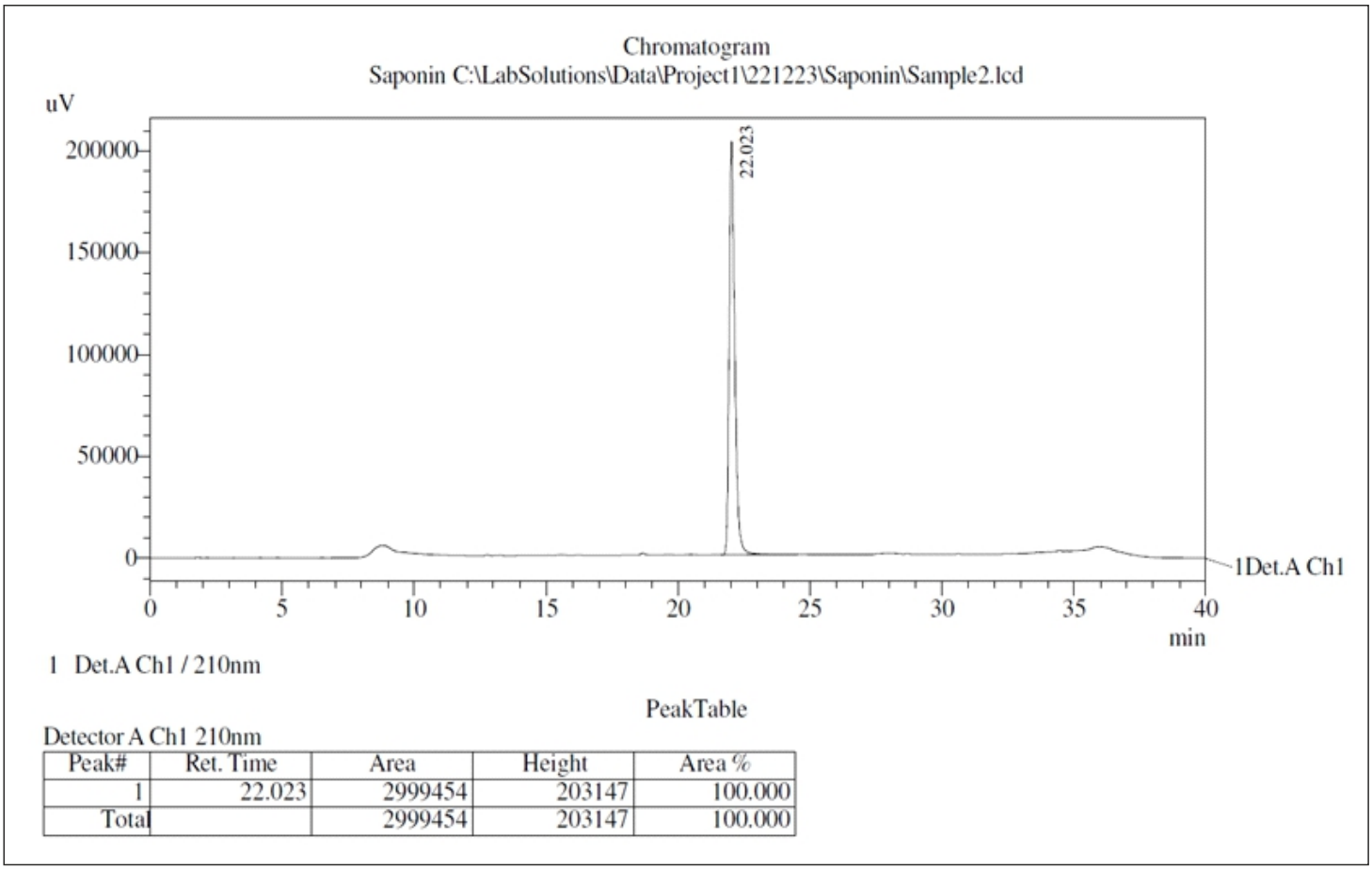
HPLC analysis of acalifa indica plant extract chromatogram, the run time was matched with standard saponin. The purity calculation was revealed that the aclifa indica plant extract is having 43.12% of saponin concentration.

3.5 HPLC analysis of Carica papaya



PeakTable

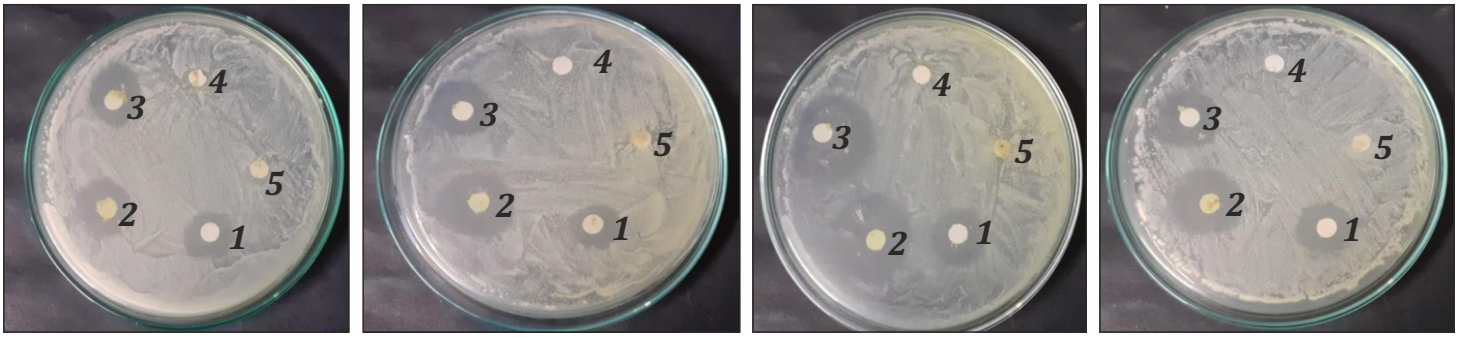
Detector A Ch1 210nm

Peak#	Ret. Time	Area	Height	Area %
1	22.023	2999454	203147	100.000
Total		2999454	203147	100.000

Figure 6: Carica papaya plant extract chromatogram by using HPLC.

HPLC analysis of Carica papaya plant extract chromatogram, the run time was matched with standard saponin. The purity calculation revealed that the Carica papaya plant extract is having 23.67% of saponin concentration.

3.6 Antibacterial Activities:



Pseudomonas fluorescens MTCC 9768 | ***E.coli: MTCC 424*** | ***Staphylococcus aureus: MTCC 96*** | ***Bacillus subtillis: MTCC 305***

Figure 7:Antibacterial activity against bacterial strains

Here 1: Standard saponin 2: Plant extract of AI with methanol, 3: Plant extract of CP with methanol, 4: Plant extract of AI with water, 5: Plant extract of CP with water.

Table 3: Antibacterial activity of plant extracts

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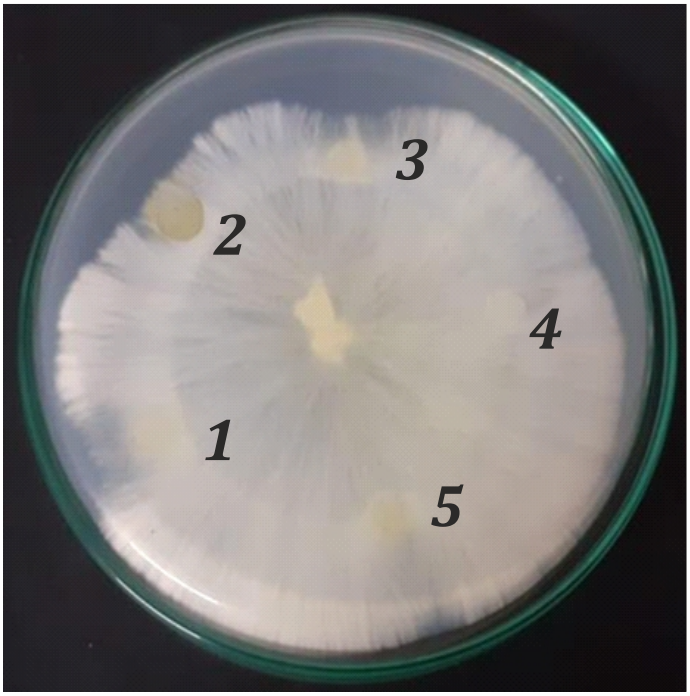
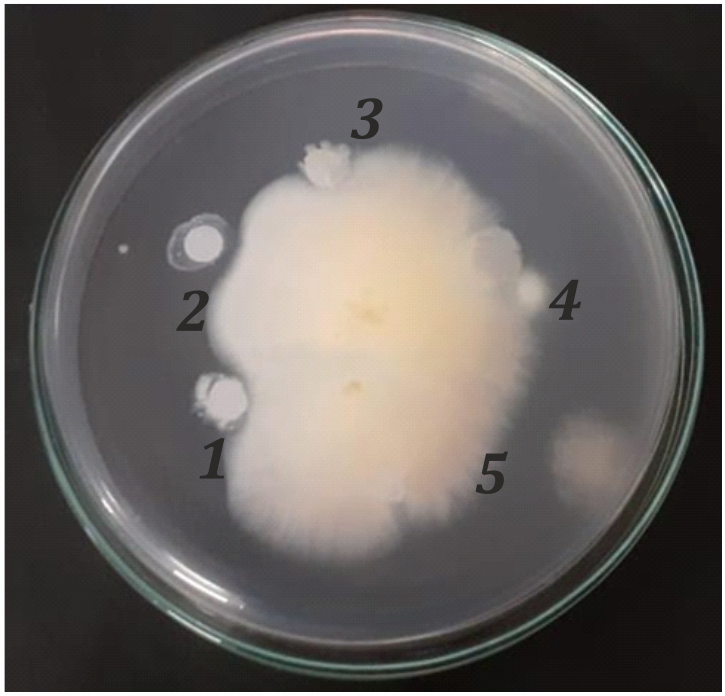
	Standard saponin (Zone in cm)	Plant extract of AI with methanol (Zone in cm)	Plant extract of CP with methanol (Zone in cm)
Pseudomonas fluorescens: MTCC 9768	0.8± (0.01)	1.2 ± (0.02)	1.3 ± (0.02)
E.coli: MTCC 424	0.9 ± (0.01)	1.3 ± (0.02)	1.2 ± (0.02)
Staphylococcus aureus: MTCC 96	0.5 ± (0.01)	1.1 ± (0.02)	1.3 ± (0.02)
Bacillus subtillis: MTCC 3053	0.7 ± (0.02)	1.2 ± (0.02)	1.2 ± (0.02)

Antibacterial potentials of Acalifa indica (AI) and Carica papaya (CP) are appraised using minimum inhibitory concentration (MIC). in vivo, antibacterial potential of AI was assessed against Pseudomonas fluorescens and found the MIC as 1.2 cm whereas CP showed the MIC as 1.3cm, the least activity was observed with the standard as 0.8cm. The E.coli bacteria showed significant activities due to the saponins analysed, the highest activity was observed in AI (1.3cm) followed by CP (1.2cm) the least concentration was observed with standard (0.9cm) (CP) displayed significant MIC of 1.3cm against clinical isolates of staphylococcus aureusbacteria, whereas AI displayed as 1.1cm zone and lowest was observed in 0.5cm Bacillus subtilliswas also checked for its antibacterial activity and the results revealed that the maximum antibacterial activity was observed with both plant extracts (1.2cm), least was observed in standard as 0.7cm.

3.7 Antifungal Activities



Fussariumoxysporum NCIM1008



Phytophtherainfestans MTCC 8707

Figure 8: Antifungal activity against Fussariumoxysporum andPhytophtherainfestans

Here 1: Standard saponin 2: Plant extract of AI with methanol, 3: Plant extract of CP with methanol, 4: Plant extract of AI with water, 5: Plant extract of CP with water.

Table 4: Antifungal activity of plant extracts

	Standard saponin (Inhibition %)	Plant extract of AI with methanol (Inhibition %)	Plant extract of CP with methanol (Inhibition %)
*Fussariumoxysporum*NCIM1008	40± (1.8)	46 ± (1.2)	55 ± (1.8)
Phytophtherainfestans MTCC 8707	50 ± (1.5)	45 ± (1.6)	50± (1.4)

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The plant extracts isolated from Acalifa indica (AI) and Carica papaya (CP) exhibit strong antifungal activities. antifungal activities of Acalifa indica against Fussariumoxysporumwas found as 46%, whereas Carica papaya was shown 55% of inhibition lowest inhibition was observed with standard saponin (40%). The fungal strain Phytophtherainfestanswas inhibited 50% by Carica papaya plant extract and standard saponin activity, and the lowest inhibition was observed with Acalifa indica (45%).

Conclusions

The study's findings indicated that Acalifa indica and Carica papaya have saponins as bioactive constituents, which contribute to their antibacterial properties and antifungal properties. The saponin quantities were identified and conformed with HPLC and GCMS analysis. The antibacterial activity could inhibit Pseudomonas fluorescens, E.coli, Staphylococcus aureus and Bacillus subtillis and antifungal activity could inhibit Fussariumoxysporum and Phytophtherainfestanswell with methanolic Acalifa indica and Carica papaya extract.

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