Analysis of Phytochemical compounds from Ethyl acetate and Methanol extract of *Trachyspermum ammi* (Ajwain) with reference to Antibacterial activity and Subacute toxicity effect.

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Abstract

Aim:

The present study deals with the analysis of phytochemical compounds, antimicrobial activity and cytotoxicity screening from the *seeds* extract of *Trachyspermum ammi* (Ajwain).

In this study, two types of extract were prepared, Methanol and ethyl acetate extract. The phytochemical compounds were analysed. The antibacterial assay for the identified bacteria was done. It was observed that the ethyl acetate extract was found to be more effective than the methanol extract. Three strains of bacteria *Bacillus spp., Enterobacter spp*. and *Streptococcus spp.* were isolated from the soil. Among these three bacteria, *Enterobacter spp.* shows a weak sensitive against the Ajwain compared to the other bacteria, showing a large zone of inhibition. The cytotoxic effect after treating with Ajwain shows normal liver functioning enzymes and live functioning in earthworm.

Keywords: Phytochemical compounds, Antibacterial assay, cytotoxic screening, bacteria

Introduction:

The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents. Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defence mechanism and protect from various diseases. This study is aimed in analysing the phytochemical compounds used in anti-bacterial activities and in analysing the cytotoxic screening of earthworm using Ajwain. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoid alkaloids and phenolic compounds. Terpenoids exhibit various important pharmacological activities i.e., anti-inflammatory, anticancer, anti-malarial, inhibition of cholesterol synthesis, anti-viral and anti-bacterial activities. Terpenoids are very important in attracting useful mites and consume the herbivorous insects .Alkaloids are used as anaesthetic agents and are found in medicinal plants.(Wadood *et al.,2013*).

Trachyspermum ammi commonly known as Ajwain belonging to Apiaceae is a plant growing in India, Pakistan, South East and Near East of Iran. It is distributed throughout in India, and it is mostly cultivated in Gujarat and Rajasthan, where the seeds are used as a spice.

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs. A wide range of medicinal plant parts is used for extract as raw drugs and they posses varied medicinal properties (Srivastav *et. al.,* 1996). Medicinal plants are believed to be important source of new chemical substances with potential therapeutic effects (Katrin Basha *et. al.,* 2011). The secondary metabolites of plants were found to be source of various phytochemicals that could be directly used as intermediates for the production of new drugs. Traditional medicine should be able to play an even greater role in the modern primary healthcare system of the developing countries. The natural medicines are believed to be more acceptable to the human body, when compare to modern synthetic drugs. Thus the most important factor needed is to derive the maximum benefit from the traditional system of medicine for providing adequate healthcare service to rural people (Ghani, 1990).

MATERIALS AND METHODS

1.1 COLLECTION OF SAMPLE:

The medicinal plants *Trachyspermum ammi* (Ajwain) was collected from the local market of Tiruchirappalli district, Tamil Nadu, India. It was then used for the extraction of its bioactive compounds.

1.2 PREPARATION OF PLANT EXTRACT:

The dried seeds of Ajwain were crushed into fine powder with the help of a mechanical grinder .10g of the seeds powder was placed in a glass container and soaked in a 80% of methanol. The container with its content was sealed and kept for 7 days. The entire mixture then underwent a coarse filtration by a piece of clean, white cotton material. The extract then was filtered through Whatman filter paper. The extract was dried at room temperature and both the aqueous solution and solidified extract was stored under refrigeration for further studies.

The same method was used for the preparation of Ethyl acetate extract.

1.3 ANALYSIS OF PHYTOCHEMICAL COMPOUNDS: (Wadood *et al*;2013)

Screenings of medicinal plants for various phytochemical constituents were carried out using the following tests:

1.3.1 TEST FOR PHLOBATANNINS:

Plant powder sample was mixed with distilled water in a test tube, then was shaked well, and filtered to take plant extract. Then, 1% aqueous hydrochloric acid was added and it was boiled with the help of Hot plate stirrer. Formation of red colour precipitate confirmed a positive result.

1.3.2 TESTS FOR REDUCING SUGAR:

An amount of 0.50 g of plant sample was added in 5 ml of distilled water. Then 1 ml of ethanol mixed in plant extract. After that, 1 ml of Fehling solution A and 1 ml of Fehling solution B was added in a test tube, heated it to boiling and then poured it in the aqueous ethanol extract. When color reaction was observed, it shows a positive result.

1.3.3 TEST FOR TERPENOIDS:

An amount of 0.8 g of plant sample was taken in a test tube, 10 ml of methanol was added, it was shaked well and filtered to take 5 ml extract of plant sample. Then 2 ml of chloroform were mixed in extract of plant sample and 3 ml of sulphuric acid were added in sample extract. Formation of reddish brown color indicates the presence of terpenoids in the selected plants.

1.3.4 TEST FOR FLAVANOIDS:

0.5 g of plant extract was added in a test tube and 10 ml of distilled water, 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of plant extract followed by addition of 1 ml concentrated H2S04. Indication of yellow color shows the presence of flavonoid in each extract.

1.3.5 TEST FOR ALKALOIDS:

0.2 g of the plant samples was added in the test tube and 3 ml of hexane were mixed in it, it was shaken well and filtered. Then took 5 ml of 2% HCl and poured in a test tube having the mixture of plant extract and hexane. The test tube was heated, filtered it and poured few drops of picric acid in a mixture. Formation of yellow color precipitate indicates the presence of alkaloids.

1.3.6 TEST FOR TANNINS: (Watal *et al,*2014)

2ml of extract sample was taken in a test tube, then 2ml of H_2O was added in the test tube and 2-3 drops of $FeCl_3$(5%) was added in the mixture. Green precipitate shows a positive result.

1.3.7 TEST FOR SAPONINS (Foam test):

5ml extract was taken and it was mixed with 5ml of water and it was heated. Froth formation shows a positive result.

1.3.8 TEST FOR STEROIDS (Salkowski test):

2ml of plant extract was taken in a test tube and 2ml of $CHCL_3$ was added and then 2ml of H2SO4(conc.) was added to the test tube .Reddish brown ring at the junction indicates positive result.

1.3.9 TEST FOR GLYCOSIDES:

2ml of extract was taken and 2ml of $CHCl_3$ was added and 2ml of CH_3COOH was added to the test tube.Violet to blue color formation indicates a positive result.

1.3.10 TEST FOR COUMARINS:

6.10 2ml of extract was taken and 3ml of NaOH(10%) was added .Yellow coloration indicates a positive result.

1.3.11 TEST FOR PROTEINS(Xanthoproteic test):

1ml of extract was taken in a test tube and mixed with a 1ml of H_2SO_4 (conc.).White precipitate will shows a positive result.

1.3.12 TEST FOR LEUCOANTHOCYANINS TEST:

5ml of extract was taken in a test tube, and 5ml of Isoamyl alcohol was added. Organic layer into red will shows positive layer.

1.4 ISOLATION OF BACTERIA FROM SOIL: (Cappuchino & Sherman, 2014)

The soil sample was collected from the campus of Bishop Heber College, Tiruchirappalli District, Tamil Nadu, India .

1.4.1 SERIAL DILUTION AND POUR PLATE METHOD:

The test tubes, conical flask and the nutrient agar media were autoclaved at 15lb/inch^2 for 30 minutes.9ml of distilled water was added into a test tube.10gm of soil was added into the test tube. The flasks were shaken to get a homogeneous suspension. Another 4 test tubes were taken and were labelled from, $S10^{-2}$, $S10^{-3}$, $S10^{-4}$, and $S10^{-5}$. 9ml of distilled water was added to each test tube. 1ml of the sample was taken from the 1:10 dilution and added to the test tube labelled $S10^{-2}$ containing 9ml of distilled water and mixed thoroughly to get a dilution of 1:100(10^{-2}) .Again, 1ml of the sample was taken from $S10^{-2}$ and added to the second test tube labelled $S10^{-3}$ containing 9ml of distilled water. All the steps were repeated till the last test tubes. The plating was performed from appropriate dilution by pour plate method.1ml of the diluted sample were poured on to the petriplates and 15-20ml of media was poured and rotates clockwise and anticlockwise direction or the eventual distribution of the sample. Incubate all the plates at appropriate temperature. Observe plates for growth.

1.4.2 SUBCULTURING OF COLONIES:

The serially diluted colonies further characterized by inoculating the bacterial colonies in a nutrient agar medium. The colonies were subcultured by pour plate method. The further dilutions were selected by serial dilution sample 10^{-2} , 10^{-3} ,10^{-4} to perform plating from the approximately dilution by pour plate method.

1.4.3 STREAK PLATE METHOD:

The petriplates, media etc. were sterilized in an autoclave. Prepared nutrient agar is poured into the petriplates. Allow the plates to solidify. Sterilize the inoculation loop by using flaming technique. Transfer microbial mixture from a tube to the edge of an agar plate with an inoculation loop for quadrant streak and T streak. And incubate it 37°C for 24hours.

1.4.4 BROTH CULTURE METHOD:

Nutrient broth is prepared in a required quantity. The test tubes, broth were sterilized in autoclave. The nutrient broth is poured into the test tubes. And the colonies were isolated from plates and inoculated into test tubes by using inoculation loop. Inoculated test tubes were incubated for 24 hours at 37°C. After 24 hours the growth of microbes is seen in surface of the broth.

1.4.5 CHARACTERISATION OF COLONIES:

When microbes were grown in different media it shows differences in the macroscopic appearance of their growth. These difference called cultural characteristic and are used as the basis for separating microorganism into taxonomic groups. These are determined by culturing the organism on nutrient agar.

Shape : Circular, Rhizoid, Irregular, Filamentous, Spindle.

Margin : Entire, Undulate, lobate , curled, Rhizoid, Filamentous.

Elevation : flat, raised, convex, pulvinate, Umbonate.

Size : punctiorm, small, moderate, large.

Texture : smooth or rough

Appearance : glistening (shiny) or dull.

Pigmentation : Non pigmented (eg.,cream, tan, white) pigmented (e.g., purple, red).

1.6 ANTIBACTERIAL ASSAY:

1.6.1 PLATE METHOD:

The three different strains of bacteria (Streptococcus, Enterobacter, and Bacillus) were subjected on agar media in a separates petriplates. Each species of bacteria had three petriplates which were treated with different concentrations of Ajwain aqueous extract 5ml, 10ml and 15ml respectively. The antibacterial assay was performed using the two extract of Methanol and Ethyl acetate separately. It was kept in the incubator for 48 hours and the Minimum Inhibition Concentration (MIC) was measured. The zone of inhibition was compared with the control using Gentamycin.

1.7 HPLC TEST:

High performance liquid chromatography (HPLC) is a versatile, robust, and widely used technique for the isolation of natural products (Cannell, 1998). Natural products are frequently isolated following the evaluation of a relatively crude extract in a biological assay in order to fully characterize the active entity.

The biologically active entity is often present only as minor component in the extract and the resolving power of HPLC is ideally suited to the rapid processing of such multicomponent samples on both an analytical and preparative scale. Many bench top HPLC instruments now are modular in design and comprise a solvent delivery pump, a sample introduction device such as an auto-sampler or manual injection valve, an analytical column, a guard column, detector and a recorder or a printer.

1.8 CYTOTOXIC SCREENING: (Ukpabi C. F *et al*., 2013)

The Earthworm samples were collected from the campus of Bishop Heber College. Each earthworm was rinsed with distilled and deionized water; a buffer solution was added and was cut and crushed using a laboratory mortar. The resulting crushed tissues were centrifuged 4000r/min for 5min and the supernatant was removed and stored in a refrigerator .Serum were tested for Bilirubin (Total), Bilirubin (Direct), T.Protein, Albumin, AST/SGOT, ALT/SGPT, ALP and GGT.

The same species of earthworm was collected and kept in a container, which was treated with the powder of *Trachyspermum ammi* (Ajwain). After 2 days, no mortality was observed. And the treated earthworm were undergo the same procedure for the following test; Bilirubin (Total), Bilirubin (Direct), T. Protein, Albumin, AST/SGOT, ALT/SGPT, ALP and GGT.

Result

Fig-2.1.1 Presence of Tannins compounds showing green coloration in *Trachyspermum ammi* extract.

Biochemical Analysis

TABLE 3.1. BIOCHEMICAL ANALYSIS:

SL.NO.	BIOCHEMICAL TESTS	OBSERVATION	RESULTS	ORGANISM
1	Indole Test (Tryptone broth)	Red layer at the top	Negative(-)	-
2	Methyl Red Test (MRVP broth)	Red layer at the top	Negative (-)	-
3	Macconkey Agar	Bright pink red colonies	Positive(+)	*Enterobacter spp.*
4	Vogues proskauer Test	Pink/Red	Negative(-)	-
5	Mannitol Fermentation	Red to yellow	Negative(-)	-
6	Catalase Test	Gas bubbles	Positive(+)	*Bacillus spp.*
7	Citrate Agar Test	Yellow to cerise	Negative(-)	-

8	Starch	A clear zone	Negative(-)	-
9	Blood Agar		Positive(+)	*Streptococcus spp.*
10	Eosin Methylene Blue	Pink colonies	Positive(+)	*Enterobacterter spp.*

3.2. PLATE METHOD OF ZONE OF INHIBITION (Ethyl acetate and Methanol extract)

CONTROL: Gentamycin against Streptococcus *spp.*, *Enterobacter spp.* and *Bacillus spp.*

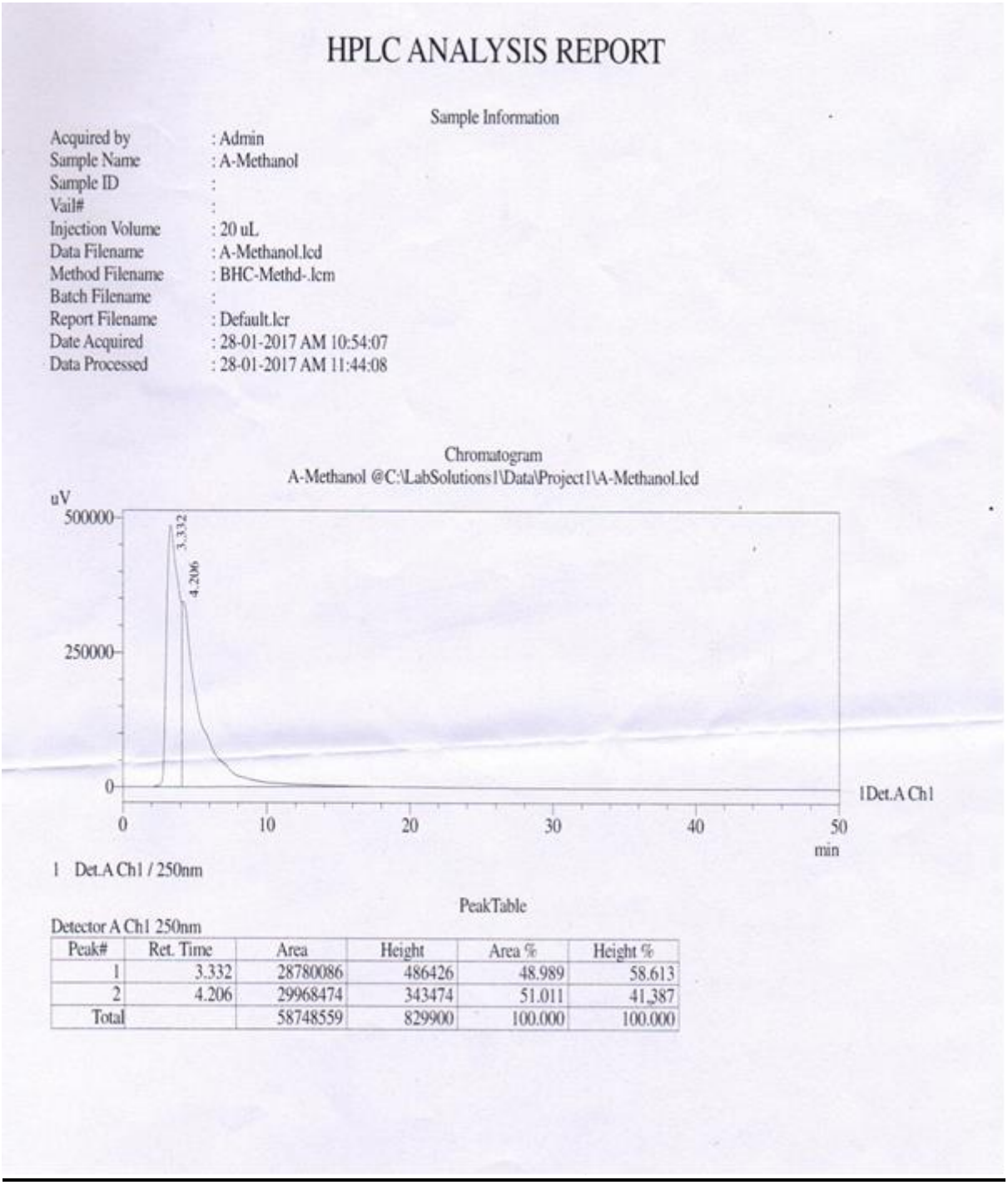
SL.NO.	ORGANISMS	Radius of Zone of Inhibition
1	*Streptococcus spp.*	20mm
2	*Enterobacter spp.*	15mm
3	*Bacillus spp.*	15mm

Table 3.3.CYTOTOXI

C TEST ON EARTHWORM:

SL.NO.	TEST	RESULT (Before treated)	RESULT(Treated with Ajwain extract)	NORMAL RANGE	UNITS
1.	Bilirubin (Total)	<0.2	<0.10	0.3-1.9	mg/dl
2.	Bilirubin (Direct)	<0.1	0.18	0-0.3	mg/dl
3.	T.Protein	<1.0	<0.50	6-8.3	gm/dl
4.	Albumin	<0.5	<0.20	3.5-5.3	gm/dl
5.	AST/SGOT	430	23	8-48	U/l
6.	ALT/SGPT	20	6	7-55	U/l
7.	ALP	454	351	45-115	U/l
8.	GGT	18	17	9-48	U/l

Fig 4 HPLC Assay of Methanol extract



HPLC ANALYSIS REPORT

Sample Information

Acquired by	: Admin
Sample Name	: A-Methanol
Sample ID	:
Vail#	:
Injection Volume	: 20 uL
Data Filename	: A-Methanol.lcd
Method Filename	: BHC-Methd-.lcm
Batch Filename	:
Report Filename	: Default.lcr
Date Acquired	: 28-01-2017 AM 10:54:07
Data Processed	: 28-01-2017 AM 11:44:08

Chromatogram

A-Methanol @C:\LabSolutions1\Data\Project1\A-Methanol.lcd

1 Det.A Ch1 / 250nm

PeakTable

Detector A Ch1 250nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.332	28780086	486426	48.989	58.613
2	4.206	29968474	343474	51.011	41.387
Total		58748559	829900	100.000	100.000

Discussion

Medicinally, *Trachyspermum ammi* (Ajwain) has been proven to possess various pharmacological activities like antimicrobial, antifungal, cytotoxic activity, hypolipidaemic, antioxidant, anti-nociceptive, antihypertensive, antispasmodic, antilithiasis, diuretic , abortifacient, nematicidal, anthelmintic and antifilarial activity.(Jeet *et al.,2012).*

The results of this study reveals that the seeds extract of *Trachyspermum ammi* (Ajwain) contains a phytochemical compounds steroids, tannins and coumarins in the extract of both Methanol and Ethyl acetate

extract(,Fig. 2.1.1-2.1.3). In addition, it also shows an effective result on the antibacterial activity and cytotoxic effect. Among the two extract it was observed that, Ethyl acetate extract of *Trachyspermum ammi* was more effective than Methanol extract against the bacteria *Streptococcus, Enterobacter and Bacillus*. (Fig 2.2).

Ajwain contains a large number of bioactive compounds, whereas in this study we observed three compounds i.e. steroids, tannins, and Coumarins. Steroids are responsible for cholesterol-reducing properties. Steroids also help in regulating the immune response (Shah *et al*.,2009). Tannins have shown potential antiviral (Lin *et al*., 2004), antibacterial (Akiyama *et al*., 2001; Funatogawa *et al*., 2004), and antiparasitic effects (Bhagavathi *et al*.,1999; Yang *et al*, 2000; Tanimura *et al*, 2005).Tannins have amazing stringent properties. They are known to hasten the healing of wounds and inflamed mucous membrane. It also helps in maintaining diabetes induced oxidative stress. (Rabi and Bishayee,2009, Wagner and Elmadfa,2003). Coumarins are very significant in the treatment of cancer and are used in the treatment of prostate cancer, renal cell carcinoma and leukemia (Rohini K and Srikumar PS, 2014). Plants containing Coumarins are known to exert a beneficial action on immune system by increasing body strength and hence are valuable as dietary supplements. It can also be suggested to be beneficial for hyper proliferative skin diseases on the basis of their antimicrobial and anti-inflammatory effects (Theis and Lerdau, 2003).

Seeds contain an essential oil containing about 50% thymol which is a strong germicide, anti-spasmodic and fungicide. Ajwain seed analysis has revealed it to contain fiber (11.9%), carbohydrates (38.6%), tannins, glycosides, moisture (8.9%), protein (15.4%), fat (18.1%), saponins, flavone and mineral matter (7.1%) containing calcium, phosphorous, iron and nicotinic acid (Bairwa *et al*;2012).The active principles thought to be responsible for the antimicrobial activity of ajwain were reported to be carvacol and thymol. Thymol kill the bacteria resistant to even prevalent third generation antibiotics and multi-drug resistant microbial pathogens and thus work as a plant based 4^{th} generation herbal antibiotic formulations.(Kamal Jeet *et al*.,2012). There are nearly 16 compounds in Ajwain, but Thymol, the most important and main phenolic compound in Ajwain has been reported to be a germicide (anti-bacterial and anti-fungal) and an antispasmodic agent. Also, ethanol and acetone extracts are found to be effective against many bacteria, including Pseudomonas species, *E. coli*, *Bacillus subtilis* and *S.aureus*. (Sharifi-Mood *et al.*, 2014).

In this present study, the bacteria were isolated from the soil which was collected from the Bishop Heber College campus, Trichy, Tamil Nadu, India. Using the Serial Dilution Method, the soil sample was used for isolation of the bacteria. Biochemical analysis was done for all the dilution containing the bacteria. It was observed that the bacteria present in the soil sample were *Streptococcus spp.*, *Enterobacter ssp.* and *Bacillus spp*.(Table 3). Gram staining was performed for the three strains of bacteria. Among the three bacteria, two shows Gram-positive (+) i.e. *Streptococcus spp.* and *Bacillus spp*. whereas *Enterobacter spp*.shows gram-negative (-).

This present study reveals that the seeds extract of *Trachyspermum ammi* (Ajwain) ethyl acetate and methanol extract has antimicrobial properties. In this study, the ethyl acetate extract was more effective than the methanol extract against the bacteria *Streptococcus sp.*, *Enterobacter sp.* and *Bacillus sp*. Sensitivities of Ajwain was studied using the agar method and broth method. In the agar method, the inhibition zone of Ajwain ethyl acetate extract against Enterobacter was higher (39%) , followed by Streptococcus(36%) and then Bacillus(25%) was the least inhibition.(Table 3.2).The inhibition zone of the methanol extract of Ajwain against *Enterobacter* was higher (30%), followed by Streptococcus (23%) and then Bacillus(21%).(Table 3.2). The MIC test on each bacteria were tested with three different concentrations (5ml,10ml,15ml) of Ajwain extract both ethyl acetate and methanol extract. In this study, we observed that the extract of 10ml and 15 ml concentration of both ethyl acetate and methanol extract of Ajwain shows a great antibacterial activity.

Cytotoxic screening was done on earthworm using the extract of Ajwain (*Trachyspermum ammi*). The collected samples of earthworm were cut and crushed using a laboratory mortar. The resulting crushed tissues were centrifuged 4000r/min for 5min and the supernatant was removed and stored in a refrigerator (Ukpabi C. F *et al*., 2013). And the serum sample was used for Protein liver Function test. In this screening, liver function test; Bilirubin (Total), Bilirubin (Direct), T. Protein, Albumin, AST/SGOT, ALT/SGPT, ALP and GGT were done on the serum sample of earthworm. The test was performed before treated with Ajwain extract, the test showed a result (<0.2, <0.1,<1.0,<0.5, 430,20,454,18) and after treated with the Ajwain extract, the result of this liver function test was decreased (<0.10 ,0.18,<0.50, <0.20 ,23,6,351,17) compared with the result of before treated with Ajwain.(Table 4). This shows that there is a decrease in the liver enzymes by treating it with the Ajwain powder. Increase in these liver enzymes causes liver damage and liver cirrhosis. By treating with *Trachyspermum ammi (*Ajwain) powder, the liver enzymes were decreased which shows normal liver function and effective result(Table 3.3).

HPLC test were also done for both the methanol and ethyl acetate seed extract of *Trachyspermum ammi (*Ajwain) in which the presence of compounds were confirmed.(Graph Fig.4).

Conclusion:

The methanol and ethanol plant extracts of Ajwain shows anti-bacterial activity against the wide range of bacteria isolated from soil. The plant extracts of Ajwain contains many phyto chemical compounds which shows enhanced removal of toxins in the cellular level and shows normal functioning of liver and other organs.

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List of Figures & tables

Fig-2.1.1 Presence of Tannins compounds showing green coloration in *Trachyspermum ammi* extract.

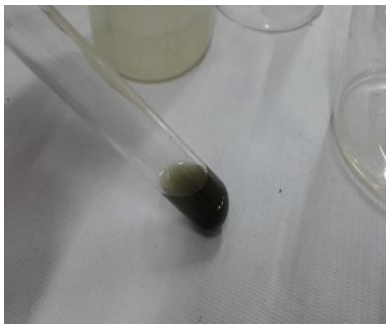


Fig 2.1.1

Fig 2.1.2

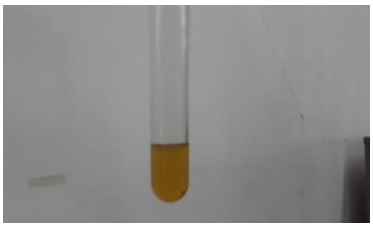
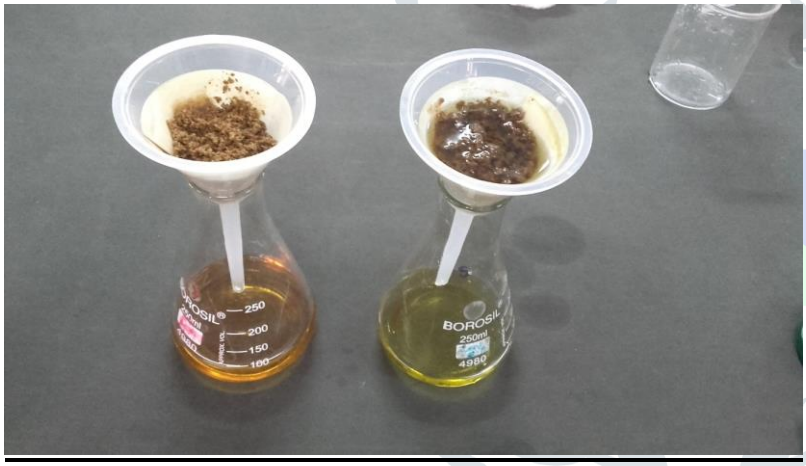


Fig 2.1.3

Fig-2.1.2 Presence of Steroids compounds showing red brown ring at the junction of *Trachyspermum ammi* extract.**:**

Fig-2.1.3.Presence of Coumarins compounds showing yellow coloration in *Trachyspermum ammi* extract.

Fig-2.2. METHANOL AND ETHYL ACETATE EXTRACT OF AJWAIN SEEDS:



1)Methanol extract 2)Ethyl acetate extract.

Biochemical Analysis

Fig-3.1 Macconkey agar showing the presence of *Enterobacter spp.*

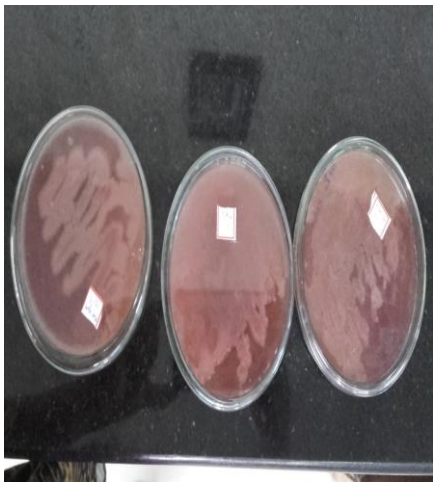


Fig.3.2 Eosin Methylene Blue shows the presence of *Enterobacter spp*

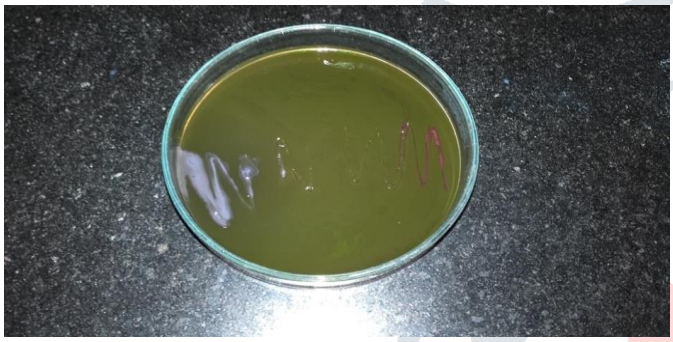


Fig-3.3 Catalase test shows the presence of *Bacillus* spp.

TABLE 3.1. BIOCHEMICAL ANALYSIS:

SL.NO.	BIOCHEMICAL TESTS	OBSERVATION	RESULTS	ORGANISM
1	Indole Test (Tryptone broth)	Red layer at the top	Negative(-)	-
2	Methyl Red Test (MRVP broth)	Red layer at the top	Negative (-)	-
3	Macconkey Agar	Bright pink red colonies	Positive(+)	*Enterobacter spp.*
4	Vogues proskauer Test	Pink/Red	Negative(-)	-

5	Mannitol Fermentation	Red to yellow	Negative(-)	-
6	Catalase Test	Gas bubbles	Positive(+)	*Bacillus spp.*
7	Citrate Agar Test	Yellow to cerise	Negative(-)	-
8	Starch	A clear zone	Negative(-)	-
9	Blood Agar		Positive(+)	*Streptococcus spp.*
10	Eosin Methylene Blue	Pink colonies	Positive(+)	*Enterobacterter spp.*

3.2. PLATE METHOD OF ZONE OF INHIBITION (Ethyl acetate and Methanol extract)

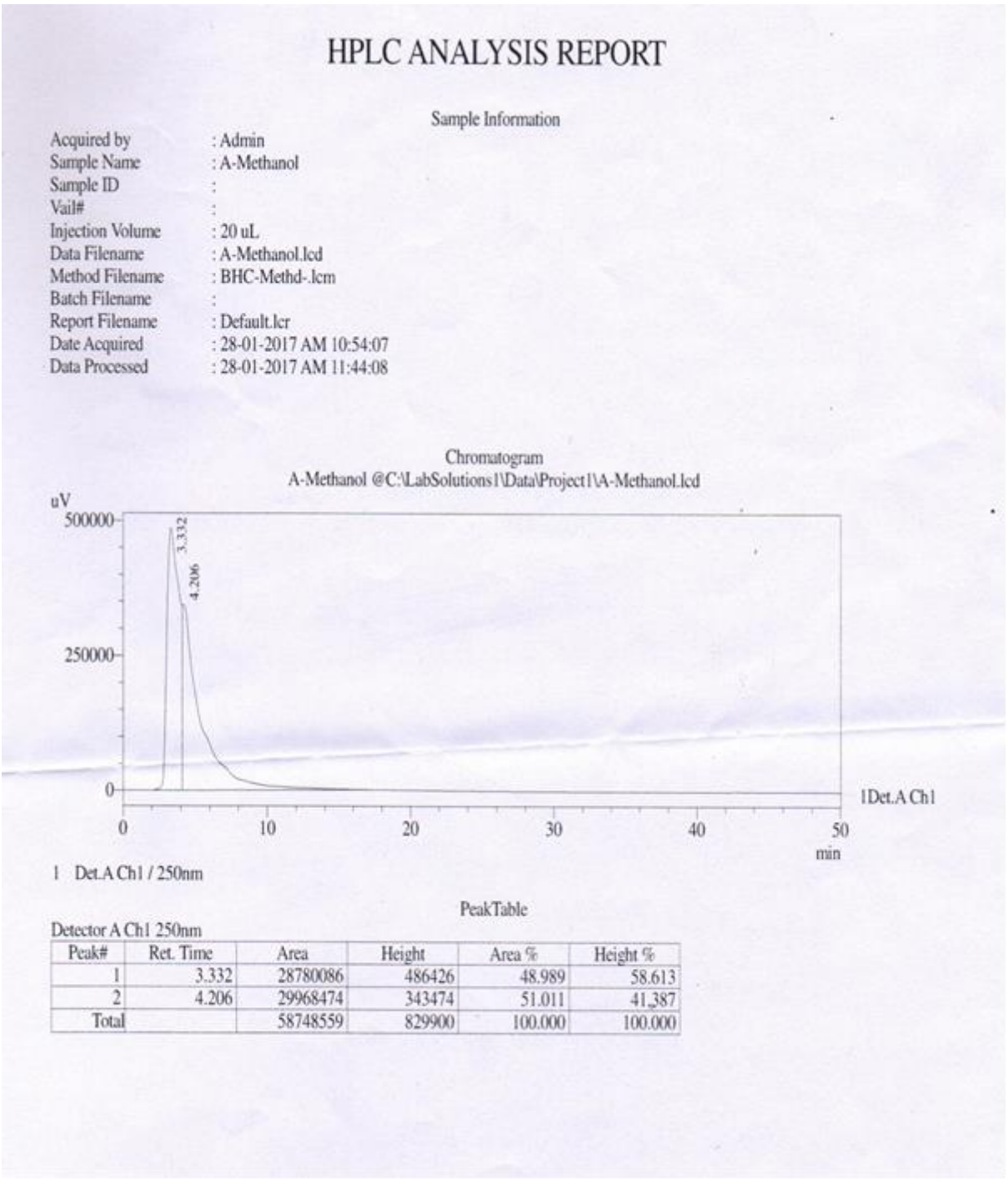
CONTROL: Gentamycin against Streptococcus *spp.*, *Enterobacter spp.* and *Bacillus spp.*

SL.NO.	ORGANISMS	Radius of Zone of Inhibition
1	*Streptococcus spp.*	20mm
2	*Enterobacter spp.*	15mm
3	*Bacillus spp.*	15mm

Table 3.3.CYTOTOXIC TEST ON EARTHWORM:

SL.NO.	TEST	RESULT (Before treated)	RESULT(Treated with Ajwain extract)	NORMAL RANGE	UNITS
1.	Bilirubin (Total)	<0.2	<0.10	0.3-1.9	mg/dl
2.	Bilirubin (Direct)	<0.1	0.18	0-0.3	mg/dl
3.	T.Protein	<1.0	<0.50	6-8.3	gm/dl
4.	Albumin	<0.5	<0.20	3.5-5.3	gm/dl
5.	AST/SGOT	430	23	8-48	U/l
6.	ALT/SGPT	20	6	7-55	U/l
7.	ALP	454	351	45-115	U/l
8.	GGT	18	17	9-48	U/l

Fig.4.Methanol extract of Ajwain- HPLC Analysis



HPLC ANALYSIS REPORT

Sample Information

Acquired by	: Admin
Sample Name	: A-Methanol
Sample ID	:
Vail#	:
Injection Volume	: 20 uL
Data Filename	: A-Methanol.lcd
Method Filename	: BHC-Methd-.lcm
Batch Filename	:
Report Filename	: Default.lcr
Date Acquired	: 28-01-2017 AM 10:54:07
Data Processed	: 28-01-2017 AM 11:44:08

Chromatogram

A-Methanol @C:\LabSolutions1\Data\Project1\A-Methanol.lcd

1 Det.A Ch1 / 250nm

PeakTable

Detector A Ch1 250nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.332	28780086	486426	48.989	58.613
2	4.206	29968474	343474	51.011	41.387
Total		58748559	829900	100.000	100.000