Antibacterial Activity, Phytochemical Studies of Medicinal Plants(*Euphorbia hirta* and *Achyranthesaspera)* against Diabetic Wound Pathogens

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ABSTRACT: *Diabetic foot infections are most frequent and serious complications of diabetes mellitus and responsible for most of the non-traumatic lower and limb amputations. Hundreds of plants species have been tested for antimicrobial properties and the medicinal value of these plants depends on bioactive phytochemical constituents that produce physiological action in the human body. In the present study 19 diabetic wound samples which are suspected for bacterial infection were analyzed. From these samples, 31 isolates were isolated and identified as Staphylococcus aureus, Escherichia coli, Pseudomonas sps and Klebsiellasps. The antibacterial activity of Achyranthesaspera and Euphorbia hirta were evaluated using different extracts viz., Petroleum ether, Methanol, and Di -ethyl ether extract against the isolated bacteria, Staphylococcus sps, Klebsiellasps, Escherichia coli, and Pseudomonas sps. Phytochemical study and antibacterial activity of Achyranthesaspera and Euphorbia hirta were evaluated against different pathogensand GC-MS analysis was carried out based on the antimicrobial activity of the selected medicinal plants and zone of inhibition against isolated diabetic wound pathogens. Among the Euphorbia hirta plant extracts, compared to methanol and Petroleum ether extracts, Di-ethyl ether extracts of Euphorbia hirta highly inhibit the growth of the isolated organisms of Staphylococcus aureus, Klebsiellasps, Escherichia coli, and Pseudomonas sps at 15µl concentration whereas among theAchyranthesaspera plant extracts, compared to methanol and Petroleum ether extracts, Di-ethyl ether extracts of Achyranthesaspera highly inhibit the growth of the isolated organisms of Staphylococcus aureus, Klebsiellasps, Escherichia coli, and Pseudomonas sps at 15µl concentration. From the GC-MS analysis of di-ethyl ether extract of Euphorbia hirta plant, four predominant compound were identified.*

Key Words: *Diabetes mellitus, Diabetic Foot Ulcer (DFU), Euphorbia hirta, Achyranthesaspera, Phytochemicals, Antibacterial activity*

I. Introduction

Diabetes mellitus, commonly referred to as diabetes was first identified as a disease associated with "sweet urine," and excessive muscle loss in the ancient world (Manisha *et al.,* 2011). Diabetes mellitus is a heterogeneous group of disorders characterized by elevated blood glucose levels (hyperglycemia) resulting from defects in insulin secretion, insulin action or both. (American Diabetes Association,2012) Diabetes mellitus, commonly referred to as a metabolic disorder characterized by hyperglycemia, glycosuria, hyperlipidemia, negative nitrogen balance and sometimes ketonemia. It is a chronic medical condition, meaning that although it can be controlled (Aneese*t al.,* 2013).

There are different causes of diabetes but the majority of cases are classified as either type 1 or type 2 diabetes. Type 1 diabetes accounts for about 10-15% of all diabetics. (Ganie and Kotwal, 2012). It is estimated that there are approximately 33 million adults with diabetes in India. This number is likely to increase to 57.2 million by the year 2025. It remains as the third leading cause of death and second leading cause of blindness in developed countries it is also in the rise in India. (Ramachandran *et* al., 2010). Amongst diabetes, NIDDM (Non-Insulin Dependent Diabetes mellitus) is common disease and most prevalent all over the world. It is estimated that at least 1 in 20 deaths, globally and across all ages, are attributable to diabetes. (Olokoba*et al*., 2012).

Diabetic Foot Infections (DFIs) are a common and serious problem in persons with diabetes and typically begin in a wound, most often a neuropathic ulceration. Diabetic foot infections are commonly encountered problems in the practice of clinical medicine today. (Hobizal and Wukich, 2012). DFIs are most frequent and serious complications of diabetes mellitus and responsible for most of the non-traumatic lower and limb amputations. In India, Diabetic foot infections are the most common diabetes related cause of hospitalization. Every year, more than a million diabetic patients require limb amputation (Casqueiro*et al.,* 2012)

Numerous factors related to diabetes can impair wound healing, including wound hypoxia (inadequate oxygen delivered to the wound) infection, nutrition deficiencies, and the disease itself. Fluctuating blood sugar and hypoxia from poor circulation may impair the ability of white blood cells to destroy pathogenic bacteria and fungi, increasing infection risk. The presence of infection depends mainly on the number of microorganisms residing in the wound, whereas the healing process depends on the type of bacterial strains and their pathogenicity (Han and Ceille, 2017).

Footulcer is the leading cause of hospitalization in diabetic patients and is one of the most feared complications of diabetes. Infected foot ulcer is a common cause of morbidity in diabetic patients, ultimately leading to dreaded complications such as gangrene and amputations (Al-Rubeaan*et al*., 2015).

The large number of synthetic drugs produced from pharmaceutical industries from time to time has led to develop resistant to microorganisms that become major global issue in the treatment of infectious diseases. At present, there is an urgent and continuous need of exploration and development of cheaper as well as effective new plant based drugs with better bioactive potential with least side effects. Antimicrobials of plant origin have been proved to be effective in the treatment of infectious diseases. Simultaneously with lesser side effects, which are often associated with synthetic antibiotics (Alain, 2017).

Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents. Therefore, there is a need to develop alternative antimicrobial drugs from medicinal plants for the treatment of infectious diseases. In addition to this problem, antibiotics are sometimes associated with adverse effects, on the host including hypersensitivity immune suppression and allergic reactions. The situation forced scientists to search for new antimicrobial substances. There are several reports on the antimicrobial activity of different herbal extracts in different regions of the world.

II. Materials And Methods

Collection of Samples:

Twenty-onePus and wound samples from diabetic patients were collected from Government Hospital in and aroundNamakkal area. Sterile cotton swab were inserted into the deep wound and samples were collected in sterile screw capped tubes and immediately transported to laboratory.

Processing of Samples:

The collected samples were inoculated into the nutrient broth and incubated at 37ºC for 24 hours. After incubation the samples were streaked on the nutrient agar medium and the colonies developed were isolated and purified into pure cultures as per the standard procedure. The isolated bacteria were identified based on microscopic and biochemical characterization.

Identification of Isolates:

Cultural Characteristics and Biochemical Tests:

The bacterial cultures were inoculated on to the surface of the different media to study the cultural characteristics of isolates. The culture media includes Nutrient agar, MacConkey agar, Mannitol Salt agar, Cetrimide agar and Eosin Methylene Blue agar. The isolated bacteria was identified bybased on microscopic and biochemical characters. The biochemical testsincludesIMViC, Catalase test, Oxidase test, Carbohydrate fermentation test, Coagulase test, Urease test and Triple sugar iron agar test (Aneja, 2006)

Antibiotic Sensitivity Test (Kirby Bauer Method):

Sterile Muller-Hinton agar plates were prepared and isolated bacteria was inoculated by swapping. Antibiotic disc were placed on the Muller Hinton Agar plates. Then the plates were kept in an incubator at 37ºCfor 24 hours. After incubation the Muller-Hinton Agar plates were observed for the development of zone of inhibition. The zones of inhibition was measured (Bauer *et al.,* 1966).

Collection of Plant Sample:

The fresh plant *Euphorbia hirta* and *Achyranthesaspera* were collected in and around Namakkal area. The plant were freshly collected and were dried. The dried plant materials were ground well using mechanical blender into fine powder and then transferred into airtight containers for further studies.

Extraction of Plant:

The plant extract was prepared with Petroleum ether, Methanol, and Diethyl ether as solvent for 45 hours by soxhlet extractor. The extract was filtered while hot and concentrated in vacuum under reduced pressure using rotary flask evaporator.

Effect of Medicinal Plant Extracts Against Pathogens (Agar Well Diffusion Method):

The antibacterial activity was carried out by well diffusion method. The sterile Muller Hinton agar plate was prepared. The isolated test organisms were spread over the Muller Hinton Agar plates by using

separate sterile cotton buds. A sterile cork-borer was used to make a well on the Muller Hinton agar plates. The plant extract was introduced into the well at various concentrations (5µl, 10µl, 15µl) and the plate were incubated at 37ºC for 24 hours. Effect of plant extract on pathogens was determined by measuring the diameter of the zone of inhibition (Perez *et al.,* 1990).

Phytochemical Analysis of Plant Extracts:

Qualitative chemical tests were carried out using extract from plant to identify the phytochemicals. A 10 mg/ml plant extract was used for the tests. The plant extract were analyzed for the Alkaloids, Flavonoids, Steroids, Terpenoids, Carbohydrate, Phenols, Tannins, and Glycosides etc. (Tiwari *et al.,* 2011).

GC-MS Analysis of Plant Extract:

GC-MS analysis was carried out in SITRA, Coimbatore, to identify the active principle in the extract of the plant *Euphorbia hirta*by Thermo GC-Trace Ultra Version: 5.0, ThermoMsDsq II using DB 35-Mscapillary standard non- polar column. (dimension: 30 Mts, ID: 0.25 µm) and the Carrier Gas In Helium, with the Flow rate; 1.0 ML/Min and the Temperature 70ºC at raised to 25ºC at 10 min hold.

III. Results

In the present study, 21 wound samples were collected from the diabetic patients from government hospital at Namakkal. From these samples, 31 bacterial cultures were isolated and identified by microscopic examination and biochemical tests and the results are presented below.

Isolation and Identification:

Colony Morphology:

Among the 31 isolates, 10 isolates were large, circular, convicts, smooth, shiny, opaque colonies on nutrient agar. Sevenisolates were showed large opaque irregular fluorescent greenish pigmented colonies. Seven isolates were showed small, smooth colorless and opaque colonies. Another 7 isolates were showed large dome-shaped mucoid colonies.

Microscopic Observation:

Gram staining:

Among the 31 isolates, 21isolates were Gram Negative rod and the remaining 10 isolates were Gram positive cocci. The results are presented in Table-1.

Motility Test:

Among 31 isolates, 14 isolates showed motility and remaining 17 isolates are non -motile. The results are presented in Table-1.

Based on biochemical test and microscopic examination, 10 isolates were tentatively identified as *Staphylococcus aureus*,7 isolates as *Escherichia coli*,7 isolates as *Pseudomonas sps*and 7 isolates as *Klebsiellasps*. The results are presented in Table-2 and Figure 1.

Antibiotic Sensitivity of the Isolated Organisms:

Antibiotic sensitivity of the isolated organism was analyzed and the results are presented in Table-3, Figure 2 and Figure 3.

Staphylococcus aureus shows sensitivity to Chloramphenical, Vancomycin, Erythromycin, Tetracycline and resistant to Pencillin. *Escherichia coli* shows sensitivity to all antibiotics. *Pseudomonas sps* shows sensitivity for Erythromycin, Chloramphenical and resistant for Tetrachycline, Pencillin, Vancomycin. Chloramphenical showed sensitive against *Klebsiellasps*whereas other antibiotics did not inhibit the organism.

Antibacterial activity of Euphorbia hirta extract and Achyranthesaspera extract:

The antibacterial activity the *Euphorbia hirta* and *Achyranthesaspera* were tested using different solvents viz., Petroleum ether, Methanol, and Di -ethyl ether extract were tested against the isolated bacteria, viz., *Staphylococcus aureus*, *Klebsiellasps*, *Escherichia coli*anda *Pseudomonas sps* and the results were depicted in Table-4 and Table-5.

In *Euphorbia hirta*, compared to Methanol and Petroleum ether extracts, Di-ethyl ether extracts of *Euphorbia hirta* highly inhibit the growth of the isolated organisms viz., *Staphylococcus aureus*, *Klebsiellasps*, *Escherichia coli*, and *Pseudomonas sps* at 15µl concentration.

In *Achyranthesaspera*, compared to Methanol and Petroleum ether extracts, Di-ethyl ether extracts of *Achyranthesaspera* highly inhibit the growth of the isolated organisms of *S. aureus*, *Klebsiellasps*, *Escherichia coli*, and *Pseudomonas sps* at 15µl concentration.

Phytochemical Analysis of Euphorbia hirta Plant Extract and Achyranthesaspera Plant Extracts:

Phytochemical analysis of *Euphorbia hirta* plant extract was carried out and the results are presented in Table-6.

Alkaloids, flavonoids, glycosides, steroids, tannins, carbohydrates, phenols, Terpenoids were detected in Methanol extracts of *Euphorbia hirta* plant.

Petroleum ether extract of *Euphorbia hirta*contain Alkaloids, flavonoids, glycosides, steroids, tannins, terpenoids. Carbohydrates, phenols were not detected from the extract.

Diethyl ether flower extract of *Euphorbia hirta* contain Alkaloids, flavonoids, glycosides, steroids, tannins, phenols, terpenoids. Carbohydrates were not detected from the extract. Table-5.

Phytochemical analysis of *Achyranthesaspera* plant extract was carried out and the results are presented in Table-6.

Methanolic plant extracts shows that alkaloids, flavonoids, phenols, and tannins, terpenoids, steroids, whereas glycosides, carbohydrates were absent.

Flavonoids, glycosides, terpenoids, tannins are detected in petroleum ether solvent of *Achyranthesaspera*. Alkaloides, steroids, carbohydrates, phenols were not detected from the extract.

The diethyl ether extract of *Achyranthesaspera* contain alkaloids, flavonoids, and glycosides, terpenoids, phenols whereas carbohydrates, steroid and tannins were not detected.

GC-MS Analysis of Euphorbia hirta:

In the present study the Di-ethyl ether extract of *Euphorbia hirta* plant was subjected to GC-MS analysis for the identification of active metabolites and the predominant compounds were found to be Cyclohexane, 1,4-dimethyl-2-octadecyl., Tricyclo [3.2.1.0(2„4)]oct-6-ene, 8-methylene-, (1à,2à,4à,5à)1h-Pyrrole-3,4-diacetic acid., 2-acetoxymethyl-5-methoxycarbonyl-, dimethyl ester 1-(3-Spiro-cyclopentane-3,4-dihydro-isoquinolin-1-ylmethyl)-3,3., and dimethyl-3,4-dihydro-isoquinolinethe. The result was indicated in Table-8and Fig.1.

IV. Discussion

Most of the people with diabetes will develop a foot ulcer and 85% of major leg amputations being with a foot ulcer, carbuncles, boils and other skin infections may be hazardous, if not properly treated. Even a small cut may progress to a deep, open sore, called an ulcer. In most cases ulceration is a consequence of the awareness of trauma that can cause the breakdown of the skin.

In the present study21 diabetic wound samples which are suspected for bacterial infection were analysed. From these samples, 31 isolates were isolated and identified as *Staphylococcus aureus, Escherichia coli, Pseudomonas spsandKlebsiellasps.*This is in accordance with Kavishankar, (2011). They isolated *Staphylococcus aureus, Pseudomonas sps, Escherichia coli, and Klebsiellasps* in addition with from the diabetic wound sample.

In the present study, leaves of *Euphorbia hirta* were analyzed qualitatively. Secondary metabolites such as alkaloids, flavonoids, terpenoids, tannins, steroids, glycosides, phenols and carbohydrates were present in *Euphorbia hirta Linn*. But protein, fats andsaponins are absent in methanolic extract of *Euphorbia hirta.*

In the present study, leaves of *Achyranthesaspera* were analysed qualitatively. The diethyl ether extract of *Achyranthesaspera* contain alkaloids, flavonoids, and glycosides, terpenoids, phenols whereas carbohydrates, steroid and tannisns were not detected.

Bacterial pathogens isolated from wound samples *Staphylococcus aureus*, showed sensitive for Chloramphenical and resistant to Penicillin. Tetracycline, Erythromycin and Vancomycin showed moderate zone formation for *Staphylococcus aureus. Escherichia coli* showed sensitive for Chloramphenicol and resistant for Penicillin. Erythromycin, Tetracycline, and Vancomycin showed moderate zone formation for *Escherichia coli. Pseudomonas sps* showed sensitive only for Chloramphenicol and showed resistant for Penicillin, Tetracycline and Vancomycin. It shows moderate zone for Erythromycin. *Klebsiellasps* showed sensitive only for Chloramphenicol and resistant for Penicillin, Erythromycin, Tetracycline and Vancomycin. Similar results were reported by Orji *et al.,* (2009).

The isolated test organisms were tested with selected medicinal plants by agar diffusion method. Antibacterial activity of the *Euphorbia hirta* plant extracts, compare to methanol and Petroleum ether extracts, Di-ethyl ether extracts of *Euphorbia hirta* highly inhibit the growth of the isolated organisms of *Staphylococcus aureus, Klebsiellasps, Escherichia coli*, and *Pseudomonas sps* at 15µl concentration.

Various extracts of *Euphorbia*hirta exhibited antimicrobial activity against various microbes including those causing burn and wound infections like *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Sudhakar*et al*., 2006; Rajeh*et al.,* 2010).

Antibacterial activity of the *Achyranthesaspera* plant extracts, compare to methanol and Petroleum ether extracts, Di-ethyl ether extracts of *Achyranthesaspera* highly inhibit the growth of the isolated

organisms of *Staphylococcus aureus, Klebsiellasps, Escherichia coli,* and *Pseudomonas sps* at 15µl concentration.

Narendhiran*et al.,*(2014) reported the various extracts of *Achyranthesaspera* tested for antibacterial activity against some bacteria are *Escherichia coli, Proteus mirabilis, S.typhiandE.aerogenes* and antifungal activity against some fungus like*Aspergillus niger, Aspergillus flavus*, *Fusariumverticillioides*. It shows mild activity.

GC-MS analysis was carried out based on the antimicrobial activity of the selected medicinal plants and zone of inhibition against isolated diabetic wound pathogens.

From the GC-MS analysis of di-ethyl ether extract of *Euphorbia hirta*, plant totally 26 compounds were identified from this compound only 4 compoundare identified as predominant compound like Cyclohexane, 1,4-dimethyl-2-octadecyl., Tricyclo[3.2.1.0(2,,4)]oct-6-ene, 8-methylene-, (1à,2à,4à,5à)1h-Pyrrole-3,4-diacetic acid., 2-acetoxymethyl-5-methoxycarbonyl-, dimethyl ester 1-(3-Spiro-cyclopentane-3,4-dihydro-isoquinolin-1-ylmethyl)-3,3., and dimethyl-3,4-dihydro-isoquinoline.(Sharma *et al.,* 2011).

The present study was carried out to scientifically evaluate the use of extract *Euphorbia hirta* against isolated bacteria. Same results were observed in the study conducted by Mei Fen Shih1 and Jong YuhCherng, (2012). In their studies they observed that the extracts of *Euphorbiahirta*have been reported to have various pharmacological effects like wound healing activity and antibacterial effects.

V. Summary and Conclusion

In the present study 21 diabetic wound samples which are suspected for bacterial infection were analyzed. From these samples, 31 isolates were isolated and identified as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas sps*and*Klebsiellasps*.

The isolated organism were tested against standard antibiotics. The antibacterial activity of *Euphorbia hirta* was tested against the isolated bacteria which reveals that Di-ethyl extract was recorded maximum inhibition zone against organism tested.

Secondary metabolites such as alkaloids, flavonoids, terpenoids, tannins, steroids, glycosides, phenols and carbohydrates are present in the di-ethyl ether solvent of *Euphorbia hirta* plant extract.

Di-ethyl ether plant extract was subjected to GC-MS analysis for the identification of active metabolites in *Euphorbia hirta*.

From the GC-MS analysis of di-ethyl ether extract of *Euphorbia hirta* totally 26 compounds were identified from this compound only 4 compoundare identified as predominant compound.

Table-1. Isolation and Identification of bacterial isolates from wound samples

S. No	Isolate No	Preliminary Verification				Biochemical Tests						Sugar Fermentation Test			Probable Organism
		Gram Staining	Motility	Catalase	Oxidase	Indole	MR	VP	Citrate	TSI	Urease	Glucose	Sucrose	Lactose	
1	S1	G+ve cocci	Non Motile	+	-	-	+	-	-	A/A. H_2S· Gas -ve	-	+	+	+	*S.aureus*
2	$S1_1$	G-ve rod	Motile	+	-	+	+	-	-	A/A. H_2S· Gas -ve	-	+	+	+	*E.coli*
3	$S1_2$	G-ve rod	Motile	+	-	+	+	-	-	A/A. H_2S· Gas -ve	-	+	+	+	*E.coli*
4	S2	G-ve rod	Motile	+	+	-	-	-	+	Ak/Ak	-	+	-	-	*Pseudomonas sps*
5	$S2_1$	G+ve cocci	Non Motile	+	-	-	+	-	-	A/A. H_2S Gas -ve	-	+	+	+	*S.aureus*
6	$S2_2$	G-ve rod	Non Motile	+	-	-	-	-	+	A-/A-.H_2S Gas +ve	-	+	+	+	*Klebsiellasps*

7	$S2_3$	G+ve cocci	Non Motile	+	-	-	+	-	-	A/A. H_2S Gas -ve	-	+	+	+	*S.aureus*
8	S3	G-ve rod	Motile	+	+	-	-	-	+	Ak/Ak	-	+	-	-	*Pseudomonas sps.*
9	S4	G-ve rod	Non Motile	+	-	-	-	+	+	A+/A+. HzS· Gli+Ve	+	+	+	+	*Klebsiellas ps*
10	S5	G-ve rod	Motile	-	+	-	-	-	+	Ak/Ak	-	+	-	-	*Pseudomonas sps.*
11	$S5_1$	G-ve cocci	Non Motile	+	-	-	+	-	-	A/A. H_2S· Gas -ve	-	+	+	+	*S.aureus*
12	S6	G-ve rod	Motile	+	+	-	-	-	+	Ak/Ak	-	+	-	-	*Pseudomonas sps.*
13	$S6_1$	G-ve rod	Non Motile	+	-	-	-	+	+	A+/A+ H_2S· Gas +ve	+	+	+	+	*Klebsiellas ps*
14	$S6_2$	G+ve cocci	Non Motile	+	-	-	+	-	-	A/A. H_2S· Gas +ve	-	+	+	+	*S.aureus*
15	$S6_3$	G-ve rod	Non Motile	+	-	-	-	+	+	A+/A+ H_2S Gas +ve	+	+	+	+	*Klebsiellas ps*
16	S7	G+ve cocci	Non Motile	+	-	-	+	-	-	A/A. H_2S· Gas -ve	-	+	+	+	*S.aureus*
17	$S7_1$	G-ve rod	Motile	+	-	-	-	+	+	Ak/Ak	-	+	-	-	*Pseudomonas sps.*
18	S8	G+ve rod	Non Motile	+	-	-	+	-	-	A/A. H_2S· Gas -ve	-	+	+	+	*S.aureus*
19	S9	G-ve rod	Motile	+	-	+	+	-	-	A/A. H_2S Gas -ve	+	+	+	+	*E.coli*
20	S10	G+ve cocci	Non Motile	+	-	-	+	-	-	A/A. H_2S· Gas -ve	-	+	+	+	*S.aureus*
21	S11	G-ve rod	Non Motile	+	-	-	-	+	+	A-/A-. H_2S Gas +ve	+	+	+	+	*Klebsiellas ps*
22	S12	G-ve rod	Non Motile	-	-	-	-	-	+	A/A-. Gas +ve	+	+	-	-	*Klebsiellas ps*
23	$S12_1$	G-ve rod	Motile	-	+	-	-	-	-	Ak/Ak	-	+	-	-	*Pseudomonas sps.*
24	S13	G-ve rod	Motile	+	-	+	+	-	-	A/A H_2S Gas -ve	-	+	+	+	*E-coli*
25	S14	G-ve rod	Non Motile	+	-	-	-	+	+	A+/A+. H_2S Gas -ve	+	+	+	+	*Klebsiellas ps*
26	S15	G+ve cocci	Non Motile	+	-	-	+	-	-	A/A. H_2S Gas -ve	-	+	+	+	*S.aureus*

27	S16	G+ve rod	Non motile	+	-	-	+	-	-	A/A. H_2S· Gas -ve	-	+	+	+	*S.aureus*
28	$S16_1$	G-ve cocci	Motile	+	+	-	-	-	+	AK/AK	-	+	-	-	*Pseudomonas sps.*
29	S17	G-ve rod	Motile	+	-	+	+	-	-	A/A. H_2S Gas -ve	-	+	+	+	*E.coli*
30	$S17_1$	G-ve rod	Motile	+	-	+	+	-	-	A/A. H_2S Gas -ve	-	+	+	+	*E.coli*
31	$S19_2$	G-ve rod	Motile	+	-	+	+	-	-	A/A. H_2S· Gas -ve	-	+	+	+	*E.coli*

Table- 2. Organisms Isolated From Diabetic Wound Samples

Organisms	No of isolates
Staphylococcus aureus	10
Escherichia coli	7
Pseudomonas sps	7
Klebsiellasps	7

Table-3. Antibiotic Sensitivity of Organisms Isolated From Diabetic Wound Samples

Organism	Zone of inhibition(diameter in mm)				
	Tetracycline	Vancomycin	Chloromphenical	Penicillin	Erythromycin
Staphylococcus aureus	31	13	32	-	23
Escherichia coli	20	13	22	10	21
Pseudomonas sps	-	-	19	-	13
Klebsiellasps	-	-	22	-	-

Table -4 Antibacterial Activity of *Euphorbia hirta* (Amman pacharisi) Against Bacteria Isolated from Diabetic Wound Sample

Organisms	Concentration of extract and zone of inhibition (mm)								
	Petroleum ether			Methanol			Di -ethyl ether		
	5µl	10 µl	15µl	5µl	10 µl	15µl	5µl	10 µl	15µl
Staphylococcus aureus	6	9	13	7	9	16	29	30	31
Klebsiellasps	-	4	11	6	8	14	16	18	22
Escherichia coli	5	8	14	9	12	17	14	17	21
Pseudomonas sps	-	6	9	-	7	15	18	19	22

Table-5 Antibacterial Activity of *Achyranthesaspera* (Naayuruvi) Against Bacteria Isolated from Diabetic Wound Sample

Organisms	Concentration of extract and zone of inhibition (mm)								
	Petroleum ether			Methanol			Di-ethyl ether		
	5µl	10 µl	15µl	5µl	10 µl	15µl	5µl	10 µl	15µl
Staphylococcus aureus	7	9	12	6	8	14	18	22	28
Klebsiellasps	-	6	9	-	-	6	20	22	24
Escherichia coli	6	8	13	7	9	11	11	12	14
Pseudomonas sps	4	7	12	5	8	11	18	20	22

Table -6 Phytochemical Characteristics of *Euphorbia hirta*and *Achyranthusaspera*Plant Extracts

Plant	*Euphorbia hirta*			*Achyranthusaspera*		
Phytochemical test	Methanol extract	Petroleum ether extract	Di-ethyl ether extract	Methanol extract	Petroleum ether extract	Di-ethyl ether extract
Test for Alkaloids Mayer Test	+	+	+	+	-	-
Test for flavonoids Lead acetate test	+	+	+	+	+	+
Test for glycosides Borntrager's Test Aqueous NaoH	+ +	+ -	+ -	- -	+ -	+ +
Test for steroids Salkowshi test	+	+	+	+	-	-
Test for carbohydrates Benedict"s test	+	-	-	-	-	-
Terpenoids test	+	+	+	+	+	+
Tannins test	+	+	+	+	+	-
Phenols test	+	-	+	+	-	+

Table-7 GC-MS Analysis of *Euphorbia hirta*Di-Ethyl Ether Extract

S. No.	Retention time (min)	Compound Name	Molecular Formula	Molecular Weight	Area %
1	18.61	Cyclohexane, 1,4-dimethyl-2-octadecyl-	$C_{26}H_{52}$	364	3.33
2	25.84	Tricyclo[3.2.1.0(2,,4)]oct-6-ene, 8-methylene-, (1à,2à,4à,5à)	C_9H_{10}	118	1.42
3	28.60	2-Furancarbothioamide	C_5H_5NOS	127	3.56
4	29.47	3-Bromo-thiophene-2-carboxamide	C_5H_4BrNOS	205	3.15
5	29.77	1,2-Bis[1-(2-hydroxyethyl)-3,6-diazahomoadamantantydene-9]hydrazine	$C_{22}H_{36}N_6O_2$	416	1.69
6	30.66	Olean-12-ene-3,15,16,21,22,28-hexol, (3á,15à,16à,21á,22à)	$C_{30}H_{50}O_6$	506	2.42
7	30.90	Acetic acid, 17-acetoxy-4,4,10,13-tetramethyl-7-oxo-2,3,4,7,8,9,10,11,12,13,1 4,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl (ester)	$C_{25}H_{36}O_5$	416	2.25
8	31.71	9-Desoxo-9-x-acetoxy-3,8,12-tri-O-acetylingol	$C_{28}H_{40}O_{10}$	536	3.57
9	31.94	9H-Carbazole-1-carboxylic acid, 4-(1H-indol-3-yl)-, methyl ester	$C_{22}H_{16}N_2O_2$	340	5.97
10	32.43	Mercury, chloro(3,17-dioxoandrosta-1,4,6-trien-2-yl)-	$C_{19}H_{21}ClHgO_2$	518	3.67
11	32.73	Acetamide, N-[5-(diethylamino)-2-[(2,4-dinitrophenyl)azo]-4-methoxypheny l]-	$C_{19}H_{22}N_6O_6i$	430	1.68
12	32.90	psi.,.psi.-Carotene, 3,4-didehydro-1,2,7',8'-tetrahydro-1-methoxy-2-oxo-	$C_{41}H_{58}O_2$	582	1.28
13	33.32	Cyclohexane, 1,3,5-trimethyl-2-octadecyl-	$C_{27}H_{54}$	378	7.66
14	33.66	Ketone, 3á-(difluoromethoxy)-16à,17à-epoxy-2a,2a,6a,6a-tetrafluoro-1,2 á,2a,3,6,6a,7á,8á,9à,10,11,12,13,14à,16,17-hexadecahydro-5á,10 á,13á,16-tetramethyl-15H-dicyclopropa[2,3:6,7]cyclopenta[a]phe nanthren-17-yl methyl Ketone,	$C_{26}H_{30}F_6O_3$	504	5.85
15	33.83	Pregnane-3,11,12,14,20-pentol, 3,12,20-triacetate 11-(hydroxyacetate), (3á,11à,12á,14á)-	$C_{29}H_{44}O_{10}$	552	3.17
16	34.35	1-(3-Spiro-cyclopentane-3,4-dihydro-isoquinolin-1-ylmethyl)-3,3 -dimethyl-3,4 -dihydro-isoquinoline	$C_{25}H_{28}N_2$	356	4.87
17	34.86	1h-Pyrrole-3,4-diacetic acid, 2-acetoxymethyl-5-methoxycarbonyl-, dimethyl ester	$C_{15}H_{19}NO_8$	341	4.90

18	35.43	3-(4-(3-Aminophenoxy)-2,3,5,6-tetrafluorophenoxy)aniline ditms	$C_{24}H_{28}F_4N_2O_2Si_2$	508	6.70
19	36.32	9-Desoxo-9-xi-hydroxy-3,7,8,9,12-pentaacetate ingol	$C_{30}H_{42}O_{11}$	578	3.43
20	36.62	3H-Pyrazol-3-one, 4-chloro-1,2-dihydro-5-methyl-2-phenyl-1-(trimethylsilyl)-	$C_{13}H_{17}ClN_2OSi$	280	5.07
21	37.00	Terbutaline, N-trifluoroacetyl-o,o,o-tris(trimethylsilyl)deriv.	$C_{23}H_{42}F_3NO_4Si_3$	537	1.92
22	37.25	5á)Pregnane-3,20á-diol, 14à,18à-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)]-, diacetate	$C_{19}H_{38}O_4$	489	3.57
23	37.55	Sarcosine, N-(2-methoxybenzoyl)-, propyl ester	$C_{14}H_{19}NO_4$	265	2.75
24	38.17	à,á-Methyl-2-deoxy-D-ribofuranoside	$C_6H_{12}O_4$	148	3.92
25	38.31	Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)-	$C_{27}H_{42}O_4$	430	5.61
26	39.07	4H-Cyclopropa[5',6']benz[1',2':7,8]azuleno[5,6-b]oxiren-4-one, 8,8a-bis(acetyloxy)-2a-[(acetyloxy)methyl]-1,1a,1b,1c,2a,3,3a,6a, 6b,7,8,8a-dodecahydro-6b-hydroxy-3a-methoxy1,1,5,7-tetramet hyl-, [1aR-(1aà,1bá,1cà,2aà,3aá,6aà,6bà,7à,8á,8aà)]-	$C_{27}H_{36}O_{10}$	520	6.59

Fig.1.Organisms Isolated From Diabetic Wound Samples

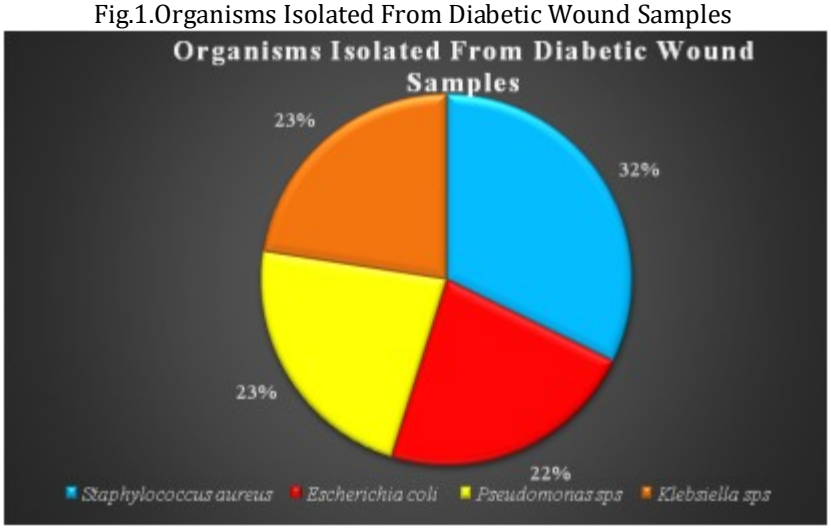


Fig. 2. Antibiotic Sensitivity of Organisms Isolated From Diabetic Wound Samples

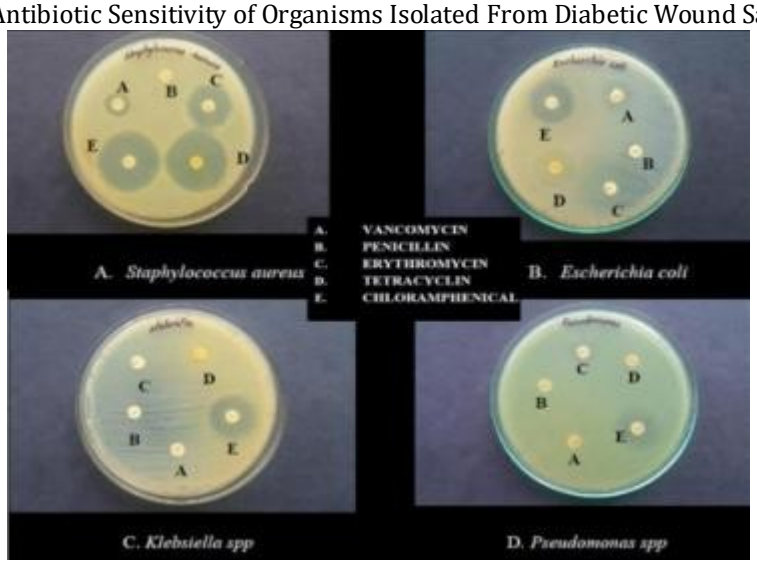


Fig. 3. Antibiotic Sensitivity of Organisms Isolated From Diabetic Wound Samples

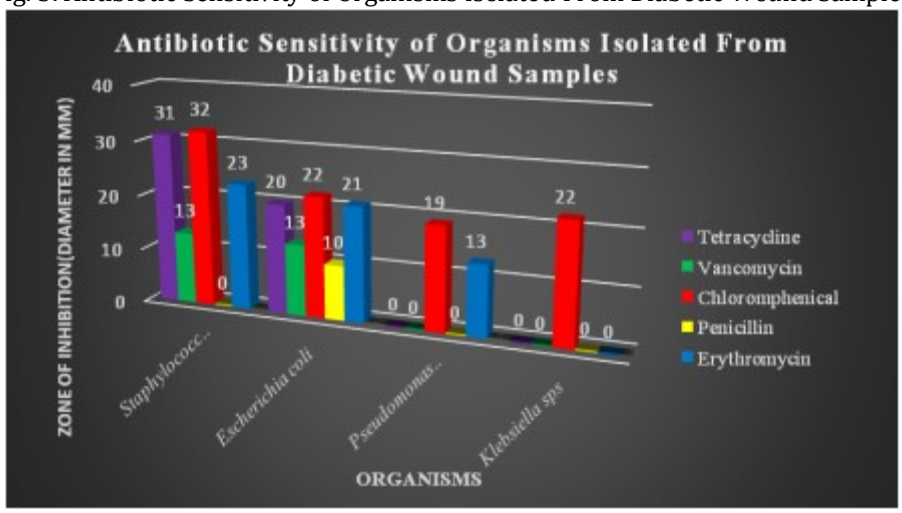
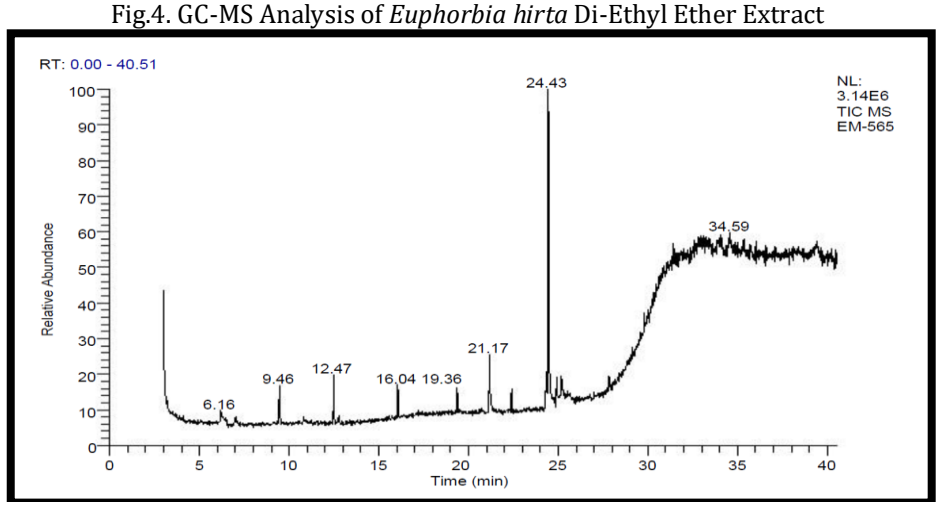


Fig.4. GC-MS Analysis of *Euphorbia hirta* Di-Ethyl Ether Extract



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