WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 11, Issue 2, 2282-2302. Research Article ISSN 2277– 7105

EVALUATION OF ANTIBACTERIAL ACTIVITY OF TULSI AND POMEGRANATE GEL FOR ORAL BACTERIA

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Article Received on
20 Dec. 2021,

Revised on 10 Jan. 2022,
Accepted on 30 Jan. 2022

DOI: 10.20959/wjpr20222-23115

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ABSTRACT

Plants have been used from thousands of years to treat health diseases and to conserve food. In Ayurveda many plants have been mentioned potential medicinal values. *Punica granatum* known as Pomegranate is widely used as a folk remedy in India to treat pathogenic microbes. Nowadays, there has been an increasing interest in extracting relevant natural antimicrobial agents as a potent as the chemical antibiotic to be used as an alternative approach, Pomegranate has gained a wider attention for its potent antimicrobial activity as bacteria are getting resistance to synthetic antibiotics. *Ocimum sanctum*, sometimes known as holy basil, tulsi it is a fragrant plant native to Asia and Africa's tropics and a medicinally important plant in the Lamiaceae family. The family Lamiaceae is one of the most used medicinal plants but also with its valuable essential oils being used as spices and flavours for food products. *Ocimum sanctum's* antibacterial activity reveals that the plant has significant antimicrobial capabilities, and *Ocimum* is widely used in India. In the present study, the aqueous peel extract of *Punica granatum* and volatile oil of *Ocimum sanctum* been tested to evaluate its antimicrobial activity comparing with the standard antibiotics. [Tetracycline, Ceftriaxone, Cefotaxime]. The tulsi volatile oil, gel and *Punica granatum*, tetracycline

showed prominent antimicrobial activity with the zone of inhibition compared to the Ceftriaxone and Cefotaxime. The antimicrobial efficacy of *Ocimum sanctum* gel and *Punica granatum gel* indicates that the plant poses potent antimicrobial activity.

KEYWORDS: *Punica granatum*, *Ocimum sanctum*, volatile oil, Aqueous peel extract, Gel, Antimicrobial activity.

INTRODUCTION

Gelling agents

The agents when dissolved in a liquid phase as a colloidal mixture forms a weakly cohesive internal structure are called as gelling agents. This is may be of organic hydrocolloids or hydrophilic inorganic substances. The gelling agents are used at a concentration of 0.5-10% in semisolid dosage forms.[1] Gelling agents also function as stabilizers and thickeners to provide thickening without stiffness. polymers have been widely used as gelling agents in the semisolid dosage form, which are synthetic macromolecular polymers of acrylic acid known as Carbomers. Carbomers are most commonly used as they show high thickening ability in a wide pH range. Usually a polymer, the gel forming agent in a small concentration produces a semisolid consistency within the formulation which reduces the drainage rate of formulation and prolongs the residence time at the administration site.[2]

Eg: Natural gelling agents: Tragacanth, pectin, agar, gelatin, etc.

Synthetic gelling agents: Carbomers, poly vinyl alcohol, etc.

Semi-synthetic agents: Hydroxy propyl cellulose, Carboxy methyl cellulose, etc.[3]

Gels: The USP defines gels as a semisolid system consisting of dispersion made up of either small inorganic particle or large organic molecule enclosing and interpenetrated by liquid.[4]

Properties of gels

- It should be convenient in handling and its applications.
- To prevent from microbial attack gels should possess suitable anti-microbial activity.
- It should not affect the biological nature of drug.
- The topical gel should be tacky.
- The gelling agents should not react physically or chemically with other formulation components and should be safe.
- It should be non-toxic, compatible and inert with other additives.
- It should possess properties such as non-greasy, emollient, thixotropic, non-staining, etc.,

- Gels should be stable at storage conditions.

Classification of gel[5]

Gels can be classified based on the-

1) Based on the nature of solvent used

a) Hydrogel: It contains water as their continuous liquid phase.

Eg: Gelatin, derivatives of cellulose and Poloxamer gel.

b) Organic gel (with a non- aqueous solvent): It contains continuous phase as non- aqueous solvent.

Eg: Plastibase (low molecular weight polyethylene dissolved in mineral oil and short cooled)

c) Xerogels: They are solid gel produced by evaporation of solvent or freeze drying with low solvent concentration.

Eg: Tragacanth ribbons, Dry cellulose and polystyrene.

2) Based on colloidal system

a) Two phase system (Inorganic): If the size of the particle of the dispersed phase is relatively large and forms the 3D structure throughout the gel such a system consist of floccules of small particles rather than large molecule and structure of gel in this system is not always stable.

Eg: Aluminium Hydroxide gel USP

b) Single phase system (Organic): This system consist of large organic molecules existing on the twisted stands dissolved in continuous phase.

Eg: Carbopol, Tragacanth.

3. Based on the physical nature

a) Rigid gels: It can be formed from macromolecule in which the framework is linked by primary valance bond.

Eg: The molecules of silica gel-silic acid are held by Si-O-Si-O bond to give a polymer structure possessing a network of pores.

b) Elastic gels: Elastic behavior is exhibited by gels of agar, pectin, guar gum and alginates.

4. Based on rheological properties: The gels exhibit usually non-newtonian flow properties. They are classified into-

a) Plastic gels

Eg: flocculated suspension of Bingham bodies, Aluminium hydroxide exhibit a plastic flow and the rheogram plot gives the yield value of gels above which the elastic gel distorts and start to flow.

b) Pseudo plastic gels

Eg: Pseudo plastic flow is exhibited by liquid dispersion of tragacanth, sodium alginate. The viscosity of pseudo plastic gels decreases with the increasing rate of shear with no yield value.

c) Thixotropic gels

In these gels, the bond between the particles are very weak and can be broken down by shaking. The resultant solution will come back to gel due to the particles colliding and linking together again.

Eg: Kaolin, Agar, Bentonite.

Methods of preparation of gel

1. Cold method: In this method, the entire ingredients are mixed together to form a homogenous mass under low temperature between 4 to 10^0c. In this method polymer and penetration enhancer are mixed together to form a solution A then drug and solvent are mixed together to form a solution B and with constant stirring pour solution B into solution A.

2. Dispersion method: In this method, the polymer is dispersed over water for 2 hrs till all the polymer is soaked with water then remaining ingredients are added with stirring until a homogenous mass is formed.

3. Chemical reaction: In this method gel is produced by chemical interaction between solute and solvent.

Eg: Preparation of Silica gel.

4. Temperature effect: The most lipophilic colloid is soluble with decrease in temperature.

Eg: Gelatin, Agar is reduced. So, that when cooled, hot concentrated sol gel is produced.

5. Flocculation: In this method, Gelatin is produced by just adding sufficient quantity of salt to precipitate to produce age state but insufficient to bring about complete precipitation.

Antimicrobial properties

Ocimum sanctum contains pharmacological properties like anti-toxic, anti-oxidant, anti-cancer, anti-microbial, antihypertensive, anti-inflammatory, anticoagulant, analgesic and anti-thyroid. The essential coil is effective against gram-positive and gram-negative bacteria. The

tulsi leaf extract shows anti-oxidant activity and it inhibits the growth of *E.coli, Klebsiella, Staphylococcus aureus* and proteus.[6] The tulsi extract has great potential as anti-microbial agent for the treatment of water. This treatment is simple, cost effective, ecofriendly, reachable for all and the components present in *Ocimum sanctum* leaves have no side effects to human as it compared to chemical treatment. As the water treated with tulsi extract serve not only as germ free but also as medicinal water. The microbial activity causes serious damage to living and non-living organisms. So, tulsi is used as anti-microbial which is very effective.[7]

Extracted essential oils have also been shown to contain biologically active constituents that are insecticidal, nenaticidal and fungiastatic. Tulsi is used due it's antimicrobial activities against many pathogens and it's used as mouthwash, for wound healing and preservation of food stuff.[8,9,10] tulsi essential oil could be a valuable topical antimicrobial agent for management of skin infections caused by these organisms.[11,12,13]

Pomegranate is an ancient nutritional fruit with a wide range of biological activity because to its high concentration of natural bioactive ingredients. Pomegranate has grown in popularity in recent years as a result of its versatility and nutritional value in the human diet. The presence of tannins, flavonoids, alkaloids, and organic acids in *Punica granatum* peel has been associated with numerous of health advantages.[14,15,16]

Pomegranate has a wide range of therapeutic characteristics, including cancer treatment and prevention, cardiovascular disease treatment and prevention, diabetes treatment and prevention, and dental diseases, treatment and prevention. Infant brain ischemia, Alzheimer's disease, male infertility, arthritis and obesity are some other applications. Furthermore, pomegranate constituents applications have been used for their anti-inflammatory properties to treat dental conditions, bacterial infections, antibiotic resistance, intestinal parasites in addition to the use of antimicrobial properties of the juice against *E.coli, Bacillus subtilis*. Pomegranate peel is known for its numerous health-promoting effects as well as its apparent wound-healing abilities.

Recently, pomegranate has been an interesting subject as a medicinal agent with a wide range of therapeutic indications. Investigations presented that pomegranate may be used as a natural alternative against different microbial pathogens. Approximately, a different fraction of the pomegranate has been evaluated for antimicrobial activities. The efficacy of pomegranate

peel has been indicated in several studies. There are numerous phytochemical components in pomegranates that have been shown to have antimicrobial activity. Ellagic acid, hydrolyzable tannins, and punicalagin contain most of the activities.

MATERIALS AND METHODS

Authentication of the plant

The plant was identified and authenticated as *Punica granatum and Ocimum sanctum.*

Preparation of the fruit peel extract and tulsi volatile oil

A.1.Collection of the plant material(Pomegranate)[17,18,19]

The fresh peels of Pomegranate were collected from the region of Bangalore. The collected peels were washed thoroughly with the distilled water. The cleaned peels were shade dried for 3-4 days. Then the peels were powdered by grinding them in a grinder. Then the grounded powder was passed through Sieve No.24 to get the appropriate size of the fine particles of peel powder was obtained for better results.

2. Preparation of the extract: pomegranate peel powder (100gms) was subjected to defatting with Petroleum ether (250 ml) in a Soxhlet apparatus for around 4 hours.

3. Aqueous extraction: The defatted peel powder (50gms) was extracted with distilled water with continuous boiling until it was reduced to half of the quantity. Then it was filtered using the Buchner funnel. The filtrate was taken in a china dish and then evaporated to dryness. Then the crystals of the aqueous extract of peel powder were scraped and it was preserved by adding 1ml of alcohol as a preservative for further use.

4. Hydroalcoholic extraction: The defatted peel powder (50gms) was extracted with 70% alcohol by using the Reflux method by refluxing for 6hrs. Then the extract was transferred to a china dish and was evaporated to dryness. Then the crystals were scraped and preserved.

B. Tulsi: The tulsi leaves were collected and washed with distilled water. It is then allowed to shade dry for 3-4 days till they were completely dried. The dried tulsi leaves was subjected for the Clevenger's extraction because tulsi leaves contain the volatile oil.[20]

Formulation of the gel: 2gms of Carboxy methyl cellulose and 50ml distilled water was taken in a beaker. Then the solution was subjected to homogenization by using a homogenizer. After homogenization, the remaining ingredients like Sodium benzoate was

added for preservation, and Citric acid was used for adjusting the pH and then the above solution was homogenized to get the desired gel consistency refer table no-1.

Formula of the pomegranate and tulsi gel[21]

Table No 1: Formula of the Pomegranate gel and tulsi gel.

Sl.No.	Ingredients	Quantity Required for Pomegranate gel	Quantity Required for Tulsi gel
1	Carboxy methyl cellulose	2 gms	2 gms
2	Sodium Benzoate	0.012 mg	0.012 mg
3	Pomegranate peel extract	1 gm	-
4	Tulsi gel	-	1 ml
5	Citric acid	To adjust pH 7	To adjust pH 7
6	Distilled water	q.s. to 50ml	q.s. to 50ml

Formulation of the gel

a) Placebo: It was prepared by dissolving 2 grams of Carboxy methyl cellulose in 50 ml of distilled water and stir gently with a stirring rod for 15 to 20 minutes. Then with the help of the homogenizer mix the solution until a gel consistency is obtained. Then add 0.012 mg of Sodium Benzoate was used as a preservative and homogenize again. Citric acid was used for adjusting the pH to 7.

b) Pomegranate gel: It was prepared by adding 1 gm of fruit peel extract to a beaker containing 2gms of carboxy methyl cellulose in 50 ml of distilled water. Then 0.012mg of sodium benzoate was added as a preservative and stirred for 15-20 minutes with a stirring rod and then it was subjected to a homogenizer to homogenize all the ingredients to get a gel-like consistency. Citric acid was used for adjusting the pH to 7 refer fig 1.

c) Tulsi gel: It was prepared by dissolving 2gms of carboxy methyl cellulose in 50 ml of distilled water in a beaker and stir gently. Then add 0.012mg of sodium benzoate as a preservative and stirred for 15-20 minutes with stirring road and then it is subjected to homogenizer to homogenize all the ingredients and at last the tulsi volatile oil is added and given a homogenization to get a gel like consistency refer fig 2.

Analysis of physicochemical parameters

Physical parameters

The formulated Pomegranate gel and tulsi gel was prepared and has been subjected to different physical properties such as texture, color, odour, spreadability refer table no 2 and 3.

Chemical evaluation

The aqueous peel extract of *Punicagranatum and Ocimum sanctum* volatile oil has been subjected to phytochemical screening for evaluating different phytochemical constituents refer table no 4,5,6.

Isolation of oral bacteria from saliva

Oral bacterial sample was collected using a sterile cotton bud and dissolved into 500µl phosphate buffered saline (PBS) buffer (0.12M NaCl, 0.01M Na_2HPO_4, 5mM KH_2PO_4 [pH 7.5]). An aliquot (100µl) of bacterial suspension was spread on Agar plate. Agar media shows blue colonies in case of Streptococcus mitis, S. salivarius, S. mutans, S. sanguis and Enterococcus and subsequently cultured on fresh agar medium and identified with stain test to observe the colonies of organisms. Biochemical tests used in this study was Gram staining refer fig 3.

Preparation and sterilization of media: Ingredients in their required quantities was dissolved in an appropriate volume of distilled water. The pH of the dissolved medium was adjusted. The medium is disposed into suitable container whose mouth was then closed with the cotton plug or metal cap. The medium was then sterilized by moist heat by autoclave. All the ingredients were weighed accurately and was mixed well in a conical flask and at last water was added and was heated to 100^{o}C to dissolve all the ingredients. Then 30ml of the preparation was transferred into the conical flask and was autoclaved for sterilization. After the sterilization was completed the whole 30ml medium was transferred into a sterile petri plate aseptically. Then it was allowed for solidification.

Determination of anti-microbial activity[22,23]: The antimicrobial activity of the pomegranate peel aqueous extract and gel were evaluated using two different methods namely, Agar disk diffusion and Agar well diffusion method.

Agar disk-diffusion method: Agar plates was inoculated with standardized inoculums of the test microorganism in this well-known process. Then, filter paper discs of approximately 6mm in diameter, containing the test substance at the desired concentration, was placed on the surface of the agar. Under ideal conditions, the Petri dishes was incubated. In general, an antimicrobial substance diffuses into the agar and inhibits the test microorganism's growth and development, after which the diameters of the inhibition growth zones were determined. The results of zone of inhibition determination are mentioned in Table no.7 and Figure

no.4,5,6,7,16,17,18 and 19.

Agar well diffusion method: The antimicrobial activity of plants or microbial extracts is commonly evaluated using the agar well diffusion method. Similar to the disk diffusion method, the agar plate surface was inoculated by spreading a volume of microbial inoculum over the entire agar surface, a hole with a diameter of length 6 to 8mm was punched aseptically with a tip or sterile cork borer, and a volume (20–100 ml) of an antimicrobial agent or extract solution at the desired concentration was poured into the well. Then, depending on the test microorganism, agar plates were incubated under the appropriate conditions. The antimicrobial substance diffuses across the agar medium and inhibits the bacterial growth of the microbial strain tested. The antimicrobial zone of inhibition was measured in millimeters. The results of zone of inhibition determination are mentioned in Table no.8 and Figure no.8,9,10,11,12,13,14 and 15.

RESULTS AND DISCUSSION

RESULTS

Authentication details

The plant was identified and authenticated as *Ocimum sanctum L*. belonging to the family Lamiaceae, Ref No: Authentication/SMPU/CARI/BNG/2020-21/1253A.

The plant was identified and authenticated as *Punicagranatum L*. belonging to the family Lythraceae, Ref No: Authentication/SMPU/CARI/BNG/2020-21/1253.

at Central Ayurveda Research Institute at Uttarahalli, Kanakapura Main Road, Talaghattapura Bengaluru – 560109.

Physical parameters of the gel

Table no. 2 Physical parameters of tulsi gel.

Sl.no	Physical characters	Result of pomegranate gel	Result of tulsi gel
1.	Colour	Brown	Transparent
2.	Odour	Odourless	Characteristic
3.	Taste	Mucilaginous	Mucilaginous
4.	Appearance	Viscous	Viscous
5.	Texture	Fine	Fine
6.	Spreadability	Uniform and even	Uniform and even

Phytochemical screening

Physical properties of aqueous extract of *P. granatum* peel and tulsi volatile oil: Table no:3

PHYSICAL PROPERTIES	AQUEOUS EXTRACT OF *P. GRANATUM* PEEL	TULSI VOLATILE OIL
COLOUR	Brown colour crystal flakes	Green
ODOUR	Odourless	Characteristic
TASTE	Mucilaginous	Mucilaginous

Chemical evaluation

Table no. 4: Aqueous extract of *Punica granatum* peel.

Sl.no.	Tests	Chemical constituents	Inference
1.	Fehling's test	Carbohydrates	+++
2.	Benedict's test		++
3.	Iodine test	Non-reducing polysaccharides	-
4.	Foam test	Saponin Glycosides	+++
5.	Dragendorff's test	Alkaloids	-
6.	Hager's test		-
7.	Wagner's test		-
8.	5 % Ferric chloride test	Tannins and Phenolic compounds	+++
10.	Lead acetate test		++
11.	Acetic acid solution		-
12.	Bromine water		+
13.	Potassium Dichromate		-
14.	Dilute Iodine test		-
15.	Dilute Nitric acid test		+
16.	Dilute Potassium Permanganate solution		+
17.	Shinoda test	Flavonoids	+++

Table no 5: Hydroalcoholicextract of *Punicagranatum* peel.

Sl.no.	Tests	Chemical constituents	Inference
1.	Fehling's test	Carbohydrates	+++
2.	Benedict's test		++
3.	Iodine test	Non-reducing polysaccharides	-
4.	Salkowski reaction	Steroids	+
5.	Liebermann- Burchard reaction		+
6.	Liebermann's reaction		-
7.	Shinoda test	Flavonoids	+++
8.	Dragendorff's test	Alkaloids	-
9.	Hager's test		-
10.	Wagner's test		-

11.	5% Ferric chloride test	Tannins and phenolic compounds	+++
12.	Lead acetate test		++
13.	Bromine water		+
14.	Acetic acid solution		-
15.	Potassium dichromate		-
16.	Dilute Iodine Test		-
17.	Dilute Nitric acid test		-
18.	Dilute Potassium Permanganate solution		+

Table no. 6: Tulsi volatile oil chemical test.

Sl.no.	Tests	Inference
1	Solubility test	Soluble in 90% alcohol
2	Filter paper test	Not permanently stained with volatile oil
3	Odour	Characteristic odour

Table no 7: Antimicrobial activity of *Punica granatum* aqueous peel extract and gel comparing with the standard antibiotics by agar disk diffusion method.

Punicagranatum	**Zone of inhibition (mm)**		***Ocimum sanctum***	**Zone of inhibition (mm)**	
	Diameter	**Radius**		**Radius**	**Diameter**
Aqueous peel extract	16	8	Volatile oil	10	6
Gel	7	4	Gel	13	7
Tetracycline	6	3	Tetracycline	11	5
Cefotaxime	No Inhibition	No Inhibition	Cefotaxime	No inhibition	No inhibition
Ceftriaxone	No Inhibition	No Inhibition	Ceftriaxone	No inhibition	No inhibition

Table no 8: Antimicrobial activity *of Punica granatum* aqueous peel extract and gel *Ocimum sanctum* volatile oil and gel comparing with the standard antibiotics by agar well diffusion method.

Punicagranatum	**Zone of inhibition (mm)**		***Ocimum sanctum***	**Zone of inhibition (mm)**	
	Diameter	**Radius**		**Diameter**	**Radius**
Aqueous peel extract	16	8	Volatile oil	13	7
Gel	20	11	Gel	15	9
Tetracycline	15	8	Tetracycline	12	5
Cefotaxime	No Inhibition	No Inhibition	Cefotaxime	No Inhibition	No Inhibition
Ceftriaxone	No Inhibition	No Inhibition	Ceftriaxone	No Inhibition	No Inhibition

Fig 1: Pomegranate peel gel.

Fig 2: Tulsi gel

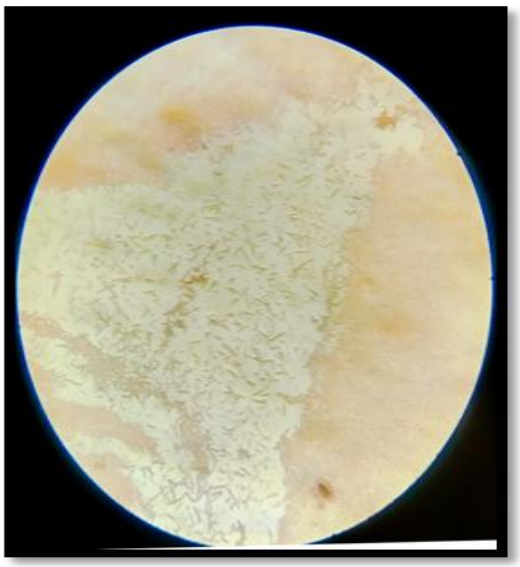

Fig 3: Isolation of oral bacteria from saliva identified as Gram negative bacteria by Gram staining.

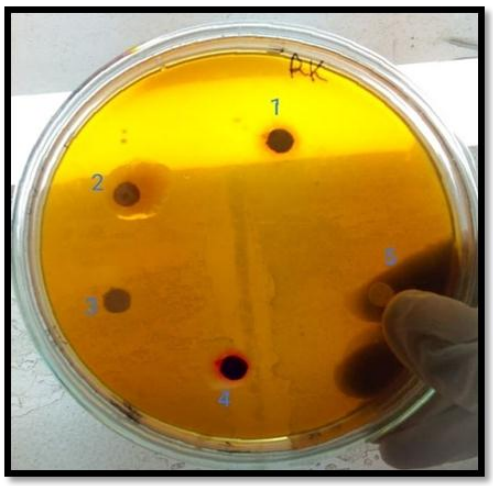

Fig 4: Antimicrobial activity of *P. granatum* aqueous peel extract and gel by Agar disk bacteria by diffusion method.

1. Pomegranate aqueous peel extract
2. Pomegranate gel
3. Cefotaxime
4. Tetracycline
5. Ceftriaxone

Fig 5: Pomegranate aqueous peel inhibition.

Fig 6: Pomegranate gel zone of inhibition.

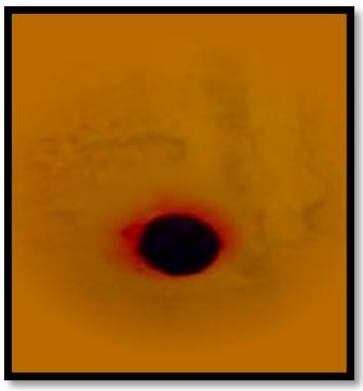

Fig 7: tetracycline zone of extract zone of inhibition.

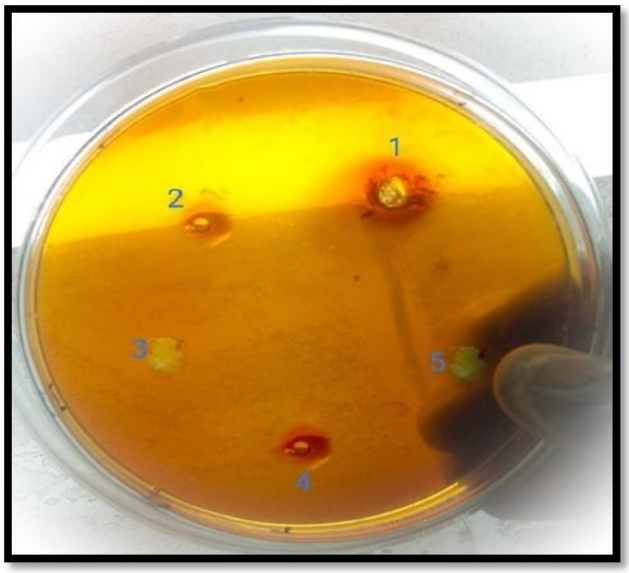

Fig 8: Antimicrobial activity of *Punica granatum* aqueous peel extract and gel by the agar well diffusion method.

1. Pomegranate gel
2. Pomegranate aqueous peel extract
3. Ceftriaxone
4. Tetracycline
5. Cefotaxime

Fig 9: Pomegranate gel Zone of inhibition.

Fig 10: Pomegranate aqueous peel extract zone of inhibition.

Fig 11: Tetracycline zone of inhibition.

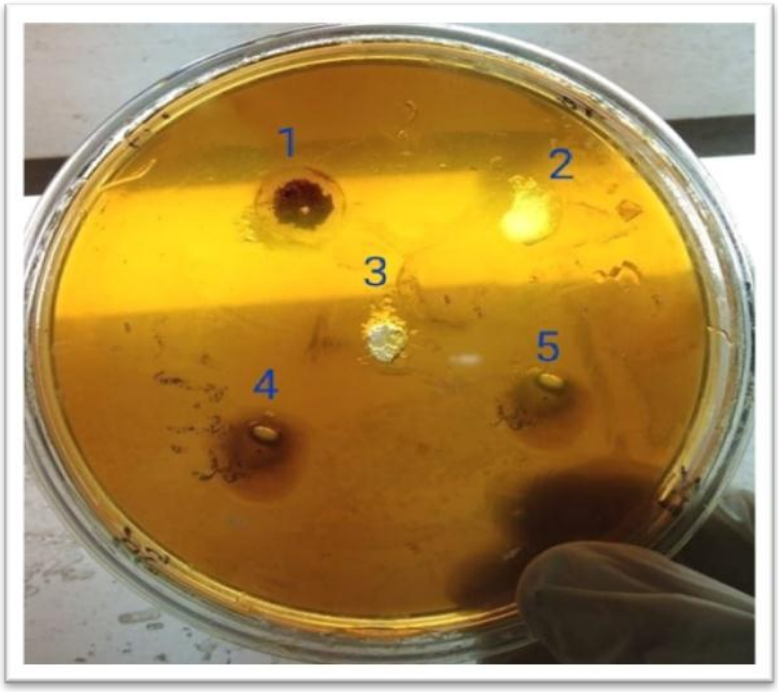

Fig 12: Antimicrobial activity of *Ocimum sanctum* by agar well diffusion method.

1.Tulsi volatile oil
2.Ceftriaxone
3.Cefotaxime
4.Tulsi gel
5. Tetracycline

Fig 13: Tulsi volatile oil Zone Of Inhibition.

Fig 14: Tetracycline zone of inhibition.

Fig 15: Tulsi gel zone of inhibition.

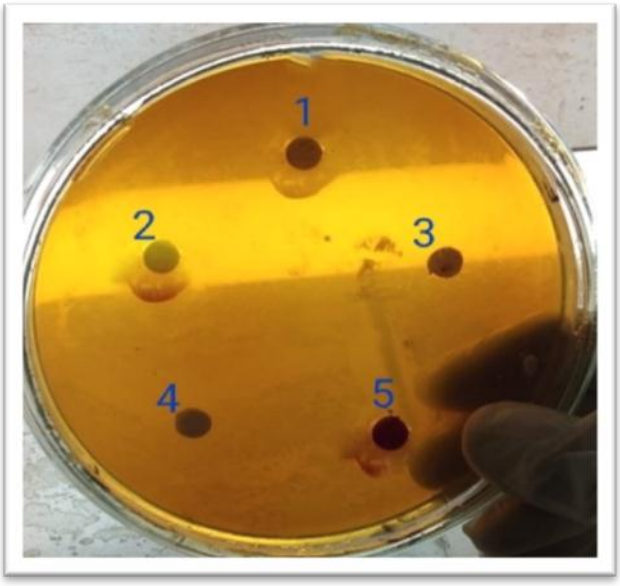

Fig 16: Antimicrobial activity of *Ocimum sanctum* by agar disk diffusion method.

1.Tulsi volatile oil 4.Cefotaxime

2. Tulsi gel
3.Ceftriaxone
5. Tetracycline

Fig 17: Tulsi gel zone of inhibition.

Fig 18: Tulsi volatile oil zone of inhibition.

Fig 19: Tetracycline zone of inhibition.

DISCUSSION

Medicinal plants are used to treat different diseases, since ancient times. The increasing resistant pattern and associated side effects of antibiotics have the evolved the importance of medicinal plants to be used as an antimicrobial agent. This property is due to the production of several secondary metabolites by different parts of the plant. Therefore, for all the present day medical complications these metabolites act as an alternative source. Among, these *P. granatum* is known for its pharmacological properties. In the present study it describes that the physicochemical parameters of *P. granatum* aqueous and hydroalcoholic peel extract showed the presence of Phenols and Tannins, and Carbohydrates and Flavonoids and the determination of antimicrobial activity of *P. granatum* peel aqueous extract and gel in comparison to the standard antibiotics [Tetracycline, Ceftriaxone, Cefotaxime]. Pomegranate aqueous peel extract, gel and Tetracycline showed the zone of inhibition compared to the Cefotaxime and Ceftriaxone did not show any considerable zone of inhibition and *Ocimum* species are used as medicines in ancient medicinal traditions. In present study, the *Ocimum sanctum* volatile oil and gel antimicrobial activity is compared with standard antibiotics. [Tetracycline, Ceftriaxone, Cefotaxime]. The tulsi volatile oil, gel and tetracycline showed antimicrobial activity with the zone of inhibition compared to the Ceftriaxone and Cefotaxime.

CONCLUSION

The attempt has been made to determine the antimicrobial activity of Pomegranate gel and tulsi gel results suggests that the aqueous peel extract and gel showed better antimicrobial activity when compared to the standard Tetracycline drug. Furthermore, study is required with respect to the isolated compound present in the aqueous peel extract to know the active constituents responsible for the antimicrobial activity against the particular species of oral microorganisms.

ACKNOWLEDGEMENT

We take this privilege and pleasure to acknowledge the contribution of many individuals who have been inspirational and supportive throughout our work and we would be thankful to the management of St. John's Pharmacy college, Bangalore.

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