

**EFFECT OF SYZYGIUM AROMATICUM FLOWER BUDS OIL AGAINST AEROBIC BACTERIA  
ISOLATED FROM DENTAL INFECTION**

**REVIEW ARTICLE**

**K. GUNASEKARAN<sup>1</sup>, A. HEMALATHA<sup>1</sup> AND T. KOKILAN<sup>1</sup>**

**<sup>1</sup>PG and Research Department of Microbiology,  
Kanchi Shri Krishna College of Arts and Science,  
Kilami, Kancheepuram-631551, (T.N.), India.**

**Dr. K. UMASANKAR<sup>2</sup>**

**<sup>2</sup>Assistant Professor, PG and Research Department of Microbiology,  
Kanchi Shri Krishna College of Arts and Science, Kilami, Kancheepuram-631551, (T.N.), India.**

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

---

**ABSTRACT**

*Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels. The onset of symptoms is rapid in type 1 diabetes, and includes the classic triad of polyphagia, polydipsia and polyuria, as well as weight loss, irritability, drowsiness and fatigue. Symptoms of type 2 diabetes develop more slowly, and frequently without the classic triad; rather, these patients may be obese and may have pruritus, peripheral neuropathy and blurred vision. Collection of sample diabetic patients included in this study was of age group (35-45). The dental 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 aerobic 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.*

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

---

**INTRODUCTION**

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and failure of various organs, especially, Eyes, Kidneys, Nerves, Heart, Blood vessels (American Diabetes Association., 2005). DM results when one of the following conditions occurs: insulin released from the pancreas is impaired or insulin action in peripheral tissues is impaired (Golla *et al.*, 2004). A deficiency in insulin or a problem with its metabolic activity can result in an increased blood glucose level (ie, hyperglycemia). Hyperglycemia leads to an increase in the urinary volume of glucose and fluid loss, which then produces dehydration and electrolyte imbalance (Guthrie and Guthrie., 2004). Based on the pathogenic processes, four types of diabetes are identified (Gray and Rahilly., 1994).

- Type 1 diabetes: 5% of diabetics.
- Type 2 diabetes: 90% of diabetics.
- Gestational diabetes.
- Other: caused by various metabolic disorders, drugs or surgery.

---

**Corresponding Author: Dr. K. Umasankar**

**Assistant Professor, PG and Research Department of Microbiology,  
Kanchi Shri Krishna College of Arts and Science, Kilami, Kancheepuram-631551, (T.N.), India.**

The onset of symptoms is rapid in type 1 diabetes, and includes the classic triad of polyphagia, polydipsia and polyuria, as well as weight loss, irritability, drowsiness and fatigue (Gray and Rahilly., 1994). Symptoms of type 2 diabetes develop more slowly, and frequently without the classic triad; rather, these patients may be obese and may have pruritus, peripheral neuropathy and blurred vision. Opportunistic infections, including oral and vaginal candidiasis, can be present. Adults with long-standing diabetes, especially those with poorly controlled hyperglycemia, may develop microvascular and macrovascular conditions that can produce irreversible damage to the eyes (retinopathy, cataracts), kidneys (nephropathy), nervous system (neuropathy and paresthesias), and heart (accelerated atherosclerosis), as well as recurrent infections and impaired wound healing. Gestational DM is defined as any degree of glucose intolerance with onset or first recognition during pregnancy (Lalla and Ambrosio, 2001). Management and treatment of diabetes, Diet, Exercise, Oral anti-diabetic drugs, Sulfonylureas, Biguanides, Thiazolidinediones, Alpha-glucosidase inhibitors, Peptide analogs, Injectable Incretin mimetics, Dipeptidyl Peptidase-4, Inhibitors (Umasankar.*etal.*,2015).

Biological properties of *Syzygium aromaticum* flower buds oil traditional medicine plant active compound oleanolic acid present in Anti-diabetic activity, Antimicrobial activity in eugenol, Anticancer activity (Castellano *et al.*, 2013, Horiuchi *et al.*, 2007, Feng *et al.*, 2009) anti diabetic oxidative stress activity (Umasankar *et al.*,2018) anti immunomodulative activity (Umasankar *et al.*,2013), Antineoplastic activity (Christina *et al.*,2017). Pharmacological activities in Anti -viral activity, Chemo- reventive, Hepato-protective activity, Anti-oxidant activity, Anti-inflammatory activity, Anti-platelet activity, Anti-stress activity, Anti-pyretic effect, Anaesthetic effect, Aphrodisiac, Mosquito repellent, Insecticidal activity (Parle Milind and Khanna Deepa., 2001). Clove is known to possess antibacterial properties and is used in various dental creams, tooth pastes, mouth washes, and throat sprays to cleanse bacteria. It is also used to relieve pain from sore gums and improves overall dental health. In dentistry, eugenol in combination with zinc oxide is used for temporary filling of cavities. Clove is an anodyne (an agent that soothes or relieves pain) for dental emergencies (Cai and Wu., 1996). Cloves are aphrodisiac (an agent for arousing or increasing sexual desire or potency).Clove is used as an anti-inflammatory agent, due to its high content of flavonoids. Aroma therapists use pure clove oil to cure the symptoms of rheumatism and arthritis.

## MATERIALS AND METHODS

Collection of sample diabetic patients included in this study was of age group (35-45). The dental 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 *Syzygium aromaticum* flower buds oil was bought from Aroma product Pvt. Ltd, Chennai in Tamilnadu.

**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, 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 35 Sample diabetics dental infected patients had been collected from Government Hospital in Kanchipuram 20 samples were collected from male and 15 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 no. 4 and 5 and antibiotic sensitivity in table number 9 and 10. The immune system of diabetic patients is compromised, and chances of infection and inflammatory diseases are more (Chawla., 2012). Most of the diabetic patients suffer from oral diseases. (Arrieta *et al.*,2003) found high gingival index, loss of insertion and gingival recession in diabetic patients as compare to the control population. Another study was conducted in Ethiopia in which Bahru and Abdu found that 79% diabetic patients were suffering from dental carries, 70.5% from gingival recession and 65.7% from inflammation. They also found that females had higher loss of teeth (Bahru and Abdu., 1992).According to (Hintao *et al.*, 2007).

The most commonly isolated pathogen was *Staphylococcus aureus*, the isolated pathogen was about 80 % the result was compatible with the findings of microorganism. The present study revealed the predominance of *Pseudomonas aeruginosa* among the Gram negative organism. The isolation was 60 %. This percentage of isolation was lesser than report of *Escherichia coli* isolation in their study. Our result showed about 20 % of *Escherichia coli* and *Proteus mirabilis* comprised about 40% of isolation. The result was compatible with the frequency of isolation was lesser than microorganism identified diabetic dental infection. The isolated bacteria were tested for their susceptibility of different antibiotics, which was compared with syzygium aromaticum flower buds oil. The syzygium aromaticum flower buds oil and ampicillin shows maximum activity than other antibiotics, amikacin, gentamicin, and vancomycin against *S. aureus*. The major active compound eugenol was present as effective antimicrobial compound in herbal medicinal plant, syzygium aromaticum. It may be shows the maximum antibacterial activity.

*Pseudomonas aeruginosa* were found to be highly sensitive to syzygium aromaticum flower buds oil compared with amikacin, gentamicin, ceftazidime and moderately sensitive to ciprofloxacin compared to syzygium aromaticum flower buds oil. The maximum zone of inhibition was observed in Syzygium aromaticum flower buds oil. The syzygium aromaticum flower buds oil shows maximum zone of inhibition was observed in Ciprofloxacin, Tetracycline against *Proteus mirabilis*, *Escherichia coli* highly sensitive for syzygium aromaticum flower buds oil than tetracycline followed by ampicillin, streptomycin and polymyxin-B.

The presents studies result indicate that, syzygium aromaticum flower buds oil has antibiotic potential and compared with commercial available antibiotics, Amoxycillin, Cefotaxime Ciprofloxacin, Tetracycline, Amikacin, Gentamicin, Vancomycin, Methicillin, Ceftazidime, Streptomycin and Polymyxin-B shows maximum activity than antibiotics against all test pathogens. Also the syzygium aromaticum flower buds oil has most effective compound, eugenol and antidiabetic compound oleanolic acid. So this oil may use as antibiotics with proper purification and development and may develop therapeutic options for treatment of targeting multidrug resistant organisms.

Cloves represent one of the Mother Nature's premier antiseptic. Clove oil found to be more effective than sodium propionate standard food preservative against some food borne microbes. Clove oil was found to be very effective against *staphylococcus species*. Amongst the fungi *Aspergillus niger* was found to be highly sensitive to the clove oil. Essential oil of clove dispersed 0.4% v/v in a concentration sugar solution, had a germicidal effect against various bacteria *S.aureus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Clostridium perfringens*, *E.coli* and *Candida albicans* (Bariozzo *et al.*, 1989). Clove has been found that a 0.05% solution of eugenol is sufficient to kill bacillus tuberculosis. Clove oil showed antimicrobial activity against some human pathogenic bacteria resistance to certain antibiotics (Lopez *et al.*, 2005). Oleanolic acid is the anti diabetic, anticancer active (Castellano *et al.*, 2013, Horiuchi *et al.*, 2007, Feng *et al.*, 2009) Clove oil and its main component eugenol show considerable antifungal activity against *Candida Aspergillus* and *dermatophyte species*. It also shows activity against clinically relevant fungi including fluconazole - resistant strains (Pinto *et al.*, 2009).

## CONCLUSION

Syzygium aromaticum flower buds oil shows maximum antibacterial activity it indicate presence of antibacterial compound majorly and also it has no side effect so Syzygium aromaticum flower buds oil may developed as commercial antibiotic with proper purification and clinical trail.

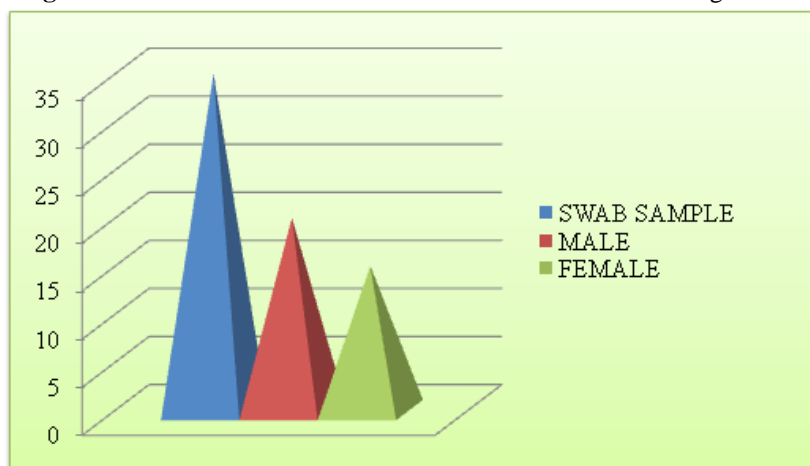
**Plate-1:** Dental Infection Diabetes



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

SWAB SAMPLE	MALE	FEMALE
35	20	15

**Figure-1:** Number Of Diabetic Dental Infected Patient According To Sex



#### CHARACTERIZATION OF *STAPHYLO COCCUS AUREUS*

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

**Table-2:** Biochemical Characterization 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 colony 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 Characterization of *Pseudomonas Aeruginosa*

NAME OF THE TEST	RESULT
Catalase	Positive
Oxidase	Positive
Coagulase	Not Done
Indole	Negative
Methyl red	Negative
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 H <sub>2</sub> S (+)

**Table-4:** Biochemical Characterization 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

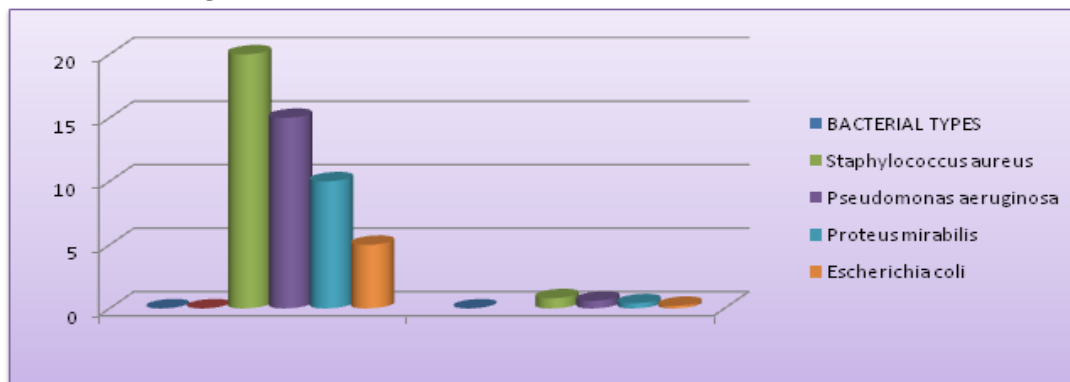
**Table-5:** Biochemical Characterization Of *Escherichia Coli*

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:** Aerobic Bacteria Isolated From Diabetic Dental Infection

BACTERIAL TYPES	NUMBER OF THE CASES	PERCENTAGE (%)
<i>Staphylococcus aureus</i>	20	80 %
<i>Pseudomonas aeruginosa</i>	15	60 %
<i>Proteus mirabilis</i>	10	40 %
<i>Escherichia coli</i>	5	20 %

**Figure-2:** Aerobic Bacteria Isolated From Diabetic Dental Infection



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

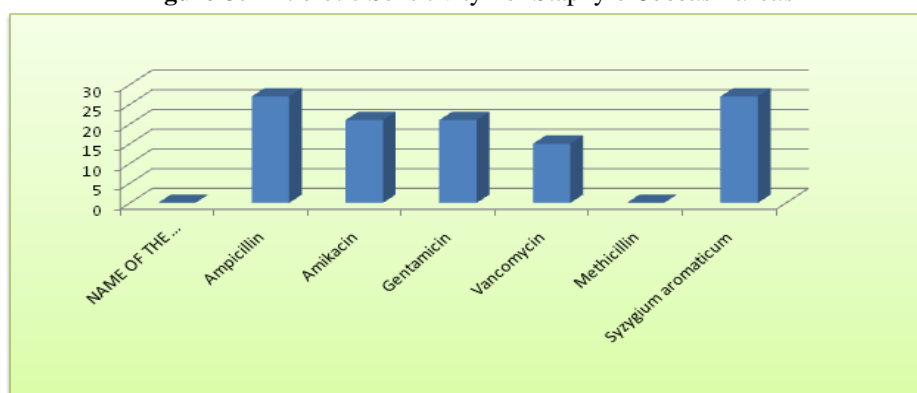
NAME OF THE ANTIBIOTIC	ZONE OF INHIBITION(MM)
Ampicillin	27 ± 1.24 (S)
Amikacin	21 ± 0.82 (S)
Gentamicin	21 ± 0.68 (S)
Vancomycin	15 ± 0.70 (S)
Methicillin	Resistant
Syzygium aromaticum	27 ± 1.35 (S)

(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.

**Figure-3:** Antibiotic Sensitivity For *Staphylo Coccus Aureus*



**Table-8:** Antibiotic Sensitivity For *Pseudomonas Aeruginosa*

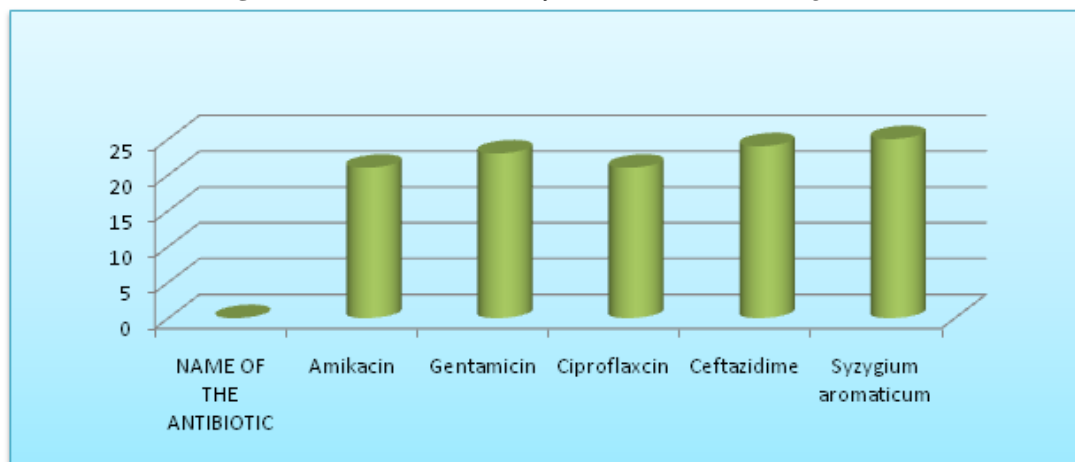
NAME OF THE ANTIBIOTIC	ZONE OF INHIBITION(MM)
Amikacin	21 ± 0.73 (S)
Gentamicin	23 ± 0.84 (S)
Ciproflaxcin	21 ± 0.67 (I)
Ceftazidime	24 ± 1.26 (S)
Syzygium aromaticum	25 ± 1.34 (S)

(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  $P < 0.05$ .

**Figure-4:** Antibiotic Sensitivity For *Pseudomonas Aeruginosa*



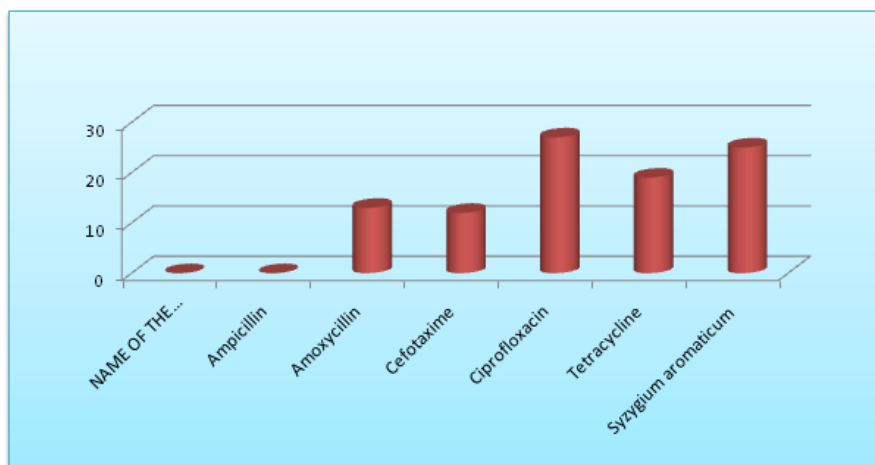
**Table-9:** Antibiotic Sensitivity for *Proteus Mirabilis*

NAME OF THE ANTIBIOTIC	ZONE OF INHIBITION(MM)
Ampicillin	12± 0.25 (R)
Amoxycillin	13± 0.28 (R)
Cefotaxime	12±0.52 (R)
Ciprofloxacin	27± 1.26 (S)
Tetracycline	19 ± 0.75 (I)
Syzygium aromaticum	25± 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  $P < 0.05$ .



**Figure-5:** Antibiotic Sensitivity For *Proteus Mirabilis*

**Table-10:** Antibiotic Sensitivity For *Escherichia Coli*

S.NO.	NAME OF THE ANTIBIOTIC	ZONE OF INHIBITION(MM)
1	Streptomycin	19±0.64 (S)
2	Ampicillin	20±1.27 (S)
3	Polymyxin-B	19±0.73 (S)
4	Tetracycline	20± 0.86 (S)
5	Syzygium aromaticum	24±1.47 (S)

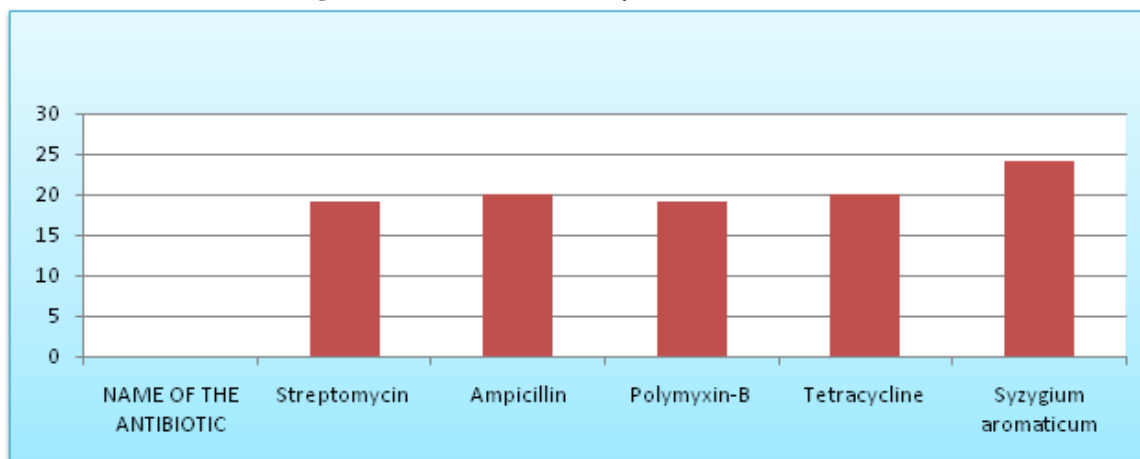
(S) - Sensitive (I) - Intermediate (R) - Resistance

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  $P < 0.05$ .

**Figure-6:** Antibiotic Sensitivity For *Escherichia Coli*



## REFERENCES

- American Diabetes Association (2005).Diagnosis and classification of diabetes mellitus.Diabetes Care. 28 (Suppl 1):S37- S42.
- Arrieta BJJ, Bartolomé VB, Jiménez ME, Saavedra VP and Arrieta BFJ (2003). Academia Iberoamericana de Patologíay Medicina Bucal,8(4): 233-247.
- Bahru Y and Abdu SS (1992).Ethiopian Medical Journal, 30(2): 95-103.
- Bariozzo J, Nunez L, chirife J, Herszage L, D, Aquino M (1989).Antimicrobial activity of clove oil dispersed in a concentrated sugar solution .J,Appl.Bacteriol. 1989; 66 (1) 69-75.
- Cai L, and Wu C.D (1996). Compounds from *Syzygium aromaticum* possessing growth inhibitory activity against oral pathogens. *J. Nat. Prod.* 59(10): 987-990.
- Castellano JM, Guinda A, Deloado T, Rada M, Cavuela J(2013). A Biochemical basis of the antidiabetic activity of oleanolic acid and related pentacyclic triterpenes. *J Diabetes* 62(6):1791-9.
- Chawla R(2012). Complications of Diabetes. New Delhi: Jaypee Brothers Medical Publishers
- Christina, K. J., alwin Nambikkairaj, Umasankar, K. and Ramya, D.R (2017). Anti neoplastic effect of selected essential oils toxicity in albino wistar rats. *International Journal of Current Research*, Dec.Vol. 9, Issue, 12, pp.63120 -63122.
- Feng JH, Chen W, Zhao Y, Ju XL (2009).Antitumor activity of Oleanolic, Ursolic and Glycyrrhetic acid. *The Open Nat Prod J* 2:48-52.
- Feng JH, Chen W, Zhao Y, Ju XL (2009).Antitumor activity of Oleanolic, Ursolic and Glycyrrhetic acid. *The Open Nat Prod J* 2:48-52.
- Golla K, Epstein JB, Rada RE, Sanai R, Messieha Z and Cabay RJ (2004).Diabetes mellitus: an updated overview of medical management and dental implications. *General Dentistry*, 52(6):529-35.
- Gray H and Rahilly SO (1994).Towards improved glycemic control in diabetes. What's on the horizon, *Arch Intern Med*.155:1137?
- Guthrie RA and Guthrie DW (2004). Pathophysiology of diabetes mellitus. *Crit Care Nurs.* Apr-Jun; 27(2): 113-25.
- Gutr. Leonard Hills (Books) Ltd., London, 1959.
- Hintao J, Teanpaisan R, Chongsuvivatwong V, Dahlen G and Rattarasarn C (2007). Community Dentistry and Oral Epidemiology, August; 35(4): 302-309.
- Horiuchi K, Shiota S, Hatano T, Yoshida T, Kuroda T and Tsuchiva T (2007). Antimicrobial activity of oleanolic acid from *Salvia officinalis* and related compounds on vancomycin-resistant *enterococci* (VRE). *J Biol Pharm Bull* 30 (6):1147-9.
- Lopez P,Sanchez C, Batle B, Nerin C (2005).Solid and vapour phase antimicrobial activites of six essential oils: susceptibility of selected food borne bacterial and fungal strains, *journals of Agriculture and Food Chemistry*.2005; 53:6338-6346.
- Parle Milind and Khanna Deepa (2001). Clove a champion spice.*IJRAP*, 2(1), 47-54.
- Pinto E, Valer- Silva L. Caveleiro C and Salguero E (2009). Antifungal activity of clove essential oil from *syzygium aromaticum* on candida, *Aspergillus* and dermatophyte species. 58: 1454- 1462.
- Umasankar K, Ramya D. R. and Balwin Nambikkairaj (2015). Antidiabetic and antidyslipidemic nature of carvacrol, a monoterpenic phenol studied in high-fat -fed and low-dose streptozotocin-induced experimental diabetic rats, *Journal of Pharmacy Research*, 9(8), 484-490.



21. Umasankar.K, Balwin Nambikkairaj, and Manley Backyavathy.D (2013). Effects of *Syzygium aromaticum* and *Rosadamascena* In Anti Oxidative Stress Activity on Diabetes Induced Rats International journals of pharmaceutical archive (IJPA), Impact factor 0.40 2319 – 7226, 2-73.
22. Umasankar.K,\* Balwin Nambikkairaj (2018). Effects on the selected herbal plant syzygiumaromaticum flower buds oil of immunomodulatory activity in albino wistar rats. International journals of science and Research (IJSR) Impact factor 7.296 7(7) ISSN2319-7064.

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

**[Copy right © 2020. This is an Open Access article distributed under the terms of the International Journal of Pharmaceutical Archive (IJPA), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.]**