International Journal of Pharmaceutical Archive-9(12), 2020, 1-12
Available online through www.ijpaonline.info ISSN 2319-7226

EFFECTS OF *CARVACROL COMPOUND FROM ORIGANUM VULGAR*E AGAINST PATHOGENS ISOLATED FROM DIABETIC FOOT INFECTION

REVIEW ARTICLE

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(Received on: 18-10-2020; Revised & Accepted on: 26-11-2020)

ABSTRACT

D*iabetes mellitus is a metabolic disorder characterized by the presence of hyperglycemia due to defective insulin secretion, defective insulin action or both. The chronic hyperglycemia of diabetes is associated with relatively specific long-term micro vascular complications affecting the eyes, kidneys and nerves, as well as an increased risk for cardiovascular disease. Diabetic foot infections are infections that can develop in the skin, muscles or bones of the foot as a result of nerve damage and poor circulation that is associated with diabetes. Diabetes mellitus is a disorder that primarily affects the microvascular circulation. Collection of sample diabetic patients included in this study was of age group (35-45).Commercial compound Carvacrol an active medicinal plant in Origanum vulgare was bought from Aroma product Pvt.Ltd, Chennai in Tamil Nadu. The foot sample of both sexes were collected from government hospitals in kanchipuram, a sterile swab was used for collecting sample from diabetic patients study in sample processing, Microscopic examination, Antibiotic sensitivity test, Statistical analysis, the samples were processed according to the standard microbiological techniques the Micro organisms were isolate from pus culture statistical analysis was done. Staphylococcus aureus was the predominant organism among the isolation the gram negative organism Pseudomonas aeruginosa was predominant other organism included in our study was Escherichia coli and Proteus mirabilis, Aspergillus fumigates, Candida albicans.*

Keywords: *Diabetic foot infection, Microscopic examination, Sample processing, Carvacrol Antibiotic sensitivity test.*

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by the presence of hyperglycemia due to defective insulin secretion, defective insulin action or both. The chronic hyperglycemia of diabetes is associated with relatively specific long-term micro vascular complications affecting the eyes, kidneys and nerves, as well as an increased risk for cardiovascular disease (CVD). The diagnostic criteria for diabetes are based on thresholds of glycemia that are associated with microvascular disease, especially retinopathy. “Prediabetes” is a practical and convenient term referring to impaired fasting glucose (IFG), impaired glucose tolerance (IGT) (American Diabetes Association.,2012) or a glycated hemoglobin (A1C) of 6.0% to 6.4%, each of which places individuals at high risk of developing diabetes and its complications.

CLASSIFICATION OF DIABETES

According to American Diabetes Association, diabetes mellitus is classified into four types (American Diabetes Association., 2013).

I. Type 1 diabetes (β-cell destruction, usually leading to absolute insulin deficiency),
II. Type 2 diabetes (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with insulin resistance),
III. Gestational diabetes mellitus,
IV. Other specific types.

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Type 1 Diabetes results from selective destruction of the insulin producing β cells in the pancreatic islets and is primarily an autoimmune response developed against one or more β cell antigens (Csorba *et al.,* 2010). The markers of immune destruction of the β-cell include islet cell auto antibodies, autoantibodies to insulin, autoantibodies to Glutamic Acid Decarboxylase (GAD65), and autoantibodies to the tyrosine phosphatases IA-2 and IA-2b. Type 1 patients need to receive insulin injections, as oral administration of insulin is ineffective. The negative aspects of insulin treatment include refrigeration, very low half life, picking of the right dose at the right time, pain due to multiple sites of administration, fat pads, and immune response against exogenous insulin. Above all, most of the patients develop insulin resistance after receiving continuous insulin injection (Fleury-Milfort., 2008).

Type 2 Diabetes is the most common type of diabetes and it accounts for 90-95% of the diagnosed diabetes and equal number of undiagnosed diabetes. Type 2 diabetes is a chronic metabolic disorder that results from both defects in insulin action and insulin secretion. Chronic hyperglycemia in diabetes is accompanied by insulin resistance, which plays a key role in the initiation and progression of type 2 diabetes (T2DM). Glucose, one of the key physiological stimuli of β cells, increases the cytoplasmic free Ca^{2+} concentration and stimulates insulin secretion. However, chronic elevation of glucose concentration as a result of peripheral tissue resistance against insulin action causes β cell dysfunction (Choi and Kim., 2010).

DIABETIC FOOT INFECTION

Definition: Infection, ulceration or destruction of deep tissues associated with neurological abnormalities and various degrees of peripheral vascular diseases in the lower limb (World Health Organization definition, 1995). Risk factors Diabetic foot ulcers are a consequence of many factors including loss of protective sensation due to peripheral neuropathy where the feet become numb and the injury goes unnoticed. Also, arterial insufficiency complicates the neuropathic ulcer which leads to poor wound healing. Foot deformity and calluses can result in high plantar pressure, which results in additional risk. Mechanical stress at the wound site is hypothesized to affect wound healing (Farahani and Kloth., 2008). Many other factors contribute to the risk of foot ulceration and its subsequent infection in patients with diabetes. Uncontrolled hyperglycemia, duration of diabetes, trauma, improper footwear, callus, history of prior ulcers/amputations, older age, blindness/impaired vision, chronic renal disease and poor nutrition have also been demonstrated to play a role in the pathogenesis and progression of diabetic foot ulceration. Infection further deteriorates the diabetic foot resulting in a non-healing chronic wound. Recently, vitamin D deficiency was proposed as a risk factor for diabetic foot infection (Tiwari *et al*., 2013).

SYMPTOMS OF DIABETES MELLITUS

Diabetes mellitus is recognized by chronic elevation in the concentration of blood glucose in the blood often termed as hyperglycemia. This is sometimes accompanied by the symptoms of severe thirst, profuse urination, weight loss, and stupor, sometimes with polyphagia (Cooke and Plotnick, 2008) culminating in coma and death in the absence of effective treatment.

PREVALENCE OF DIABETES

According to International Diabetes Federation (IDF) 382 million people worldwide, or 8.3% of adults, are estimated to have diabetes. About 80% of the diabetics are living in low- and middle-income countries. If this trend continues, by 2035, more than 592 million people, or one adult in 10, will have diabetes. This equates to approximately three new cases every10 seconds or almost 10 million per year (IDF, 2013).

DIABETES MEDICATIONS

SULPHONYLUREAS
They remain the mainstay of the treatment of type II diabetes in older people. They stimulate the release of insulin in the pancreas. Patients using sulphonylureas need counseling regarding the taking of regular meals and the signs and symptoms of hypoglycaemia, because the drug causes hypoglycaemia. If possible it should not be given to the elderly because they do not eat enough due to many problems associated with their age. Sulphonylureas causes an increase in weight and it should therefore be used by thin or normal weight patients (Anuradha Gupta e*t al*., 2015).

METFORMIN
It acts by reducing glucose production by the liver. Overweight patients can use this since there is less risk of gaining weight. It also lowers cholesterol concentrations, and does not cause hypoglycaemia.

BIGUANIDES

The term biguanide refers to a group of oral type 2 diabetes drugs that work by preventing the production of glucose in the liver, improving the body s sensitivity towards insulin reducing the amount of sugar absorbed by the intestines. The only available biguanide medication is metformin, which is commonly used as a first-line treatment for type 2 diabetes. Metformin is usually prescribed as a single treatment (monotherpay), but it can also be combined with other medication in a single tablet – for example, metformin + pioglitazone (Competact), metformin + vildagliptin Eucreas and metformin + sitagliptin (Janumet). It s also sometimes prescribed in combination with insulin for people with type 1 diabetes (Anuradha Gupta e*t al*.,2015).

α-GLUCOSIDASE INHIBITORS

The drug delays the absorption of glucose from a carbohydrate-containing meal. It reduces the fluctuation in daily blood glucose levels and can be used by noninsulin dependent diabetic patients. A patient, whose blood glucose remains high in spite of the diet and the oral antidiabetic drugs, may be given insulin injections (Anuradha Gupta e*t al*., 2015).

THIAZILIDINEDIONES (TZDS)

This is a new drug Commonly called glitazones or actos (e.g. pioglitazone), thiazolidinediones lower blood glucose by increasing the sensitivity of your body's cells to insulin, so more glucose is taken into cells for the same amount of insulin in the bloodstream. They are not usually used alone, but are an option to take in addition to metformin or a sulfonylurea. Actos is effective as monotherapy and reduces the risk of microvascular complications by 2.6% (Anuradha Gupta e*t al*.,2015).

AMYLIN ANALOGUES

Amylin analogues, or agonists, are injectable drugs used in the treatment of both type 1 diabetes and type 2 diabetes. These compounds are administered before meals, and work similarly to the hormone amylin. Amylin has a number of benefits in terms of weight loss and reducing blood glucose levels.

HUMULIN

Humulin is synthesized in a laboratory strain of Escherichia coli bacteria which has been genetically altered with recombinant DNA to produce biosynthetic human insulin. Humulin R consists of zinc-insulin crystals dissolved in a clear fluid. The synthesized insulin is then combined with other compounds or types of insulin which affect its shelf life and absorption. For example, Humulin N is combined with protamine to extend the time-activity profile of Humulin R for an extended period. Humulin by itself is short-acting insulin. Humulin R, like many other form of injectable insulin, is intended for subcutaneous injection, so it should not be used intramuscularly, since its dispersion in the rest of the body would take more time (Anuradha Gupta *et al*., 2015).

TYPES OF HUMULIN:

HUMULIN R: [Regular human insulin injection (rDNA origin)] is a short-acting insulin that has a relatively short duration of activity as compared with other insulins.

HUMULIN R REGULAR U-500: (Concentrated) insulin human injection, USP (rDNA Origin) is a stronger concentration (500 units/mL) of Humulin R.

HUMULIN N: Human NPH insulin injection (rDNA origin) is an intermediate-acting insulin with a slower onset of action and a longer duration of activity than Humulin R.

HUMULIN 70/30: [70% human insulin isophane suspension, 30% human insulin injection (rDNA origin)] is a mixture insulin. It is an intermediate-acting insulin combined with the onset of action of Humulin

HUMULIN 50/50: [50% human insulin isophane suspension, 50% human insulin injection (rDNA origin)] is a mixture insulin. It is an intermediate-acting insulin combined with the onset of action of Humulin R.

MEDICINAL PLANTS

Globally, about 85% of the traditional medicines used for primary healthcare are derived from plants. The knowledge about medicinal plants has been accumulated in the course of many centuries based on different medicinal systems such as ayurveda, unani and siddha. There has been an exponential growth in the field of herbal medicine for past few years and these drugs are gaining popularity both in developed and developing countries because of their natural origin and relatively less side effects. The World Health Organization (WHO) has provided a list of 21,000 plants, which are used for medicinal purposes all around the world. Among these, 2500 species are in India, out of which 150 species are used commercially on a large scale. India is the largest producer of medicinal herbs and is called as “botanical garden of the world” (Seth and Sharma, 2004).

HERBAL TREATMENT FOR DIABETES MELLITUS

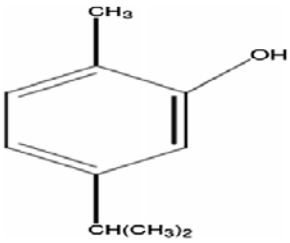
Traditional medicine is a vital source of potentially useful new compounds for the development of various therapeutic agents. India has been known to be rich repository of medicinal plants. The forest in India is the principal repository of large number of medicinal and aromatic plants, which are largely collected as raw materials for manufacture of drugs and perfumery products. There are about 2, 50,000 higher plants species are available on earth; out of which more than 80,000 are gifted with medicinal values. While the Ayurveda system of medicine uses about 700 species. Ayurveda is most developed and widely practiced in India. The emphasis of development of new biologically active molecule has been gradually replaced by the use of total herbs as medicine and food supplements (Kusum Singh and Vinita Ahirwar, 2010).

CARVACROL

Carvacrol is a monoterpenoid phenol predominantly found in oregano (Origanum vulgare), thyme (Thymus vulgaris), pepperwort (Lepidium flavum) and wild bergamot, also produced naturally by isolation of essential oil from some selected plants (Kintzios, 2002; Sokmen *et al.*, 2004; Tang *et al*., 2011; Jamali *et al*., 2012; Kim *et al*., 2013). Carvacrol plays a critical role as natural antioxidant in the reduction of lipid peroxidation which leads to oxidative destruction of cellular membranes (Rhee *et al*., 1996, Yanishlieva *et al.*, 1999).

SOURCES OF CARVACROL

Carvacrol is a component of some medicinal plants, such as black cumin (*Nigella sativa), oregano (Origanum compactum), Monarda didyma, Origanum dictamnus Origanum microphyllum, Origanum onites, Origanum scabrum, Origanum vulgare, thyme (Thymus glandulosus), savory (Satureja hortensis)* (Aligiannis *et al*., 2001; De Vincenzi *et al.*, 2004; Coskun *et al.*, 2008; Liolios *et al.*, 2009; Figiel *et al.*, 2010).



CHEMICAL AND PHYSICAL CHARACTERISTICS

Carvacrol is an isomer and derivative of phenol, the chemical formula of *carvacrol* (*cymophenol*) is C6H3CH3 (OH) (C3H7), a monoterpenoid phenol (Bouchra *et al.*, 2003; De Vincenzi *et al*., 2004). *Carvacrol* is also named 5-isopropyl-2-methylphenol or 2-Methyl-5-(1-methylethyl)-phenol according to International Union of Pure and Applied Chemistry (IUPAC). *Carvacrol* is a liquid and has the same taste of thymol. *Carvacrol* is highly lipophilic; the solubility of *carvacrol* is very high in carbon tetrachloride, ethanol, diethyl ether, acetone; but insoluble in water (Ultee *et al*., 2000).

MATERIALS AND METHODS

Collection of sample diabetic patients included in this study was of age group (35-45). The foot sample of both sexes were collected from government hospitals in Kanchipuram, a sterile swab was used for collecting sample from diabetic patients. The sample was collected by simply rolling the tip of the swab on its side for one full rotation over the infected area. Dried surface was premoistened with a saline swab which improves the yield, transportation of sample the collected swab was placed in Stuart's media and transported to the lab.

ESSENTIAL OIL

Commercial compound *Carvacrol* an active medicinal plant in *Origanum vulgare* was bought from Aroma product Pvt.Ltd, Chennai in Tamil nadu.

Processing of sample Microscopic examination, Staining method, Hanging drop method, Culture, Biochemical parameter**,** Catalase, Oxidase, Coagulase, Indole, Methyl red, Vogesproskauer, Citrate ,Triple sugar iron agar, Urease, Gelatin Hydrolysis, Nitrate reduction, Sugar fermentation, Selective media, Sabouraud's dextrose agar medium, Lacto phenol cotton blue mount, Germ tube test, Carbohydrate fermentation test, Sugar assimilation test, Clamydospore formation, Antibiotic sensitivity study. All the analysis is carried out by the method of Sigma Diagnostic kits (Sigma Chemical Company Catalogue, 1997) (Gutr. 1959). **Statistical Analysis** All the data were analyzed as per the method of Pillai and Sinha HC. (1968)

RESULTS AND DISCUSSION

The results obtained in the present investigation indicate 30 Sample diabetics dental infected patients had been collected from Government Hospital in Kanchipuram. 18 samples were collected from male and 12 samples were collected from female. The number of different isolates from total number of specimens are tabulated in table number 6. The result from microscopic examination such as staining motility test and cultural characters, colony morphology, biochemical characters for *Staphylococcus aureus* are shown in the table number 2 and for antibiotic sensitivity in table number 7. The result for *Pseudomonas aeruginosa* are shown in the table number 3 and antibiotic sensitivity in table number. 8. The result for *Proteus mirabilis* and *E.coli* are shown in the table number 4 and 5 and antibiotic sensitivity in table number 9 and 10. The result for Candida albicans are shown in the table 12, 13 and antibiotic sensitivity table number 15. The result for Aspergillus fumigatus are shown in the table 11 and antibiotic sensitivity table number 14.

Since the early 1980s, DFIs are recognized to be polymicrobial in nature. Gram-positive cocci are almost always the most commonly isolated organisms, followed by Gram-negative and anaerobic bacteria. The majority of the studies conducted during the last two decades in Western countries have shown that unless antibiotics have been used prior, cultures from acute diabetic foot wounds grow a single pathogen, which is usually *S. aureus* or *Streptococcus* spp (Kosinski and Lipsky.,2010). In the current study, 312 bacteria were isolated from 267 specimens, with a rate of 1.16 Isolates per culture (IPC). While these results compare favorably with several previous studies such as those by Hayat *et al*.,(2011) (1.24 IPC) and Viswanathan *et al*.,(2002) (1.21 Isolation per culture they differ from several others, such as the investigation by Citron *et al*.,(2007), which revealed 2.7 IPC among aerobic and 2.3 Isolation per culture among anaerobic bacteria in a diabetic population involving 433 patients with foot infections.

The most commonly isolated pathogen was *Staphylococcus aureus*, the isolated pathogen was about 83 % the result was compatible with the findings of microorganim. The present study revealed the predominance of *Pseudomonas aeruginosa* among the Gram negative organism. The isolation was 66 %. This percentage of isolation was lesser than report of *Escherichia coli* isolation in their study showed about 50 % of *Escherichia coli Proteus mirabilis* comprised about 60% of isolation. Our result showed about 40 % of *Candida albicans* and *Aspergillus fumigatus* comprised about 26% of isolation The result was compatible with the frequency of isolation was lesser than microorganism identified diabetic foot infection. The Carvacrol an active medicinal plant in *Origanum vulgare* and Ampicilin shows maximum activity than other antibiotics, Amikacin, Gentamcin and Ceftriaxone against *S. aureus*. The major active compound Carvacrol was present as effective antimicrobial compound in herbal medicinal plant, *Origanum vulgare*. It may be shows the maximum antibacterial activity.

The major active compound carvacrol was present as effective antimicrobial active compound Carvacrol in herbal medicinal plant *Origanum vulgare.* It may be shows the maximum antibacterial activity. *Pseudomonas aeruginosa* were found to be highly sensitive to carvacrol an active compound medicinal plant in *Origanum vulgare* compared with Amikacin, Ciprofloxacin and moderately sensitive to Gentamicin, Ceftazidime, compared to carvacrol an active medicinal plant in *Origanum vulgare.* Carvacrol an active compound medicinal plant in *Origanum vulgare* shows maximum zone of inhibition was observed in Piperacillin Tazobactam, Ertapenem, Ciprofloxacin, Tetracycline against *Proteus mirabilis, Escherichia coli* highly sensitive for Carvacrol an active compound medicinal plant in *Origanum vulgare* than compared tetracycline followed by ampicillin, streptomycin and polymyxin-B.

The isolated fungus were tested for their susceptibility of different antibiotics, which was compared with carvacrol an active medicinal plant in *Origanum vulgare. Aspergillus fumigatus* were found to be highly sensitive to carvacrol an active compound medicinal plant in *Origanum vulgare* compared with Fluconazole, Itraconazole, Amphotericin - B, moderately sensitive to Ketoconazole, compared to carvacrol an active medicinal plant in *Origanum vulgare.* The Carvacrol an active compound medicinal plant in *Origanum vulgare* shows maximum zone of inhibition was observed in Fluconazole, Ketoconazole, Amphotericin - B, Itraconazole against *Candida albicans.*

Our results showed the wide variation in the antimicrobial attributes of the oils with inhibition diameters ranging from 8 to 72 mm. Considered as an economical source, this study could provide useful information on the utilization of *Thymus* and *Origanum* oils as natural antimicrobial preservatives in food and pharmaceutical systems. These essential oils may find industrial applications as natural preservatives and conservation agents in the food and/or cosmetic industries, and as active ingredients in medical preparations. Oregano and thyme have been studied widely for their antimicrobial activity, due to the higher content of phenolic com pounds. The results showed that the activity of the oils could be attributed, to a considerable degree, mostly to the existence of carvacrol and thymol. Essential oils with high concentrations of thymol and carvacrol, e.g. oregano and thyme, usually in hibit patho genic bacteria (Nevas *et al.,*. 2004, Bozin *et al.,* 2006, Rota *et al*., 2008). The antimicrobial nature of the essential oils that have been studied is apparently related to their high phenolic contents, particularly carvacrol and thymol, and this finding is in agreement with a previous report (Cosentino *et al*., 1999; Boyraz and Özcan., 2006). The inhibitory activity of thyme and oregano is mainly due to a phenolic constituent (carvacrol 30.0%, 47.5%, and 54.6%). Th e same correlation was also confi rmed for oils that are only rich in carvacrol (Santoyo *et al*., 2006). Also, the inhibition of the growth of several pathogens by carvacrol was reported in various articles (Sivropoulou *et al*., 1996, Ultee *et al*., 2002). Essential oils that are rich in phenolic compounds, such as carvacrol, are widely reported to possess high levels of antimicrobial activity (Aligiannis *et al*., 2001, Baydar *et al*., 2004), which has been confirmed and extended by the present studies.

The current results also show that gram-positive bacteria were more aff ected than gram-negative ones. On the other hand, yeast and fungi were more sensitive than bacteria, and all essential oil samples were more effective than positive control (antibiotic discs). The essential oils of oregano (*Origanum acutidens* and *Origanum rotundifolium*) and thyme (*Thymus sipyleus* subsp. *sipyleus* var. *rosulans*) may be suggested as a new potential sources of a natural antimicrobial for the food industry after testing the toxic and irritating effects on humans. Therefore, further studies are necessary to estimate the potential for utilizing oregano and thyme essential oils as additives for extending the safety and shelf-life of food products.

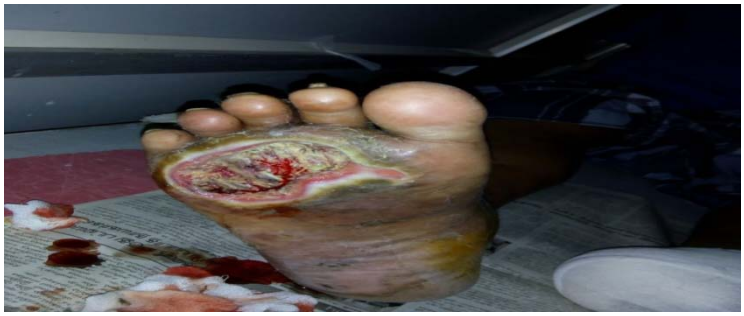
CONCLUSION

Carvacrol an active compound medicinal plant in *Origanum vulgare* shows maximum antibacterial activity it indicate presence of antibacterial compound majorly and also it has no side effect so Carvacrol may developed as commercial antibiotic with proper purification.

Table-1: Number Of Diabetic Foot Infected Patient According To Sex

SWAB SAMPLE	MALE	FEMALE
30	18	12

Diabetic foot infection



CHARACTERIZATION OF STAPHYLO *COCCUS AUREUS*

Microscope : Gram positive with more pus cells
Coloney Morphology : Circular convex smooth golden yellow coloney in nutrient agar Beta haemolytic coloney blood yellow colouration in mannitol salt agar

Table-2: Biochemical Characterzation of Staphylo *Coccus Aureus*

NAME OF THE TEST	RESULT
Catalase	Positive
Oxidase	Negative
Coagulase	Positive
Indole	Negative
Methyl red	Positive
Voges Proskauer	Positive
Citrate	Positive
Urease	Positive
Gelatinase	Positive
Nitrate	Positive
Mannitol fermentation	Positive

CHARACTERIZATION OF *PSEUDOMONAS AERUGINOSA*

Microscope : Gram negative rod with more pus cells
Motility : Motile
Cultural characters : Large opaque irregular colonies with a distinctive musty or earth smell
On Nutrient agar : Green colour coloney with diffused Pigmentation
On MacConkey agar : Forming NFL colonies
On Blood agar : Beta haemolytic colonies
Triple sugar iron : Alkaline butt alkaline slant

Table-3: Biochemical Characterzation of *Pseudomonas Aeruginosa*

NAME OF THE TEST	RESULT
Catalase	Positive
Oxidase	Positive
Coagulase	Not Done
Indole	Negative
Methyl red	Positive
Voges Proskauer	Negative
Citrate	Positive
Urease	Positive
Gelatinase	Positive
Nitrate	Positive

CHARACTERIZATION OF *PROTEUS MIRABILIS*

Microscope : Gram negative rod with more pus cells
Motility Test : Motile
Cultural Characters : Fishy or seminal odour
On nutrient agar : Pale colour swarming growth
On MacConkey agar : Smooth colour less separate colonies
On Blood agar : Non haemolytic colonies
Triple sugar iron : Acid butt alkaline slant gas Positive H2s (+)

Table-4: Biochemical Characterzation of *Proteus Mirabilis*

NAME OF THE TEST	RESULT
Catalase	Positive
Oxidase	Negative
Coagulase	Not Done
Indole	Negative
Methyl red	Positive
Voges Proskauer	Negative
Citrate	Positive
Urease	Positive
Gelatinase	Positive
Nitrate	Positive
Sugar fermentation Glucose Lactose Sucrose Mannitol	 + - - -

CHARACTERIZATION OF ESCHERICHIA *COLI*

Microscope : Gram negative straight rod with a few pus cells
Motility Test : Motile
Cultural Characters : The colonies are large thick grayish white moister smooth opaque or partially translucent discs
On nutrient agar : Large circular convex smooth white moist colonies
On MacConkey agar : Pink colonies due to lactose fermentation
On Blood agar : Non haemolytic colonies
Triple sugar iron : Acid butt alkaline slant gas production

Table-5: Biochemical Characterzation of Escherichia Coli

NAME OF THE TEST	RESULT
Catalase	Positive
Oxidase	Negative
Coagulase	Not Done
Indole	Positive

Methyl red	Positive
Voges Proskauer	Negative
Citrate	Negative
Urease	Negative
Gelatinase	Positive
Nitrate	Positive
Sugar fermentation Glucose Lactose Sucrose Mannitol	 + + - +

Table-6: Bacteria And Fungus Isolated From Diabetic Foot Infection

NAME OF THE ANTIBIOTIC	ZONE OF INHIBITION(MM)
Ampicillin	27 ±1.24 (S)
Amikacin	25 ± 0.82 (S)
Gentamicin	26± 0.68 (S)
Ceftriaxone (CTR)	25± 1.40 (S)
Carvacrol	27 ± 1.35 (S)

Table-7: Antibiotic Sensitivity For *Staphylo Coccus Aureus*

BACTERIA AND FUNGUS TYPES	NUMBER OF THE CASES	PERCENTAGE (%)
Staphylococcus aureus	25	83 %
Pseudomonas aeruginosa	20	66%
Proteus mirabilis	18	60 %
Escherichia coli	15	50 %
Candida albicans	12	40%
Aspergillus fumigatus	08	26%

(S) - Sensitive (I) - Intermediate (R) - Resistance
Results are expressed as mean ± S.E.M [n=6]. One-way ANOVA followed by post hoc test LSD. The results were compared with a Name of the antibiotic b Zone of inhibition(mm). Values are statistically significant at P<0.05.

Table-8: Antibiotic Sensitivity For Pseudomonas Aeruginosa

NAME OF THE ANTIBIOTIC	ZONE OF INHIBITION(MM)
Amikacin	21±0.73 (S)
Gentamicin	25± 0.84 (S)
Ciproflaxcin	21± 0.67 (I)
Ceftazidime	25±1.26 (S)
Carvacrol	26±1.34 (S)

(S) - Sensitive (I) - Intermediate (R) - Resistance
Results are expressed as mean ± S.E.M [n=6]. One-way ANOVA followed by post hoc test LSD. The results were compared with a Name of the antibiotic b Zone of inhibition(mm). Values are statistically significant at P<0.05.

Table-9: Antibiotic Sensitivity For Proteus Mirabilis

NAME OF THE ANTIBIOTIC	ZONE OF INHIBITION(MM)
Ciprofloxacin	`24± 0.26 (S)
Tetracycline	24± 0.27 (S)
Ertapenem	26± 1.28 (S)
Piperacillin Tazobactam	27 ± 1.25 (S)
Carvacrol	26± 1.35 (S)

(S) - Sensitive (I) - Intermediate (R) - Resistance
Results are expressed as mean ± S.E.M [n=6]. One-way ANOVA followed by post hoc test LSD. The results were compared with a Name of the antibiotic b Zone of inhibition (mm). Values are statistically significant at P<0.05.

Table-10: Antibiotic Sensitivity For Escherichia Coli

NAME OF THE ANTIBIOTIC	ZONE OF INHIBITION(MM)
Streptomycin	20±0.64 (S)
Ampicillin	25±1.27 (S)
Polymyxin-B	20±0.73 (S)
Tetracycline	25± 0.86 (S)
Carvacrol	26±1.47 (S)

S) - Sensitive (I) - Intermediate (R) - Resistance
Results are expressed as mean ± S.E.M [n=6]. One-way ANOVA followed by post hoc test LSD. The results were compared with a Name of the antibiotic b Zone of inhibition(mm). Values are statistically significant at $P<0.05$.

Table-11: Characterization of Aspergillus *Fumigatus*
Lacto phenol cotton blue mount in Microscopic examination

S.NO	CHARACTERISTIC FEATURE	RESULT OBSERVED
Lacto phenol cotton blue mount in Microscopic examination		
1.	**Colonies**	➢ Mature colonies have distinct margins ➢ They show blue green or green brown shade with velvety texture ➢ Occasionally colonies appears floccose or radically folded ➢ Surface is powdery or granular ➢ Large quantities of pigmented spore produced ➢ White apron is seen along the edge of active growth ➢ Can grow at a temperature up to 50◦C ➢ Hyaline septate hyphae are produced
2	**Conidial head**	➢ Conidial head is columnar and compact ➢ Measure 200-400 µm in length and 50 µm in width
3	**Conidiophore**	➢ Smooth and may reach up to 300 - 500 µ(I)×5-8 µ(d) ➢ Terminates in to club or flask shaped vesicle ➢ Appears green
4	**Vesicle**	➢ They are club or flask shaped ➢ Measure 20-30 µm in diameter ➢ Appears green
5	**Sterigmata**	➢ Produced on the upper half of the vesicle ➢ Present in single series (uniseriate) ➢ Measures 5-10 µm ×2-3 µm ➢ Appears green in colour and crowded ➢ The axis of the sterigmata is roughly parallel to that of conidiophores ➢ Sterigmata produced conidia in chains
	Conidia	➢ Nearly globose (2-5µ) ➢ They are green and echinulate
Colony morphology		
6	**Sabouraud's Dextrose Agar**	The surface is velvety downy or powdery showing various shades of green most commonly a blue green with a narrow white border. The colour typically darkens with age.

Table-12: Morphology Characterization of *Candida Albicans*

S.NO	CHARACTERISTIC FEATURE	RESULT OBSERVED
Microscopic examination		
1.	Grams staining	Gram positive oval yeast cells
2.	Lacto phenol cotton blue mount	Blue stained budding yeast cells
Colony morphology		
3.	Sabouraud's Dextrose Agar	White to cream colored, smooth, convex opaque, grey white colonies

Table-13: Biochemical Characterization of Candida *Albicans*

BIOCHEMICAL TEST	RESULTS
Germ tube test	Positive
Carbohydrate fermentation test	Ferments glucose and maltose
Sugar assimilation test	Assimilation glucose, maltose, and lactose
Urease Test	Negative
Chlamydospore formation	Positive

Table-14: Antibiotic Sensitivity For *Aspergillus Fumigatus*

NAME OF THE ANTIBIOTIC	ZONE OF INHIBITION(MM)
Fluconazole	25±0.65 (S)
Ketoconazole	23±1.28 (S)
Itraconazole	24±0.75 (S)
Amphotericin -B	24± 0.83 (S)
Carvacrol	27±1.45 (S)

S) - Sensitive (I) - Intermediate (R) - Resistance
Results are expressed as mean ± S.E.M [n=6]. One-way ANOVA followed by post hoc test LSD. The results were compared with a Name of the antibiotic b Zone of inhibition (mm). Values are statistically significant at $P<0.05$.

Table-15: Antibiotic Sensitivity For *Candida Albicans*

NAME OF THE ANTIBIOTIC	ZONE OF INHIBITION(MM)
Fluconazole	27±0.54 (S)
Ketoconazole	26±1.25 (S)
Itraconazole	22±0.75 (S)
Amphotericin -B	25± 0.83 (S)
Carvacrol	26±1.45 (S)

S) - Sensitive (I) - Intermediate (R) - Resistance
Results are expressed as mean ± S.E.M [n=6]. One-way ANOVA followed by post hoc test LSD. The results were compared with a Name of the antibiotic b Zone of inhibition (mm). Values are statistically significant at $P<0.05$.

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Source of support: Nil, Conflict of interest: None Declared