International Journal of Pharmacy and Biological Sciences-IJPBS™ (2021) 11 (2): 46-53
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | OA Journal | MCI Approved | Index Copernicus

Phytochemical Finger Printing of *Abutilon indicum* Extract using Chromatography Techniques and Bioautography Analysis of Active Compounds

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Received: 10 Jan 2021 / Accepted: 08 March 2021 / Published online: 01 April 2021
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Abstract

Plants are capable of producing and synthesizing a large group of organic compounds known as primary and secondary metabolites and which are used primary health care. In this study the antimicrobial activity of the medicinal plant *Abutilon Indicum* was studied. The Soxhlet method is used to obtain *Abutilon Indicum* extract. Phytochemical analysis identifies the presence of bioactive compounds like tannins, saponins, flavonoids, alkaloids, protein, steroids, anthraquinones. Thin Layer Chromatography (TLC) indicates the separation of different compounds from the extract. Gas chromatography and mass spectrometry (GC-MS) controlled the compositions and resulted in identification of 27 compounds. From High performance liquid chromatography (HPLC) the flavonoid (rutin- 1.56 mg, quercetin-6.44 mg, gallic acid-2.91 mg) was quantified and identified. By using Bioautography, the antibacterial activity of the *Abutilon Indicum* extract was studied by using *E. Coli, Salmonella sp.*, *Staphylococcus sp.*, *Vibrio sp.* This earlier report can serve as an important platform for the development of further safe and effective natural medicines.

Keywords

Abutilon Indicum, GCMS, HPLC, TLC, Bioautography and DPPH Assay.

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INTRODUCTION:

Europeans and Indians have been utilizing such plants as spices for more than 4000 years. Among old civilizations, India has been known to be a rich vault of therapeutic plants. 8000 natural meds are referenced in Ayush. Natural plant are additionally utilized as food and flavors.

The World Wellbeing Association gauges that 21,000 plants are utilized therapeutically (7). 80 % of the population utilizes it for essential medical purposes. Home grown plants are utilized as medication as well as in regular colors, food, bug control. Natural plants are used to treat illnesses like looseness of the bowels, feminine issues, covered tongue, stoppage heaps, help to filter the blood.

Secondary metabolites are metabolic items that are fundamental for the communication of plants with their current circumstance. (10, 12). Secondary metabolites are utilized by plants to endure and proliferate. These are vital for fertilization of plants. Secondary metabolites are created by numerous organism and plants (2). Alkaloids, nonprotein amino acids, steroids, saponins, flavonoids, tannis,

polyacetylenes are the secondary metabolites present in plants (20).

HPLC is a strategies used to isolate distinguish and measure every part of a combination. Thin layer chromatography is a procedure used to isolate nonvolatile blend (Harry *et al*., 1989). It is performed on a sheet of an inactive substrate, which is covered with a Thin layer of adsorbent material (1). GCMS is a logical strategy that consolidates the gas chromatography and mass spectrometry to recognize diverse substances (kiton *et al*., 201). Utilization of GCMS incorporate medication discovery ecological investigation and ID of obscure example (8).

MATERIAL AND METHODS:

Plant sample of *Abutilon indicum* were collected from Life Teck Research centers. The collected *Abutilon indicum* was dried for 1 week and afterwards powdered by invert measure.

Soxhlet Method

Utilizing soxhlet contraption, 25 grams of the example was extricated with the individual dissolvable for ten hours. The concentrates were utilizing rotational evaporator at 50 º C under decreased tension. After build up, the examples were reconstituted in their particular solvents to acquire a load of 100 mg/ml and were put away in the fridge for additional investigation. The concentrates were utilized for additional examination.

Phytochemical Analysis

Different phytochemicals including Tannins, Saponins, Flavonoids, Alkaloids, Protein, Steroids, Anthraquinones were controlled by performing fundamental subjective examination of the ethyl acetic extract derivation in *Abutilon Indicum*.

Gas Chromatography-Mass Spectrometry (GC-MS)

The GC-MS assessment of leaf ethanol removed was performed using an Agilent, 6890 GC, 5973MS furnished with Elite-5 MS 30 m × 0.25 mm × 25 μm DI - 5ms (5 % phenyl, 95 % dimethylpolysiloxane). For GC-MS, an electron ionization structure with ionization energy of 70 eV was used. Ultra-pure helium gas was used as a carrier gas at a steady stream speed of 1 mL/min. Molecule source, mass trade line, and injector temperature were set at 40° C, 260° C, and 300° C, independently. The oven temperature was changed from 50 to 150° C at a speed of 3° C / min and a short time later held in isothermal condition for 10 min ultimately raised to 300° C at 10° C / min. Debilitated models (1/100, v/v in ethyl acetic acid derivation) of 1 μL were truly imbued. Mass appalling scope range was at 40 – 650 m / z, with a dissolvable deferment of 2 min. The GC upkeep time and mass spectra helps those NIST MS Search Library Software variation 2.0 (18)

High performance Liquid Chromatography(HPLC)

Examination of methanol concentrates of leaf of E. agallocha was performed by HPLC. The HPLC framework comprises of LC-20AT conspicuousness fluid chromatograph siphon and SPD-20A unmistakable quality UV-Vis identifier and Rheodyne type injector fitted with 20 uL limit fixed circle all from Shimadzu Company, Japan. The segment utilized was Phenomenex luna 5 uL C18 (2) 100A (250 mm × 4.6 mm) at surrounding temperature. The yield signals were checked and prepared utilizing spinchrom CFR programming. The dissolvable framework improved for the examination was methanol: acetonitrile: water in the proportion 25:35:40. The stream rate was 1 mL/moment and recognition wave length was set at 232 nm. The run season of the strategy was 10 min and all analytes were isolated inside the run time.

Thin Layer Chromatography (TLC)

Compound constituents of the concentrates were isolated an aluminum-upheld Thin layer chromatography (TLC) plates (Merck, silica gel 60 F254). The TLC plates were created under immersed conditions with the eluent frameworks created in our research facility, i.e., ethyl acetic acid derivation/ methanol/ water (40:5.4:5): [EMW] (11). Isolated synthetic mixtures were recognized utilizing fermented vanillin (0.1 g vanillin: 28 ml methanol: 1ml sulphuric corrosive) as a splash. Subsequent to splashing, the chromatograms were warmed at 110 ° C in a hatchery to take into account ideal shading advancement.

Formula:

Rf = (Distance went by the solute)/ (Distance went by the dissolvable)

Bioautography

Ten µl (10 mg/ml) of each concentrate was stacked onto TLC plates in a thin band and eluted utilizing the portable dissolvable frameworks (EMW). The created plates were dried under a surge of quick air for 5 days to eliminate hints. Overnight culture of bacteria was vaccinated in Muller-Hinton agar stock. The readied chromatograms were splashed with the bacterial culture in a Laminar stream bureau. From that point, the plates were brooded for 35 °C and 100 % relative stickiness in obscurity and afterward showered with a 2 mg/ml arrangement of p-iodonitrotetrazolium violet (Sigma®) (Þ-iodonitrotetrazolium) (Begue and Klein, 1972). White group's shows decrease of INT (Þ-iodonitrotetrazolium) to the hued formazan didn't happen because of the presence of tried organic entities (17).

DPPH Assay

Aliquot 3.7 ml of outright methanol altogether test cylinders and 3.8ml of supreme methanol was added to clear. 100µl of BHT was added to tube set apart as standard and 100 µl of particular example to any remaining cylinder set apart as tests. 200 µl of DPPH reagent was added to all the test tubes. All test tubes was brooded at room temperature in dull condition for 30 minutes. The absorbance of all examples was perused at 517 nm (13).

$$\% \text{ Antioxidant activity} = \frac{(\text{Absorbance at blank}) - (\text{Absorbance at test}) \times 100}{(\text{Absorbance at blank})}$$

RESULT AND DISCUSSION:

Phytochemical analysis:

From the phytochemical analysis of ethyl acetate extract of *Abutilon indicum* shows positive results for alkaloids, flavonoids, protein, and steroids.

Table 1: Phytochemical analysis of the ethyl acetate extract of *Abutilon indicum*

S.no	Phytochemical constituents	Ethyl acetate extract of *Abutilon Indicum*
1	Tannins	-
2	Saponins	-
3	Flavonoids	+
4	Alkaloids	+
5	Protein	+
6	Steroids	+
7	Anthraquinones	-

'+' indicates the presence and '-' indicates the absence

In *Abutilon Indicum,* Phytoconstituents like alkaloid, carbohydrates, phenolic compound, saponine, proteins, amino acid and tannins of aqueous extract shows positive results in the phytochemical analysis of *Abutilon indicum* (3)

Phytoconstituents like alkaloid, carbohydrates, phenolic compound, saponine, proteins, amino acid and tannins of aqueous extract shows positive results in the phytochemical analysis of *Abutilon indicum* (3)

GCMS

GCMS is used to find the presence of chemical components and their structure, mass, and formula. From the GCMS technique, the presence of 27 compounds were identified from the Ethyl acetate extract of Abutilan indicum. In the previous study, using GCMS technique 57 components were identified in chloroform extract from abutilon indicum (16).

Table 2: GCMS analysis of various chemical compounds present in the ethyl acetate extract of *Abutilon indicum.*

Peak	RT	Names	Area	Formul a	Mass	Area%
1	4.00	Dodecane, 1-fluoro-	1165708	C12H25F	188.2	8.06
2	5.58	Benzofuran, 2,3-diydro-	144044	C_8H_8O	120.1	1.00
3	5.63	Dispiro[2.1.2.4]undecane,8-methylene-	287579	C12H18	162.1	1.99
4	5.88	1,7,7-Trimethyl-2- vinylbictclo[2.2.1]hept-2-ene	395092	C12H18	162.1	2.73
5	7.03	Methyl 8,10-octadecadiynoate	243791	C19H30 O_2	290.2	1.69
6	7.34	Methyl 10,12-octadecadiynoate	294643	C19H30 O_2	290.2	2.04
7	7.85	Limonen-6-ol, pivalate	569992	C15H24 O_2	236.2	3.94
8	7.92	1H-Indene, 3-(bromomethyl)-1,1- dimethyl-	416222	C12H13Br	236	2.88
9	8.87	1,2-15,16-Diepoxyhexadecane	850548	C16H30 O_2	254.2	5.88
10	9.10	Tricyclo[4.4.0.0(2,7)]dec-8-ene-3-methanol,.alpha.,.alpha.,6,8- tetramethyl-, stereoisomer	1894132	C15H24 O	220.2	13.10
11	9.17	10,12-Tricosadiynoic acid,methyl ester	507263	C24H40 O_2	360.3	3.51
12	9.22	Retinal	1089747	C20H28 O	284.2	7.54
13	10.43	Cholestan-3-ol, 2-methylene-, (3.beta.,5.alpha)-	244012	C28H48 O	400.4	1.69
14	10.66	Falcarinol	549941	C17H24 O	244.2	3.80

15	11.33	Neoisolongifolene, 8-oxa-	1640118	C15H22 O	218.2	11.34
16	12.03	1-penten-3-one, 1-(2,6,6-trimethyl-1- cyclohexen-1-yl)-	958781	C14H22	206.2	6.63
17	13.72	Gamolenic Acid	45402	C18H30 O_2	278.2	0.31
18	13.96	cis-5,8,11,14,17-Eicosapentaenoic acid	22888	C20H30 O_2	302.2	0.16
19	15.50	tert-Hexadecanethiol	150359	C16H34S	258.2	1.04
20	16.04	cis-5,8,11,14,17-Eicosapentaenoic acid	30129	C20H30 O_2	302.2	0.21
21	16.73	2,2-Dimethylpropanoic acid, tridec-2- ynyl ester	551992	C18H32 O_2	280.2	3.82
22	18.18	Silane, diethyl(2-phenylethoxy)tridecyloxy-	51730	C25H46 O_2Si	406.3	0.36
23	19.25	2H-pyran, 2-(7-dodecynyloxy)tetrahydro-	368310	C17H30 O_2	266.2	2.55
24	21.76	3.alpha.,5.alpha.-Cyclo-ergosta- 7,9(11),22t-triene-6.beta.-ol	331360	C28H42 O	394.3	2.29
25	22.12	Methyl 2-hydroxy-4- methoxybenzonate, trimethylsilyl ether	175176	C12H18 O_4Si	254.1	1.21
26	27.49	3.alpha.,5.alpha.-Cyclo-ergosta- 7,9(11),22t-triene-6.beta.-ol	388824	C28H42 O	394.3	2.69
27	30.81	D-Homo-24-nor-17-oxachola-20,22- diene-3,16-dione, 7(acetyloxy) - 1,2:14,15:21,23-triepoxy-4,4,8-trimethyl- , (5.alpha.,7.alpha.,13.alpha.,14.beta.,1 5.beta., 17a.alpha)-	1089165	C82H34 O_8	498.2	7.53

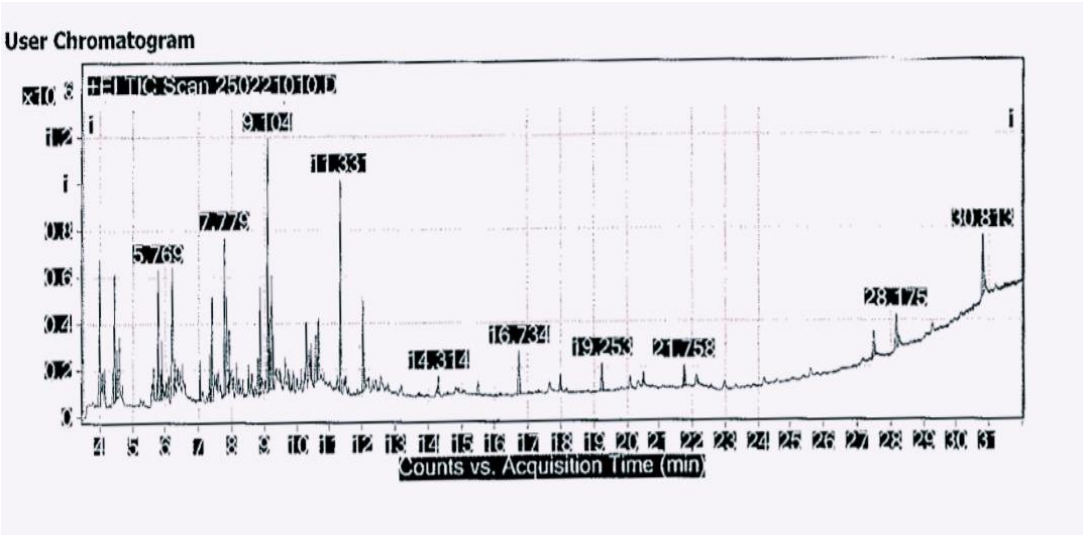


Figure 1: Graphical representation of *Abutilon indicum* through GCMS analysis.

High Performance Liquid Chromatography(HPLC)

In the current examination flavonoid (rutin-1.56mg, quercetin- 6.44mg, gallic corrosive 2.91mg) was measured by HPLC. Quescetin (flavanoid Compound) was found in the ethyl acetate extract shows a pink spot when it seen under UV Transilluminator at 366 nm. A poly phenolic compound, gallic acid was found in this concentrate. Subsequently, it shows that ethyl acetic acid derivation removal was better for TLC Separation to recognize the ideal mixtures (4). HPLC is an exact and explicit technique for the identification and measurement of alkaloids, flavonoids in plants and, henceforth dependent on this, the current investigation was completed for recognition of alkaloids and flavonoids compounds through HPLC. Flavanoids are widely used in all types of therapeutic efficacy.

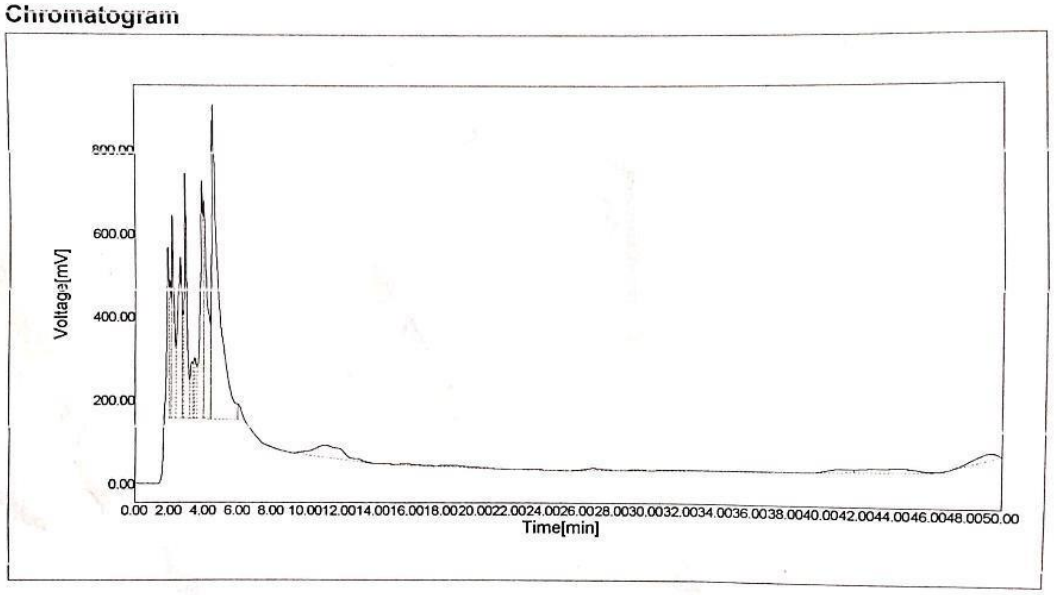


Figure 2: An overview of graph of *Abutilon indicum* in flavonoid compound by HPLC

TLC

TLC is a technique mostly used to separate compounds. *Abutilon indicum* extract on analysis showed 7 different bands or fraction and the Rf values for the bands are as follows, **0.912, 0.815, 0.843, 0.785, 0.748, 0.716, 0.70**. Under UV light all the 7 bands were clearly visible.

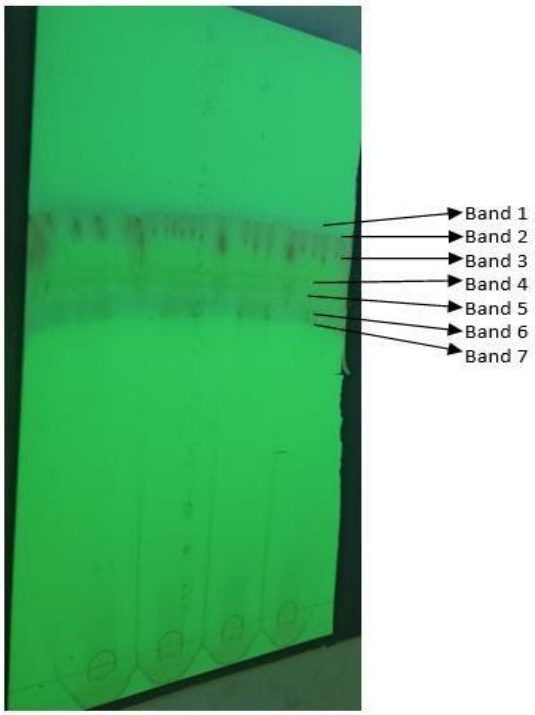


Figure 3: Thin layer Chromatography

Table 3: TLC (Thin layer chromatography) Rf values

Band	Rf Values
1	0.912
2	0.895
3	0.843
4	0.785
5	0.749
6	0.716
7	0.70

Earlier studies suggested that 21 fractions where collected out of which 7 were active fraction in **Abutilon indicum** sample. (14). The **Rf** values for the *abutilon indicum* sample were found to be **0.39**, **0.70**, **0.82**, **0.88**, **0.92**, **0.98** (3)

Bioautography

In bioautography tests, the antibacterial action of the mixtures isolated on TLC was resolved. Four distinct kinds of microbes like 1-Vibrio sp., 2- Staphylococcus sp., 3-Salmonella sp., 4-E. coli were utilized for bioautography. Critical antibacterial action was exhibited by Rf estimations of 0.749, 0.785 and 0.846 on bioautography, as apparent by the huge clear zone of restraint on a purple foundation. In the contact (backhanded) technique, just the compound with Rf 0.846 was noticeable which appeared as a huge clear zone of restraint on a purple foundation splashed with methylthiazol tetrazolium. For the Gram-negative antimicrobial action, the compound with Rf estimation of 0.846 displayed a lot of action against the given microscopic organisms. From the outcomes, the Rf estimation of 0.846 was found to have inhibitory movement against both Gram-positive and Gram-negative microorganisms. The Rf estimations of 0.86 were displayed by flavonoids and terpenoids in TLC, though Rf estimations of 0.13 were shown by alkaloids, saponins, and a few flavonoids. The bioautography being a super strategy for recognizing and restricting mixtures with antibacterial movement in Abutilon Indicum leaf. It is isolated by TLC, against microorganisms that develop straight forwardly on the TLC plate (18).

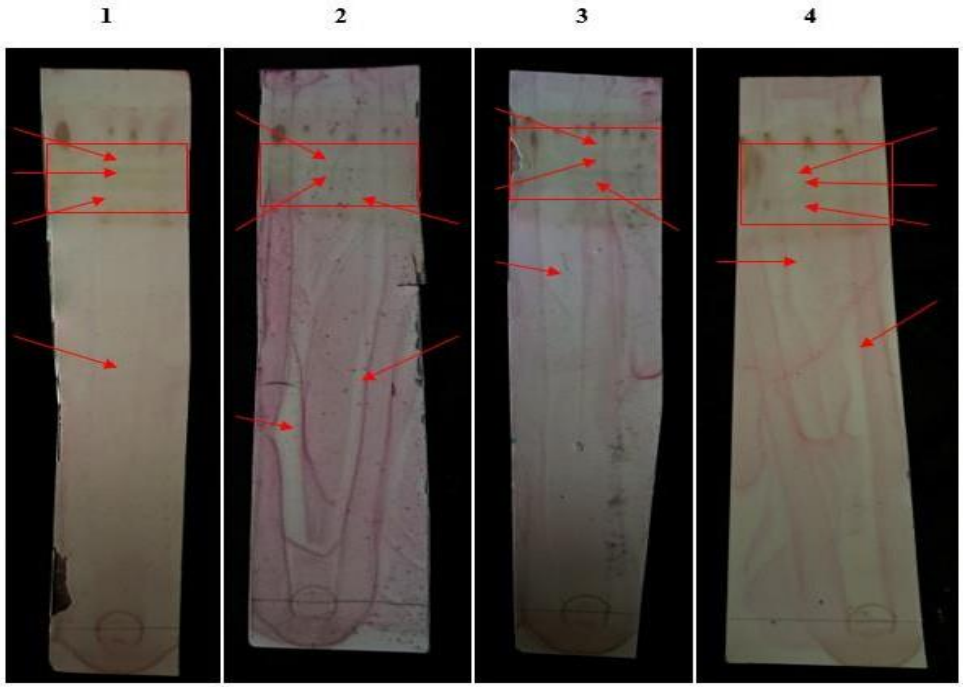
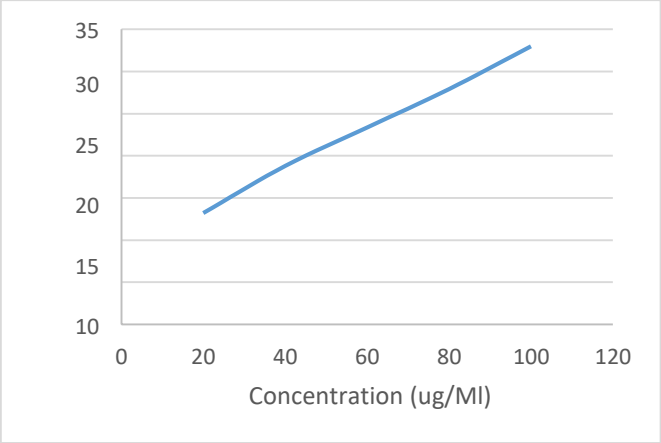


Figure 4: Bioautography for *Abutilon Indicum*

DPPH assay

Scavenging of DPPH free radicals is one of the most important antioxidant methods. The ethyl acetate extract of abutilon indicum to scavenge free radicals was assessed by the DPPH method. The highest DPPH radical scavenging activity of *Abutilon indicum* ethyl

acetate extract was 32.99 % at 100 mu gm / ml concentration. It was compared with the standard of BHT.



DPPH radical scavenging assay was performed for basic antioxidant activity of the Abutilon indicum. Results represent that methanolic extract has some excessive antioxidant activity than aqueous extract (4).

CONCLUSION:

The results of this study indicated the presence of various bioactive compounds in the *abutilon indicum* leaf extract. Analysis through GCMS has identified 27 components present in the ethyl acetate extract. The bioautography showed antimicrobial activity against vibrio sp., staphylococcus sp., salmonella sp., E.coli bacteria with RF values of 0.749, 0.785 and 0.846. Phytochemical analysis of the secondary metabolics in the *abutilon indicum* and the purified bioactive compounds and quantitative analysis can be used to improve to create new agents against bacteria's like vibrio sp., staphylococcus sp., salmonella sp., E.coli. Future studies for this experiment helps for the development of bioactive compounds that can improve to create the new drugs against bacteria.

ACKNOLEDGEMENT:

The authors are thankful to the department of Biotechnology, Adhiyamaan College of Engineering, Hosur, Tamil nadu and Life teck research centre, Arumbakkam, Chennai, Tamil nadu for providing the infrastructure facility to carry out the work.

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