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ISOLATION AND CHARACTERIZATION OF ANTIBACTERIAL ACTIVITY CONFERRING COMPOUNDS OF *SOLANUM TRILOBATUM* EXTRACTS AGAINST ISOLATED HUMAN PATHOGENIC BACTERIAL STRAINS

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ABSTRACT

Antibacterial activity of *Solanum trilobatum L* was examined against clinical human bacterial pathogens. The butanol and methanol extract of *Solanum trilobatum L* was endowed with abundance of phytochemical compounds. The zone of inhibition produced by the test organisms indicated their susceptibility to the plant extracts; it was observed that the zone of inhibition varies from one organism to another and from one plant part extract to another. The crude extracts which shows maximum inhibitory effects are subjected to TLC. All the fractions obtained through TLC of *Solanum trilobatum L* butanol, acetone, methanol and petroleum ether extracts were tested against *Streptococcus pyogenes, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus vulgaris, Bacillus subtilis, Salmonella typhimurium, Micrococcus luteus, E.coli.* by well diffusion assay. TLC profiling of butanol, acetone, methanol and petroleum ether extracts gives an impressive result that directing towards the presence of number of phytochemicals. All fractions obtained were capable of inhibiting the growth of bacteria, indicating their activity spectrum to be broad. The HPLC results revealed that, in addition to solvent peaks at 1.80 to 2.020 min, there are some compound response peaks obtained at 2.150, 2.777, 2.160 and 2.157 min with respect to butanol leaf pigment, acetone stem pigment, petroleum ether leaf pigment and butanol flower pigment accordingly.

KEYWORDS

Solanum trilobatum L, Antibacterial, HPLC and Solvents.

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INTRODUCTION

Natural products have been used as a source for antibacterial agents. The drugs developed from plant products are safe and reliable when compared with synthetic drugs which are costly and may cause side effects sometime (Gordon and David, 2001)[1]. Plant based antimicrobials gain importance by its nature of serving without causing any side effects that are often associated with synthetic antimicrobials.

Investigation of plant derived antimicrobials is needed today (Hussain and Gorsi 2004)[2]. Uses of plant derived products as medicines are comparatively safer than synthetic alternatives, providing intense therapeutic benefits and more reasonable treatment. Researcher put efforts on finding out novel antimicrobial compounds from various sources such as microorganisms, animals, and plants. Organized screening of bioactive compounds from medicinal plants may result in the discovery of valuable antimicrobial compounds (Tomoko *et al*, 2002)[3]. The use of natural products as medicines is regularly on the increase in many parts of the world (Bbosa *et al*, 2007)[4].

Solanum trilobatum plants have rich amount of calcium, iron, phosphorus, carbohydrates, protein, fat, crude fibre and minerals (Jawahar *et al*, 2004)[5]. *Solanum trilobatum* is used to treat asthma, vomiting of blood, reducing blood glucose level, rheumatism and several kinds of leprosy. This plant is used as active bioagents against bacteria, Fungus and tumors (Subramanian and Madhavan, 1983[6], Shahjahan *et al,* 2005[7], Shahjahan *et al*, 2004[8], Purushothaman *et al*, 1969[9]). Phytochemical evaluation of plant can be done by qualitative chemical analysis using specific reagents for specific constituents followed by confirmation with different chromatographic techniques, like TLC, HPLC etc. In the present study *Solanum trilobatum* root, stem, fruits, leaves and flower extracts were evaluated for different phytochemical constituents by preliminary qualitative chemical analysis. And to assess the antibacterial properties of leaves, stem, fruit, flower and root extracts of *Solanum trilobatum.*

MATERIAL AND METHODS

Collection of *Solanum trilobatum*

Solanum trilobatum plants were collected from Alanganallur, Madurai district, Tamil Nadu, India. The plants were brought to the laboratory after proper identification, fresh plant materials were washed under running tap water. Leaf, Stem, Flower, Fruit, Root parts were separated from the plants. Each of them was shade dried for 5 days, pulverized in an electric mixer and s was stored in air tight bottles.

Preparation of plant extract

The plant extracts were prepared by adopting the following procedures (Bukar *et al*, 2010[10] and Vinoth *et al*, 2012). Powdered plant parts about 20g were percolated with 60 ml of different solvents such as butanol, acetone, and methanol and petroleum ether. They were left under room temperature for two days with intermittent shaking. Using Whatman's No.1 filter paper the percolates were filtered 3 or 4 times to get the clear extracts. The resulting extracts were concentrated by evaporation at room temperature. The crude extracts were collected and stored in screw capped vials at 4ºC until further use.

Qualitative Phytochemical Analysis

The butanol, acetone, methanol and petroleum ether extracts of *Solanum trilobatum* were subjected to preliminary phytochemical analysis by the following methods (Harborne 1998[11], Siddiqui *et al*, 2009[12] and Uddin *et al*, 2011[13]).

Test for Alkaloids

2mL of crude extract was mixed with 1mL of 1% HCL. To this few drops of Mayer's reagent (5g of Potassium iodide in 50mL of distilled water) were added. Presence of creamish / brown precipitate indicated the presence of Alkaloids.

Sodium hydroxide test for Flavonoids

2mL of crude extract was mixed with few drops of 10% sodium hydroxide solution followed by the addition of dilute HCL. Formation of yellow colour indicated the presence of flavonoids in the sample. The yellow colour thus formed disappears after sometimes on standing.

Ferric chloride test for phenol

2mL of crude extract was mixed with few drops of 10% ferric chloride solution. Appearance of green-blue or violet colour indicated the presence of a phenolic hydroxyl group (Trease and Evans, 2002).

Test for Tannins

To the 2mL of crude extract few drops of 0.1% ferric chloride solution were added. Formation of blue black precipitate confirmed the presence of tannins.

Test for Saponins

5mL of plant extract was mixed with 2.5mL of water. It was shaken vigorously for the formation of

persistent froth. Persistency of froth indicated the presence of saponins.

Salkowski's test for Terpenoids

5mL of crude plant extract was mixed with 2mL of chloroform. To this solution, 3mL of concentrated H_2SO_4 was added carefully along the sides of the test tube. Formation of reddish brown interphase indicated the presence of terpenoids.

Keller-kilani test for Cardiac Glycosides

2mL of crude extract was mixed with 2mL of glacial acetic acid containing 1-2 drops of 2% $feCl_3$ solution. Transfer mixture into another test tube containing 2mL of concentrated H_2SO_4 without causing any disturbance. Formation of brown ring at the junction of the two layers indicates the presence of cardiac glycosides.

Antibacterial assay

The antibacterial screening was done by using eight bacterial strains such as *Streptococcus pyogenes, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus vulgaris, Bacillus subtilis, Salmonella typhimurium, Micrococcus luteus, E.coli.* All the bacterial strains were obtained from Bose laboratory, Madurai District; Tamil Nadu. The agar well diffusion method was followed for estimating the antibacterial activity of *Solanum trilobatum* extracts (Mariyappan *et al*, 2011[14]). The bacterial pathogens were sub cultured into Muller-Hinton Broth. After 16 hours growing bacterial culture was used for the antibacterial assay. Muller-Hinton agar medium and Petri plates were sterilized by autoclaving for 30 minutes. 20mL of the agar medium was dispensed into each petriplate to yield a maximum depth of 4mm under laminar airflow aseptically to prevent contamination. After solidification of the media, the bacterial strains were swabbed on the surface of the agar plates by using sterile buds. In each petriplate, five wells were aseptically punched by a sterile cork borer. Then 0.1mL of the plant extract was poured into each well respectively and without plant extract solvent alone added into the well for control. The plates were incubated overnight at 37°C for allowing bacterial growth. After incubation, the zones of inhibition were measured and tabulated.

Thin layer chromatography analysis

TLC studies were made to select the solvent system capable of showing better resolution. Solvent was prepared by adding butanol, acetic acid and distilled water in the ratio 4: 1: 5. Silica Gel layers of 1mm thick were used for preparative TLC separations. Silica slurry was prepared by dissolving the silica gel powder in distilled water till forms the gel like appearance. The plant extracts were applied on silica slurry coated TLC plates by using capillary tubes and developed in a TLC chamber using suitable mobile phase. Then the TLC plates were air dried and observed. The migration of the active compounds was analyzed expressed by its retention factor (R*f*). Values were calculated for different sample.

$$Rf = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

Then the coloured pigments were scrapped from the slurry plate, to this silica powder the solvent was added and the pigments were eluted out. Finally they were stored in Effendorf tubes. The eluted TLC fractions were subjected to antibacterial activity. The fraction which exhibits the best zone of inhibition was carried to the HPLC analysis.

HPLC analysis

The various fractions eluted from thin layer chromatography were subjected to HPLC analysis. In the present study the HPLC analysis were carried out in the ANJAC College, Sivakasi, Tamil Nadu. HPLC analysis was performed using the Shimadzu, LC- 10 AT VP series. C18 column was used with a flow rate of 0.8ml/ min. methanol was carried as a mobile phase and the chromatograms were developed.

RESULTS AND DISCUSSION

Phytochemical Screening

The photochemical analysis of butanol, acetone, methanol and petroleum ether extracts of Leaf, Stem, Flower, Root of *Solanum trilobatum L.* was analysed for the presence of compounds such as Alkaloids, Flavonoids, Phenols, Tannins, Saponins, Terpenoids and Glycosides, the results were presented in Table No.1 and No.2. Butanol extracts

showed the presence of Alkaloids, Flavonoids, Phenols, Tannins, and Glycosides and be deficient in Terpenoids and saponins (Table No.1 and No.2). Methanolic extracts showed the presence of Flavonoids, saponins, Phenols, Tannins, Terpenoids and Glycosides and be deficient in Alkaloids (Table No.2). The major constituents like Alkaloids, Flavonoids, Phenols, Tannins, Saponins and Glycosides were found in acetone extract of *Solanum trilobatum L* leaves and stem followed by root (Table No.1). Only Saponins and Glycosides were observed in petroleum ether extracts (Table No.2).

Antibacterial activity of crude extracts

The antibacterial activity of butanol, acetone, methanol and petroleum ether extracts was investigated using agar well diffusion method, against the selected human pathogens such as *Streptococcus progenes, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus vulgaris, Bacillus subtilis, Salmonella typhimurium, Micrococcus luteus, E.coli.* All the tested extract showed varying degrees of antibacterial activities against the pathogens. Table No.3 showed the antibacterial activity of Butanol extract of *Solanum trilobatum L. Streptococcus pyogenes* was found to be more susceptible towards the butanolic crude extracts of leaf and stem with a maximum inhibitory zone of 20mm and 16mm respectively. The flower extract exhibits a low inhibitory effect against *Pseudomonas aeruginosa* with a minimum zone of 9mm respectively. (Table No.3, Figure No.1, Plate No.1). Crude acetone extracts of leaf, shows the maximum antibacterial effect on eight bacterial pathogens whereas flower extract seems to be more sensitive to all the bacterial strains. The high inhibitory effect was showed by leaf acetone extracts against *Salmonella typhinurium and E. coli* with a zone of 20mm. Flower, fruit and root crude extract reveals no inhibitory effect against *Streptococcus pyogenes, Pseudomonas aeruginosa, Bacillus subtilis, Salmonella typhimurium, Micrococcus luteus, E.coli.* (Table No.4, Figure No.2, Plate No.2). All plant parts of methanolic crude extract represent a very high zone of inhibition. Particularly flower exhibits the higher inhibitory effect against *Staphylococcus aureus and Micrococcus luteus* and the zone of maximum inhibitory effect is 22 and 21mm. The minimum inhibitory zone is 9mm respectively. (Table No.5, Figure No.3, Plate No.3). Petroleum ether crude extracts shows very poor inhibitory effect against the pathogens. Only flower extract showed the inhibitory effect against the tested pathogen (Table No.6, Figure No.4, Plate No.4). The results obtained, revealed that the methanolic and butanolic crude extracts have shown the wide spectrum of antibacterial activity against the tested pathogens.

TLC (thin- layer chromatography) separation

Results of TLC fractionation (L1, L2, S and F) of butanol, acetone, methanol and petroleum ether extracts (Figure No.5) of *Solanum trilobatum L.* leaf, stem and fruit were recorded in (Table No.7). The Butanol extracts produces four fractions having Rf 0.85, 0.78, 0.83 and 0.82 under Butanol, Acetic acid and distilled water (4:1:5) solvent system. While Acetone extracts produces three fractions having Rf 0.82, 0.76, and 0.81 and methanol extracts yielded three fractions having Rf 0.82, 0.66 and 0.82 and petroleum ether extracts yielded two factions having Rf 0.80 and 0.80 in Butanol, Acetic acid and distilled water (4:1:5) solvent system. TLC profiling of different solvent extracts of butanol, acetone, methanol and petroleum ether gives an impressive result that directing towards the presence of number of phytochemicals. The indication of different Rf values showed that the presence of different phytoconstituents in single extracts. Their diverse Rf values of the compound also reflects an idea about their polarity. This in turn will facilitate the selection of appropriate solvent system for further separation of compound from these plant extracts.

Antibacterial activity of TLC fractions:

The crude extracts which shows maximum inhibitory effects are subjected to TLC. Six fractions such as L1, L2, S and F (Figure No.1) were eluted from the leaf, stem and fruit crude extract of four different solvents. The R_f values of leaf, stem and seed coat crude extracts of butanol, acetone, methanol and petroleum ether were represented in (Table No.5). All the fractions obtained through TLC of *Solanum trilobatum L.* butanol, acetone, methanol and petroleum ether extracts were tested against

Streptococcus pyogenes, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus vulgaris, Bacillus subtilis, Salmonella typhimurium, Micrococcus luteus, E.coli. By well diffusion assay (Table No.1-4, 6-8). All fractions obtained were capable of inhibiting the growth of bacteria (Plate 5, 6, 7 and 8), indicating their activity spectrum to be broad (Figure No.1-6). L2 TLC fractions of butanolic crude extract show maximum inhibitory effect against *Staphylococcus aureus* with a zone of 40mm (Plate No.5) respectively. The low inhibitory effect was shown by L1 pigment against *Streptococcus pyogenes* with the minimum zone of inhibition is 23 mm (Plate No.6). L1, L2 and S fraction of acetone crude extract shows a high antibacterial effect on *Staphylococcus aureus*, *Streptococcus pyogenes* and *E.coli* with a zone of 35mm (Plate No.6) respectively. In contrast to the L1 and pigment establishes very low inhibitory effect on *Proteus vulgaris* with a minimum zone of 21 mm (Table No.6). All the purified compounds of methanolic extract exhibit a very high level of inhibitory effect against the bacterial strains. Among them the L1 and S fractions inhibited the maximum inhibitory effect against *Streptococcus pyogenes* and *Staphylococcus aureus* with a zone of 40mm (Plate No.7). All the purified compounds of petroleum ether extract exhibit a high level (Plate No.8) of inhibitory effect against the bacterial strains (Table No.8).

HPLC Chromatogram

The HPLC results revealed that, in addition to solvent peaks at 1.80 to 2.020 min, there is some compound response peaks obtained at 2.150, 2.777, 2.160 and 2.157 min with respect to butanol leaf pigment, acetone stem pigment, petroleum ether leaf pigment and butanol flower pigment accordingly. Interestingly, peaks were also obtained at 6.573 and 6.547 with respect to acetone and methanol stem pigments accordingly. (Figure No.1-6).

Discussion

Most of the plants are known to have beneficial therapeutic effects as noted in the traditional Indian system of medicine, *Ayurveda*. Many studies revealed that the effects of plant extracts on bacteria were experimented by the researcher across the world, and as much as work has been done on ethno medicinal plants in India. It has been recommended that aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources of antiviral, antimicrobial agents (R. Nair *et al*, 2005)[16]. World Health Report of Infectious diseases 2000, declares that overcoming antibiotic resistance forms a major issue for the next millennium. Phytochemical screening of medicinal plants forms the first step towards identifying new sources of therapeutically and industrially essential compounds. It is very important to initiate screening of plants for secondary metabolites. Many studies declare that the presence of the secondary metabolite may be responsible for their antibacterial properties (Rojas *et al*, 2006[17], Nikitina *et al*, 2007[17], Udobi *et al*, 2008[18], Rafael *et al*, 2009[19], Adeshina *et al*, 2010[20]).

The qualitative screening of phytochemical constituents on leaf extract of *Solanum nigrum L* reveals the presence of alkaloid, saponin, tannins, flavonoids, phenols, terpenoids etc. Alkaloids and their synthetic derivatives used in medicinal field act as driving force for treating analgesic, antispasmodic and bacterial disease (Stray F 1998[21] and Okwu O E and Okwu M E 2004[22]). Tannins are known for its antimicrobial activity. Tannins prevents the growth of some microorganisms by precipitating microbial protein and making nutritional protein unavailable for them (Sodipo *et al*, 2000)[23] and it inhibits growth of many fungi, yeasts, bacteria and viruses (Chung *et al*, 1998)[24]. Phytotherapatically tannin containing plants are used to tract nonspecific diarrhoea, inflammations of mouth and throat and slightly injured skins (H. Westendary 2006). In this study, the presence of tannins might have been reported to hasten the healing of wounds and inflamed mucous membrane (Okuw O. E, 2004)[22].

Flavonoids are potent water soluble antioxidants and free radical scavengers, which prevent oxidant cell damage, have strong anticancer activity (Salah *et al*, 1995[25], Okuw O E, 2004[22]). Flavonoids in intestinal tract lower the risk of heart disease. As antioxidants, flavonoids from these plants provide anti-inflammatory activity (Okuw O E, 2004)[22]. This may be reason *Solanum nigrum* L have been used for the treatment of wounds, burn and ulcers in herbal medicine. Apart from these secondary metabolites,

due to the abundantly presence of protein in leaf of *Solanum nigrum* L can serve many of the medicinal properties exhibited by the plants. Mixture of proteins have been isolated from medicinal plants and found to be bioactive against certain complaints (Tsao *et al*, 1990[26], Mau *et al*, 1997[27]). From the reports revealed by various worker provide evidence that above said phytochemical constituents could account for the much medicinal properties of both the species for the treatment of various disease (Pronob Gogoi and M Islam 2012[28], Ravi *et al*, 2009[29] and Srivasatava R C, 2010[30]). Antimicrobial activities shown by various herbs, latex and spices in plant leaves, flowers, stems, roots or fruits have been reported by many workers in different solvent extracts (Mau *et al*, 2001[27], Uz-Zaman *et al*, 2006, Al-Nair *et al*, 2009). The present states that *Solanum trilobatum L* showed maximum antibacterial activity and so this plant can be subjected to detect the bioactive compounds and in future that may address therapeutic needs. Such screening of various natural organic compounds is required for the discovery of new drugs.

In the present study, butanol, acetone, methanol and petroleum ether extracts of leaf, Stem, Flower, Fruit Stem, Root of. *Solanum trilobatum* L were studied against *Streptococcus pyogenes, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus vulgaris, Bacillus subtilis, Salmonella typhimurium, Micrococcus luteus, E.coli.,* by well diffusion assay. The results obtained, revealed that the methanolic and butanolic crude extracts have shown the wide spectrum of antibacterial activity against the tested pathogens. Findings of biologically active compounds leads to synthesis of more useful drugs). The type of solvents used for the extraction directs the Successive isolation of botanical compounds from plant material. The traditional healers use primarily water as the solvent but in this study the plant extracts by butanol, acetone, and methanol and petroleum ether provided more consistent antibacterial activity. The trails using solvents of various polarities will explore the effect of solvent composition on extract efficacy (Romero *et al*, 2005)[31].

From the results obtained the butanol and methanol extracts of the plants screened gave better yield than acetone and petroleum ether extracts. It is evident that the Gram-positive microorganisms were more sensitive to the plant extracts than the Gram-negative microorganisms. TLC profiling of butanol, acetone, methanol and petroleum ether extracts gives an outstanding result. Difference in Rf values obtained from single extracts indicate the presence of different nature of phytoconstituents, that reflects an idea about their polarity that help in selection of appropriate solvents used for further separation of compound from these plant extracts. Pigments of *Solanum trilobatum L* displayed wide array of antimicrobial activity. Reported chemical constituents of *C. halicacabum.* Natsum *et al*, (2006)[32] also added inevitability of high concentration of methanol for the higher recovery of phytochemicals of *C. halicacabum.* In support of it, the present study revealed a peak which was approximately similar to that of the peak (Luteolin) obtained by Rajasekaran *et al*, (2013)[33]. In order to define clearly the structure of the compound, further studies like FT-IT, NMR and GC-MS and Infrared (IR) were done for identification of present active constituents, responsible for the observed activity are under consideration to perform. The type of solvent and the methods of extraction procedure followed, determines the detections of biologically active compounds from plant material.

Table No.1: Preliminary phytochemical screening of crude extracts of *Solanum trilobatum.L*
'+' = Presence of compound, '-' = Absence of compound, 'L' = Leaves, 'S' = Stem,
'FL' = Flower, 'FR' = Fruit, 'R' = Root

S.No	phytochemicals	butanol					Acetone					Methanol					Petroleum ether				
		l	S	fl	fr	r	l	s	fl	fr	r	l	s	fl	fr	r	l	s	fl	Fr	r
1	alkaloids	+	+	+	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
2	flavonoids	-	-	+	+	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-	-
3	phenols	+	+	+	+	+	+	+	+	-	-	+	-	+	+	-	-	-	-	-	-
4	tannins	+	+	+	+	+	+	-	-	-	-	+	-	+	+	-	-	-	-	-	-
5	saponins	+	+	-	-	-	+	+	-	-	+	+	+	+	+	-	-	+	+	+	+
6	terpenoids	+	-	-	-	-	-	+	-	-	+	+	+	+	+	-	+	-	-	-	-
7	glycosides	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+

Table No.2: Antibacterial effect of *Solanum trilobatum.L* crude extracts

S.No	Test Organisms	Zone of Inhibition in mm									
		Butanol extracts					Acetone extracts				
		Leaf	stem	Flower	fruit	Root	leaf	Stem	flower	fruit	root
1	*S.pyogenes*	15	20	15	16	14	18	10	-	-	-
2	*S. aureus*	12	17	18	15	15	15	10	10	16	-
3	*Pseu. aeruginosa*	16	15	9	11	-	17	-	-	-	-
4	*Proteus vulgaris*	18	13	15	16	14	17	20	-	-	15
5	*Bacillus subtilis*	18	13	19	17	14	18	10	-	-	-
6	*Sal. typhimurium*	13	18	19	18	17	20	-	10	-	-
7	*Microco. luteus*	14	11	11	12	13	18	10	-	-	-
8	*E.coli*	18	13	19		19	20	-	-	-	-

Table No.3: Antibacterial effect of *Solanum trilobatum.L crude* acetone extracts

S.No	Test Organisms	Zone of Inhibition in mm				
		Leaf	Stem	Flower	Fruit	Root
1	*Streptococcus pyogenes*	18	10	-	-	-
2	*Staphylococcus aureus*	15	10	10	16	-
3	*Pseudomonas aeruginosa*	17	-	-	-	-
4	*Proteus vulgaris*	17	20	-	-	15
5	*Bacillus subtilis*	18	10	-	-	-
6	*Salmonella typhimurium*	20	-	10	-	-
7	*Micrococcus luteus*	18	10	-	-	-
8	*E.coli*	20	-	-	-	-

Table No.4: Antibacterial effect of *Solanum trilobatum.L* crude extracts

S.No	Test Organisms	Zone of Inhibition in mm									
		Methanolic extracts					Petroleum ether extracts				
		leaf	stem	flower	Fruit	root	leaf	stem	flower	fruit	root
1	*Strep. pyogenes*	10	-	11	12	-	-	-	13	16	-
2	*Staph. Aureus*	17	10	22	12	10	-	-	15	-	-
3	*Pse. Aeruginosa*	18	9	13	12	-	-	-	-	-	-
4	*Proteus vulgaris*	10	12	11	13	10	-	-	20	-	-
5	*Bacillus subtilis*	13	-	10	14	-	-	-	18	-	-
6	*S. typhimurium*	20	11	18	17	20	-	-	15	-	-
7	*Mic. Luteus*	18	13	21	17	12	-	-	13	-	-
8	*E.coli*	15	12	21	20	-	-	-	24	-	-

Table No.5: R_f values of thin layer chromatography

S.No	Sample(extract)	Rf value of the sample
1	Butanol leaf 1	0.85
2	Butanol leaf2	0.78
3	Butanol stem	0.83
4	Butanol flower	0.82
5	Acetone leaf1	0.82
6	Acetone leaf2	0.76
7	Acetone stem	0.81
8	Methanol leaf1	0.82
9	Methanol leaf2	0.66
10	Methanol stem	0.82
11	Petroleum ether leaf	0.80
12	Petroleum ether stem	0.80

Table No.6: Antibacterial effect of TLC fractions from crude extracts

S.No	Test Organisms	Zone of Inhibition in mm						
		Butanolic crude extract				Acetone crude extract		
		L1	L2	S	F	L1	L2	S
1	*Streptococcus pyogenes*	30	30	30	22	35	35	32
2	*Staphylococcus aureus*	35	40	35	35	34	39	32
3	*Pseudomonas aeruginosa*	35	30	35	30	30	30	25
4	*Proteus vulgaris*	32	35	35	36	21	25	19
5	*Bacillus subtilis*	30	30	27	30	30	31	30
6	*Salmonella typhimurium*	23	25	27	27	30	27	22
7	*Micrococcus luteus*	25	30	25	20	35	30	24
8	*E.coli*	30	30	30	31	35	35	35

Table No.7: Antibacterial effect of TLC fractions from crude extracts

S.No	Test Organisms	Zone of Inhibition mm				
		Methanolic crude extract			Petroleum ether	
		L1	L2	S	L	S
1	*Streptococcus pyogenes*	40	33	33	27	20
2	*Staphylococcus aureus*	35	37	40	30	31
3	*Pseudomonas aeruginosa*	30	35	20	35	25
4	*Proteus Vulgaris*	27	26	27	30	20
5	*Bacillus subtilis*	25	27	25	25	27
6	*Salmonella typhimurium*	30	30	25	25	22
7	*Micrococcus luteus*	30	30	30	30	30
8	*E.coli*	30	33	30	30	22

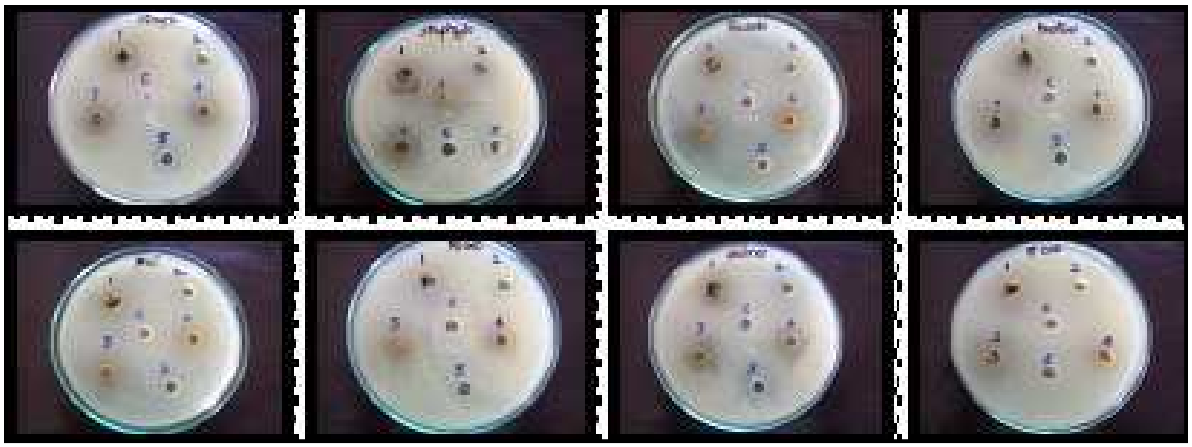


Plate No.1: Antibacterial effect of crude butanol plant parts extracts against human pathogens

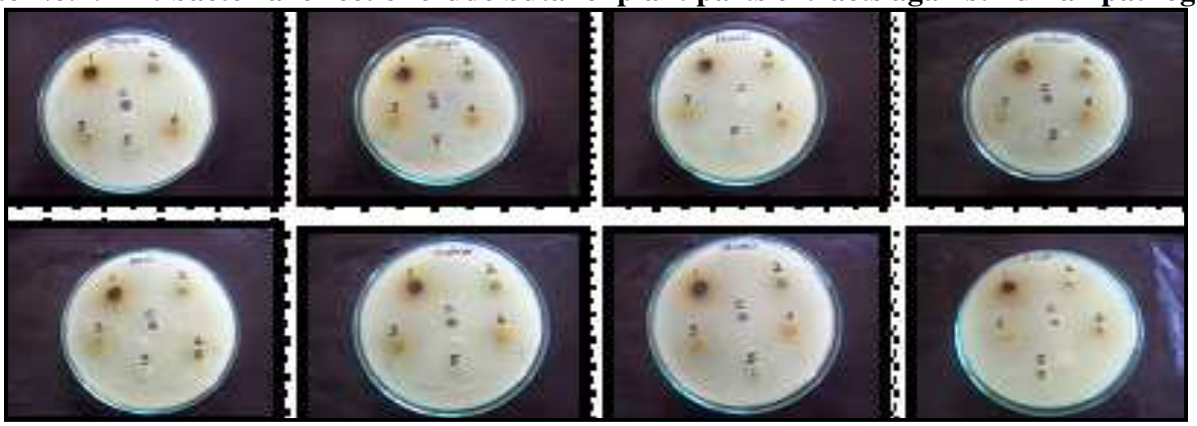


Plate No.2: Antibacterial effect of crude acetone plant parts extracts against human pathogens

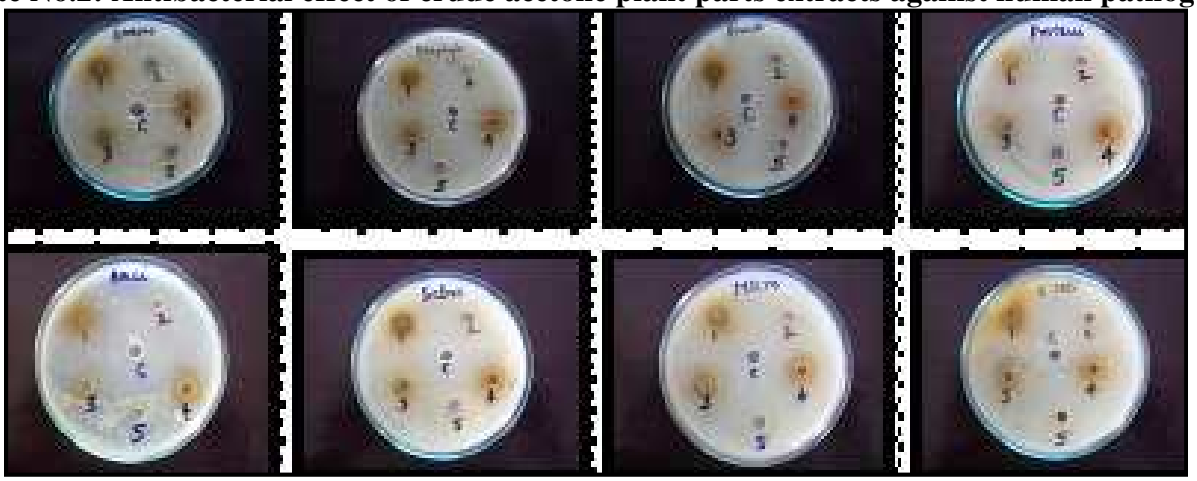


Plate No.3: Antibacterial effect of crude methanol plant parts extracts against human pathogens

Plate No.4: Antibacterial effect of crude petroleum ether plant parts extracts against human pathogens

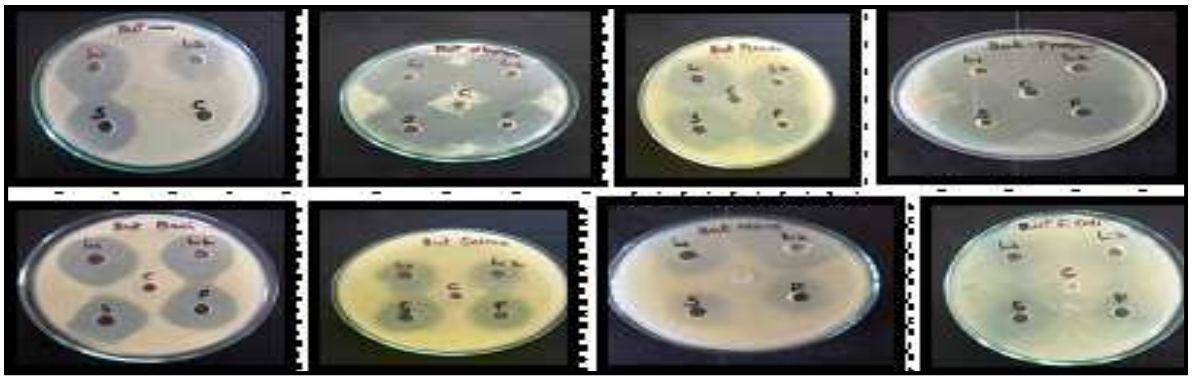


Plate No.5: Antibacterial effect of tlc fractions from butanolic crude extract

Plate No.6: Antibacterial effect of tlc fractions from acetone crude extract

Plate No.7: Antibacterial effect of tlc fractions from methanolic crude extract

Plate No.8: Antibacterial effect of tlc fractions from petroleum ether crude extract

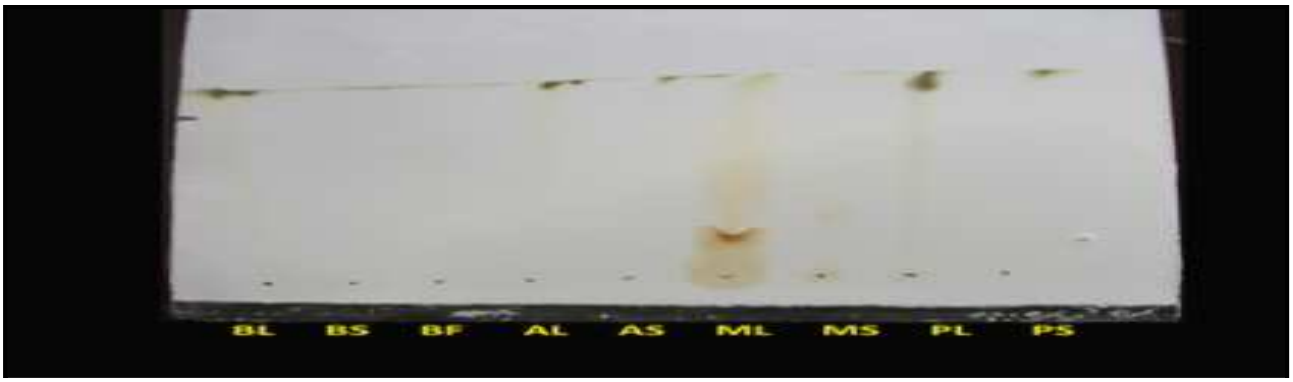


Figure No.1: Thin Layer Chromatogram

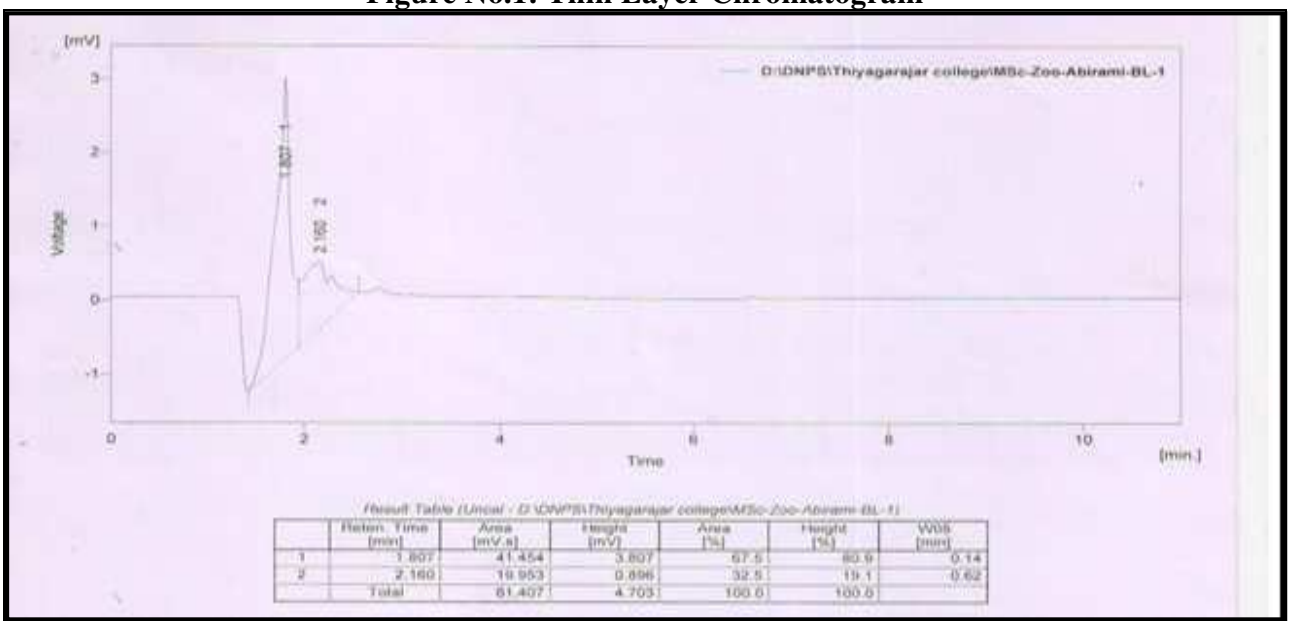


Figure No.2: HPLC Chromatograms for Butanol leaf Fractions

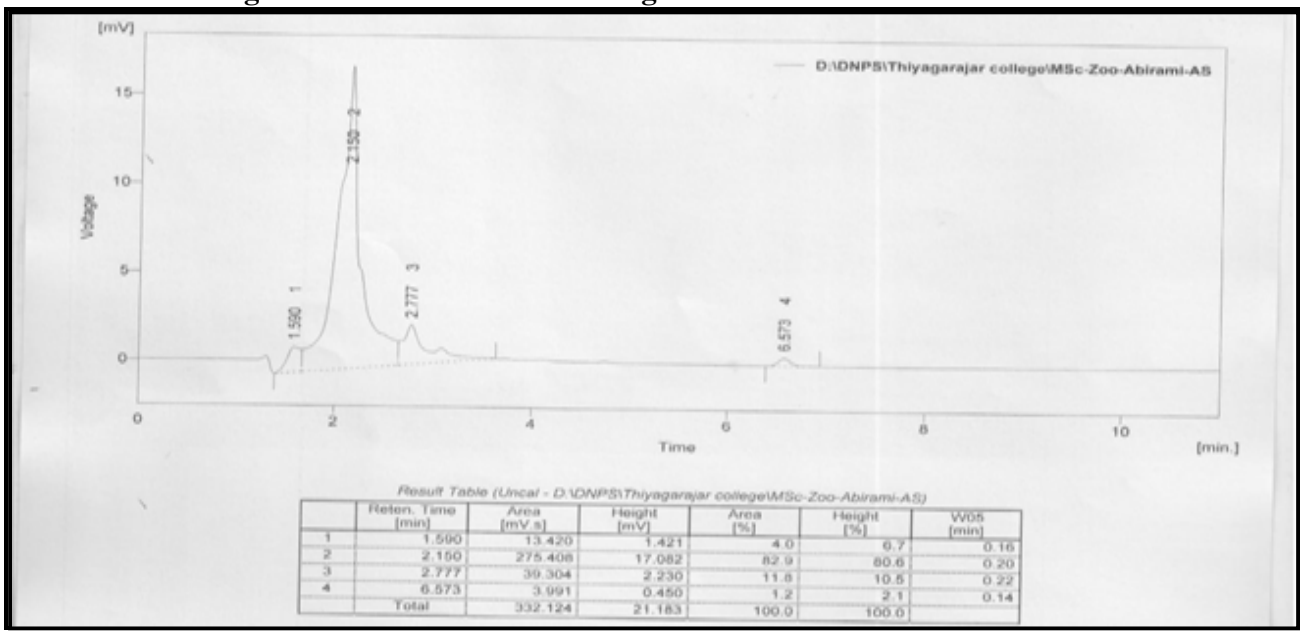
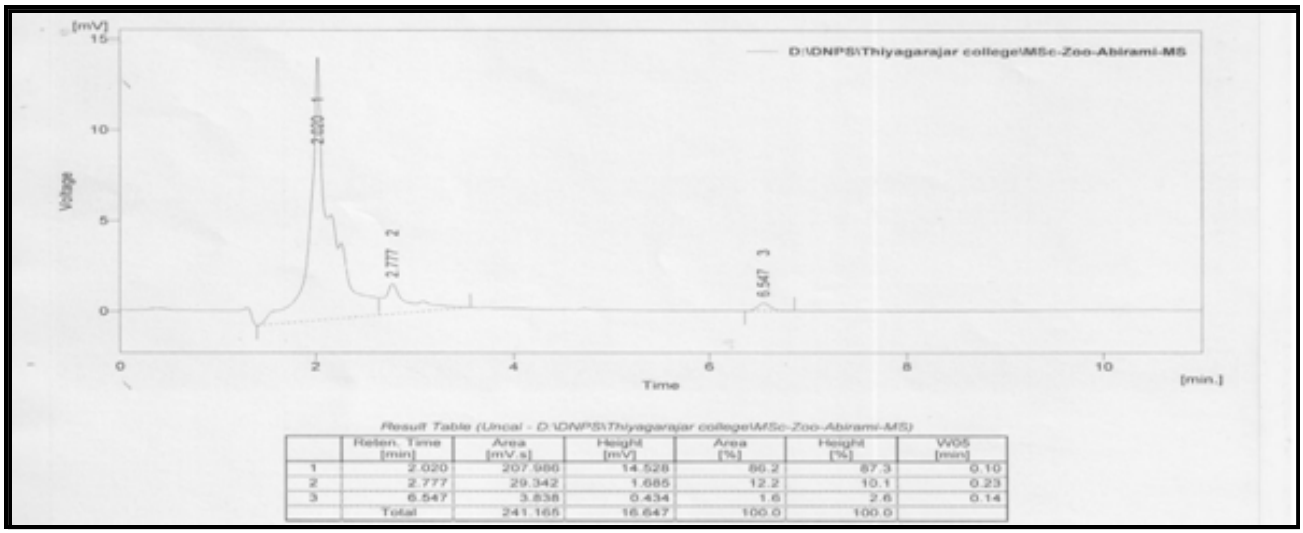


Figure No.3: AS= Acetone Stem pigment HPLC Chromatograms for AS Fractions

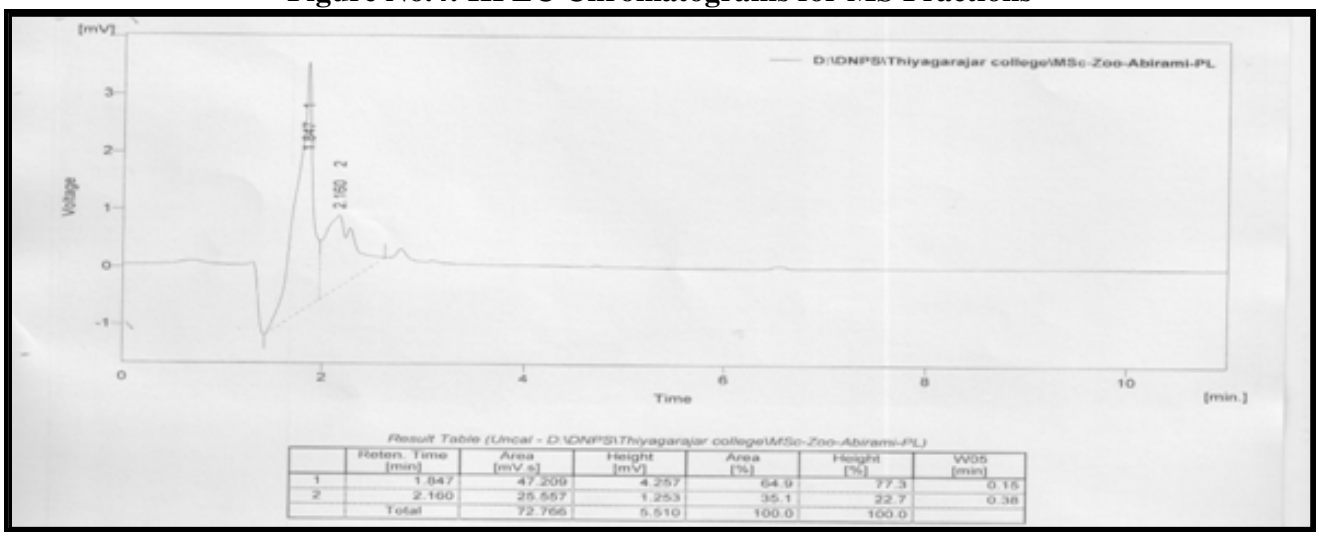


Result Table (Uncal - D:\DNPS\Thiyagarajar college\MSc-Zoo-Abirami-MS)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.020	207.986	14.528	86.2	87.3	0.10
2	2.777	29.342	1.685	12.2	10.1	0.23
3	6.547	3.838	0.434	1.6	2.6	0.14
	Total	241.166	16.647	100.0	100.0	

MS = Methanol stem pigment

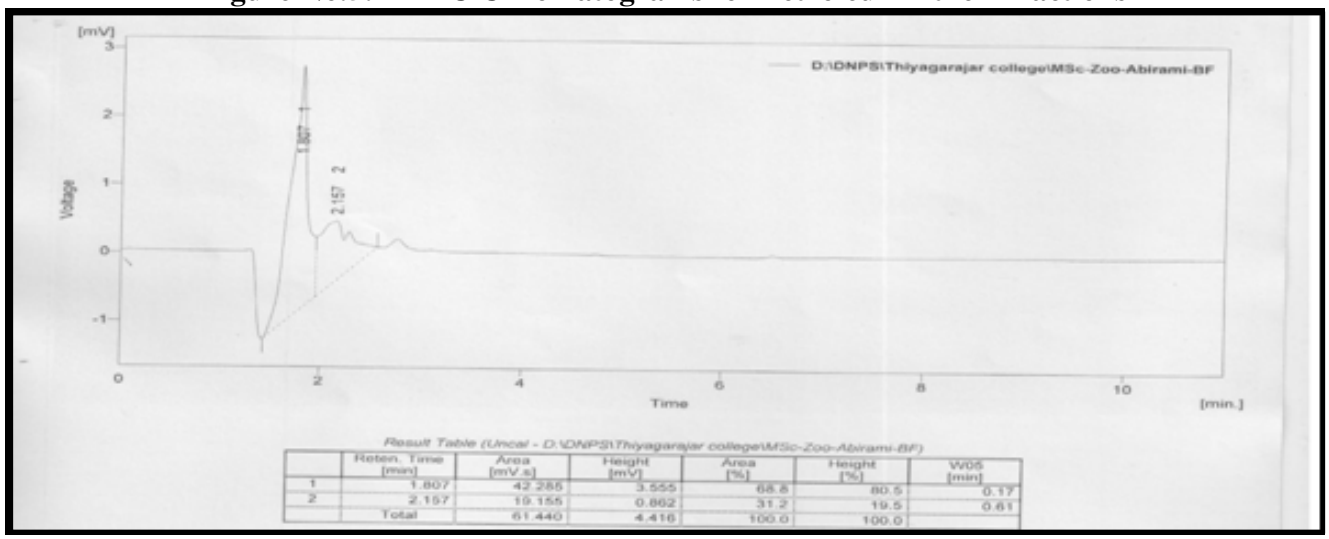
Figure No.4: HPLC Chromatograms for MS Fractions



Result Table (Uncal - D:\DNPS\Thiyagarajar college\MSc-Zoo-Abirami-PL)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	1.847	47.209	4.257	64.9	77.3	0.15
2	2.160	25.557	1.253	35.1	22.7	0.38
	Total	72.766	5.510	100.0	100.0	

Figure No.5: HPLC Chromatograms for Petroleum Ether Fractions



Result Table (Uncal - D:\DNPS\Thiyagarajar college\MSc-Zoo-Abirami-BF)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	1.807	42.285	3.555	68.8	80.5	0.17
2	2.157	19.155	0.862	31.2	19.5	0.61
	Total	61.440	4.416	100.0	100.0	

Figure No.6: HPLC Chromatograms for Butanol Flower Fractions

CONCLUSION

New approaches towards the valuation of bioactive compounds from the plant sources have need of the large scale screening process. The present study forms a base towards the isolation of some compounds, which describes the presence of active compounds and acts as effective antimicrobials. In future it would be subjected to FT-IR, NMR and GC-MS to reveal the chemical nature of the bioactive compound.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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