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FORMULATION AND EVALUATION OF HERBAL MOUTHWASH FOR CANKER SORE

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ABSTRACT

Ziziphus mauritiana (Rhamnaceae family) are reported to possess bioactive compounds, recognized for traditional use and medicinal importance. It posses various activity like antioxidant, anti-microbial, anti-diarrheal, anti-diabetic, anti-bacterial, hepatoprotective and anti-cancer, anti-inflammatory. The current study utilizes the antibacterial and anti-inflammatory properties to develop a herbal mouthwash using bark extract of *Ziziphus mauritiana* to treat Canker sore. Stem barks were extracted in aqueous solvent and analyzed by disc diffusion method. *Ziziphus mauritiana* shows greatest activity against *Staphylococcusaureus* and *E.coli* and also anti-inflammatory activity by protein-denaturation method were carried out. The results obtained in the present study suggest that stem bark of *Ziziphus mauritiana* can be used as a source for functional ingredients for pharmaceutical drug industries. Herbal mouthwash are in high demand as compared to chemical mouthwash, because they act on mouth pathogen and microbes and reduce the pain instantly and area lsohasano more side effects.

KEYWORDS

Mouthwash, Canker sore, *Ziziphus mauritiana*, Antimicrobial and Anti-inflammatory.

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INTRODUCTION

A mouth ulcer is a sore that develops in the soft issue lining of your gums, tongue, inner cheeks, lips or palate. They're usually yellow or red, and they can be quite painful and can make eating and talking difficult. Mouth ulcers are very common and they occur in association with many diseases and by different mechanisms, but usually there is no serious underlying cause[1]. Common causes of mouth ulcers include nutritional deficiencies such as

iron, vitamins especially B12 and C, poor oral hygiene, infections, stress, indigestion, mechanical injury, food allergies, hormonal imbalance, skin disease etc. They can also be triggered by several different factors, including minor injuries and emotional stress. Mouth ulcers aren't contagious and they go away on their own but there are treatments to help ease pain and discomfort[2,3]. Mouthwash, mouth rinse, oral rinse, or mouthbath is a liquid which is held in the mouth passively or swilled around the mouth by contraction of the perioral muscles and/or movement of the head and may be gargled, where head is tilted back and the liquid bubbled at the back of the mouth. Usually mouthwashes are antiseptic solutions intended to reduce the microbial load in the oral cavity, although other mouthwash esmight be given for other reasons such as for their analgesic, anti-inflammatory or anti- microbial action[4]. Herbal drugs are gaining popularity in the modern world due to their less side effects and better therapeutics as compared with modern medicine. Herb is a plant or plant part used for its scent, flavor, or therapeutic properties. People use herbal medicines to maintain or improve their health. Products made from botanicals, or plants that are used to treat diseases or to maintain health are called herbal products, botanical products, or phyto medicines. Phytogenicagents are traditionally used by herbalists and indigenous healers for the prevention and treatment of ulcer. Ethnomedical systems employs several plant extracts for the treatment of ulcer[5]. Herbal mouthwashes are mouthwashes which are prepare from natural plant extracts. The use of herbal mouthwash has grown advantage over chemical mouthwashes due to their non-irritant and non-staining properties and it does not contain alcohol. The natural extracts present in these herbal mouthwashes are obtained from various plant leaves, fruits, seeds and various tree oils. They have very minimal or no side effects and they are less harmful. Herbal mouthwashes can be used as an adjunct to various oral hygiene practices like tooth brushing, flossing.

It's proven that they have effective anti-inflammatory, anti-plaque properties and hence can be used in supportive periodontal therapy. It does not contain alcohol, artificial preservatives, flavors or colours. Hence Herbal mouthwashes can be considered an alternative to chemical mouthwashes in sustaining oral hygiene, especially because of the added advantages provided by herbal preparations[6].

Ziziphus mauritiana is a spiny, evergreen shrub or small tree upto15m high, with trunk 40cm or more in diameter; spreading crown; stipularspines and many drooping branches. Fresh Fruits contains Protein, Fat, Fiber, Carbohydrates, Reducing Sugars and Non-Reducing Sugars. The dried fruits are used as anodyne, anticancer, pectoral, refrigerant, sedative, stomach ache, styptic and tonic. They are considered to purify the blood and aid digestion. The root is used in the treatment of dyspepsia. A decoction of the root has been used in the treatment of fevers. The bitter, astringent bark decoction is taken to halt diarrhea and dysentery and relieve gingivitis. The bark pasties applied on sores. Traditionally the fruit and bark of the *Ziziphus mauritiana* obtained from the family Rhamnaceae is used for the treatment of canker sore and used as food source and helps in treatment of various diseases like malaria, asthma, diarrhea, typhoid, diabetes, skin diseases and acts as a pain killer.

In literature, many studies reported that *Ziziphus mauritiana* have some medical benefits such as antioxidant, anti-microbial, anti-diarrheal, anti-diabetic, hepatoprotective and anti-cancer. Various parts of *Ziziphus mauritiana* are used for nutritional and medical purposes.

However, leaves are employed traditionally as astringent and anti-typhoid. Mouthwashes are one of the very popular and better dosage forms. It is potentially useful means of administering drugs locally via, oral cavity. Mouthwash, mouth rinse, oral rinse, or mouth bath is a liquid that is passively kept in the mouth or swilled around the mouth by contraction of the perioral muscles and/or movement of the head, and may be gargled with the head tilted back and the liquid bubbling at the back of the mouth. Mouthwashes are used to reduce

plaque, bio film, kill bacteria in hard-to-reach places below the gums, to treat canker sores and eliminate bacteria on non-tooth oral surfaces including the cheeks, tongue and more[7]. Several antimicrobial chemical agents such as chlorhexidine, metronidazole etc have been used. However this artificial drugs have unpleasant side effects, so researchers are trying to pay more attention to herbal drugs.

The plant *Ziziphus mauritiana* is traditionally used in the treatment of canker sore. The bitter, astringent bark decoction is taken to halt diarrhoea and dysentery and relieve gingivitis. The bark paste is applied on sores. The present study attempt is to formulate and evaluate a herbal mouthwash which contains the bark extract of *Ziziphus mauritiana*.

MATERIALS AND METHODS

Collection of raw material

The stem bark of *Ziziphus mauritiana* were collected from the surrounding of Malik Deenar College of Pharmacy and were authenticated. The stem bark was dried under the shade at room temperature. The dried bark were coarsely cutted and stored in a tight container.

Extraction

The dried stem bark were submitted to aqueous extraction using Soxhlet apparatus. The extracts of *Ziziphus mauritiana* were prepared by soxhlet extraction. Then the extract was evaporated and the residues were collected and weighed.

Pre-formulation studies

Pre-formulation studies may be defined as testing of the physical and chemical properties of a drug substance alone and in combination with excipients proposed o be used in the formulation. Pre-formulation investigations are designed to deliver all necessary data. Especially physiochemical, physio-mechanical and biopharmaceutical properties of drug substances, excipients and packaging materials as well as compatability. The overall objective of the pre-formulation testing is to generate information useful to the formulator in developing stable and bio-available dosage forms, which can be produced[7].

Physical properties

Organoleptic properties

The drug were examined for its organoleptic properties like colour, odour and taste.

Solubility

The sample was qualitatively tested for its solubility in various solvents. It was determined by taking 10mg of the drug extract in 10ml solvents such as water, methanol and ethanol in small test tubes and well solubilized by shaking.

Fourier transform infra red (FT-IR) of drug

The stability of a formulation primary depends on the compatibility of the drug and the excipients. Hence, it is important to detect any possible chemical/physical interactions since they can affect bioavailability and stability of the drug. FT-IR analysis of pure drug was carried out individually. The peaks obtained in the spectrum were compared with the reference spectrum

Anti-microbial study

Disc diffusion method

Every time fresh sterile nutrient agar medium was prepared. The proceedings were carried out aseptically. All the glassware and apparatus required were sterilized. In each sterile Petridish15-20ml of agar medium was added and it is kept for sometimes to solidify. After that fresh culture of organism were transferred to each petriplate by using the help of aswab. Then sterilized Whatmann filter paper No.1 discs thoroughly moistened with the concentration of each of the compound were placed on the surface of the plate. Disc moistened with distilled water was used as control. They were allowed to diffuse in the media and then the plates were incubated at 37°C for 24 hrs. The diameter of the zones of inhibition was observed[8].

Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that will inhibit the visible growth of a micro organism after appropriate in cubation period. MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organisms. The lowest concentration of the extract

that produced no visible bacterial growth (turbidity) was recorded as the MIC of the extracts.

Procedure

Five different concentrations of the standard solution are prepared by diluting the stock solution. Take 10 test tube and label it. To each tube, 5ml of nutrient medium is added. To the first 5 test tube transfer one loop of *E.coli* bacteria and to the rest of the test tube transfer Staphylococcusaureus bacteria. To that add 1ml each concentration of standard solution. Incubate it for 24 hours at 37ºC and observed for the disappearance of turbidity[9].

Anti-Inflammatory Study

Inhibition of Protein Denaturation

The reaction mixture (0.5ml) consisted of 0.45ml bovine serum album in (3% aqueous solution) and varying concentration softest sample. The samples were incubated for 37°C for 20 min and 2.5ml phosphate buffer saline (pH6.3) was added to each tube and then heated at 80°C for 10min. The Absorbance was measured using spectrophotometer at 660nm. The percentage inhibition of protein denaturation was calculated as follows.

$$\text{Percentage inhibition} = \frac{(\text{Abs control} - \text{Abs sample/standard})}{(\text{Abs control} \times 100)}$$

Calculation of IC50 (50% of inhibitory concentration)

The concentration (µg/ml) of the drug required to denature 50% protein was calculated from the graph. The IC50 was calculated for concentration of both the sample and standard[10,11].

FTIR spectrum of drug extract and base

The compatibility studies were carried out at room temperature using FTIR spectroscopy to determine the interaction of drug with other excipients used in the formulations. The IR spectrum of drug alone was taken. Physical mixtures of the excipients in the ratio 1:1 were prepared and the samples were analyzed in IR spectra analyzer.

Formulation of Mouthwash

Weighted quantity of each ingredient will be taken based on the formulas mentioned in the Table No.1 extract were taken mixed thoroughly in mortar and pestle properly with small quantity of water. All other remaining ingredient will be gradually added with good mixing. Drop by drop clove oil is added and mixed properly taking care to avoid lump formation. PEG40 and Glycerol will then be added drop by drop and mixed well. Finally, water added to make volume and the product will be packed in an attractive, well closed container.

Evaluation of mouthwash

Organoleptic Properties

Physical parameters like colour and odour were tested by visual examination.

pH

pH of prepared mouthwash was measured by using pH meter. The pH meter was calibrated using standard buffer and about 1ml of mouthwash was weighed and dissolved in 50ml of distilled water and its pH was measured by pH meter.

***In-vitro* anti bacterial study disc diffusion method**

Every time fresh sterile nutrient agar medium was prepared. The proceedings were carried out aseptically. All the glass ware and apparatus required were sterilized. In each sterile Petridish 15-20ml of agar medium was added and it is kept for sometimes to solidify. After that fresh culture of organism were transferred to each petriplate by using the help of aswab. Then sterilized Whatmann filter paper No.1 discs thoroughly moistened with the concentration of each of the compound were placed on the surface of the plate. Disc moistened with distilled water was used as control. They were allowed to diffuse in the media and then the plates were incubated at 37°C for 24hrs. The diameter of the zones of inhibition was observed[12,13].

Test for microbial growth in formulated mouthwash

The Selected mouthwash formulation was inoculated in the plates of agar media by streak plate method and a control was prepared. The plates were placed in the incubator and are incubated at 37°C for 24 hours. After the incubation period plates were taken out and checked for microbial growth by comparing it with the control[14].

Stability studies

In any rational drug design or evaluation of dosage forms for drugs, the stability of the active

component must be a major criterion in determining their acceptance or rejection.

Stability of a drug can be defined as the time from the date of manufacture and the packaging of the formulation, until its chemical or biological activity was not less than a pre-determined level of labeled potency and its physical characteristics have not changed appreciably or deleteriously. The prepared mouthwash were placed in room temperature for 30 days. Batches were evaluated for the above mentioned parameters to check whether he lozenge shows any significant changes or not[15].

RESULTS AND DISCUSSION

The crude extract collected from the stem bark of the plant of *Ziziphus mauritiana* was prepared and the following studies were performed.

Pre-formulation studies

Physical properties

Drug identification was done by performing organoleptic properties, solubility.

Organoleptic properties

Solubility studies

Solubility of the extract was determined. The solubility of the received sample of extract was examined in various solvents aqueous and organic. The result obtained complies with the reference.

The aqueous extract of *Ziziphus mauritiana* was found to be brown in colour with characteristic odour and taste. And it is freely soluble in water and soluble in methanol an dethanol.

FT-IR Spectroscopy Studies

The FTIR spectrum of extract was shown in Figure No.3 and the peak values obtained in Table No.2. The peaks obtained were found to be similar with that of reference indicating the identity of the drug.

***In-vitro* antibacterial study of extract**

Quantitative screening of the drug extract for antibacterial activity by disc diffusion method were performed and the result obtained were shown in Table No.3.

The antimicrobial activity of the aqueous extract was determined

concentrations such as 100µg, 200µg, 400µg were taken respectively and no visible zone of inhibition was observed so the antimicrobial study with a concentration greater than 400µg were again carried out using disc diffusion method.

The zone of inhibition obtained in case of *E.coli* was found to be in the range of 13.0mm to 14.0mm and incase of *S.aureus* it was found to be in the range of 11.5 to 13.0mm. The concentration used in the assay were 600µgand 800µg and distilled water is the solvent used. Aqueous extract of *Ziziphus mauritiana* can inhibit growth of both Gram-positive and Gram-negative bacteria. The presence of flavanoids, triterpenoids, tannins may be responsible for their antimicrobial activity. This is because; phytochemical compounds such as tannins coagulate the cell wall proteins while saponinsfacilitate the entry of toxicmaterialor leakage of vital constituents from the cell. It has also been proposed that the antibacterial activity may be as a result of inhibition of cell wall formation resulting in leakage of cytoplasmic constituents by the bioactive components of the extract.

Minimum inhibitory concentration (MIC)

The lowest concentration of the extract that produced no visible bacterial growth (turbidity) was recorded as the MIC of the extracts. The visible zone of inhibition was observed above concentration 400µg. So concentration ranging from 550µg, 560µg, 570µg 580µg, 590µg was selected for conducting the MIC and the result obtained were given in Table No.5.

In the case of *E.coli* the disappearance of the turbidity was found in the range of 560µg/ml to 590µg/ml and in *S.aureus* it was found to be in range of 570µg/ml to 590µg/ml.

***In-vitro* Anti-inflammatory study of extract**

Quantitative screening of the drug extract for anti-inflammatory activity by the inhibition of protein denaturation were conducted and the result obtained was given in Table No.6. Anti inflammatory activity of plants may due to the presence of active compounds such as flavanoids and triter penoid

FTIR of drug extract and base

The FTIR spectrum of the drug extract and base was shown in Figure No.9 and the peak values

obtained were shown in Table No.7 and from the obtained spectrum and peak we can say that the reisnoin compatability between the drug extract and the base.

Formulation of mouthwash

Mouthwash was formulated as per the procedure.

Evaluation of mouthwash

Organoleptic properties

The physical parameters like color and odor were tested by visual examination and the colour was found to light brown is handithasan aromatic odour.

pH

The pH of the formulation was found to be in the range 5.9 ± 0.04 to 6.1±0.03. As the skin is having an acidic pH around 5.5. This pH range of the formulation is suitable for canker sore and other oral disorders.

***In-vitro* antibacterial activity of prepared mouthwash**

The *in vitro* antibacterial activity of the prepared mouthwash was carried by disc diffusion method and the result obtained were shown in Table No.8.

The antimicrobial activity of the mouthwash prepared from the aqueous extract was determined using disc diffusion method. The antimicrobial activity was tested against one gram positive organism namely *Staphylococcus aureus* and one gram-negative organism namely *Escherichia coli*. Marketed herbal mouthwash is used as the standard. The zone of inhibition of the formulated mouthwash (F3) were compared with that of standard. Distilled water is used as the solvent.

The result of zone of inhibition for *E. coli* was found to be in the range of 15.5mm to 17.0mm and for the standard it is 19.0mm respectively. The result of zone of inhibition for *S.aureous* was found to be in the range of 14.0mm to 16.5mm and for the standard it is 18.5mm respectively. From this it is clear that the F3 formulation posses more antimicrobial activity compared to that of both in *S. aureous* and *E. coli*. These results showed that the herbal mouthwash (F3) has significant antibacterial activity and the present preparation is able to treat cankers ore.

Test for microbial growth in formulated mouthwash

The microbial contamination tests for the mouthwash were conducted and after 24hours, no Colony Forming Units were observed at a temperature of 37°C.

From Figure No.11 it is observed that the formulation was free from microbes as they have not produced any microbial growth when they got inoculated in the agar medium.

Stability studies

The optimized batch of mouthwash were kept at room temperature for 30 days. After 30days prepared mouthwash were evaluated for pH, visual appearance, phase separation, homogeneity and odor. All parameters were within range even after 30days of study.

Table No.1: Formulation of mouthwash

S.No	Ingredients	Formulations			
		F1	F2	F3	Control/Blank
1	The aqueous stem bark extract of Ziziphus mauritiana	60mg	80mg	100mg	
2	Clove oil	1.5ml	1.5ml	1.5ml	1.5ml
3	Saccharine	0.1mg	0.1mg	0.1mg	0.1mg
4	PEG40	6ml	6ml	6ml	6ml
5	Glycerol	6.5ml	6.5ml	6.5ml	6.5ml
6	Sodium benzoate	0.2mg	0.2mg	0.2mg	0.2mg
7	Purified water	Upto 100ml	Upto 100ml	Upto 100ml	Upto100ml

Table No.2: FT-IR characterization of drug extract

S.No	Functional group	Observed peaks (cm-1)
1	OH-group	3745.5
2	Carbonyl group	1745.4
3	Unsaturated fatty acid	2939.3
4	Aromatic compound	1456
5	Carboxylic group	2580
6	Alkane compound	2939

Table No.3: Result showing the zone of inhibition of drug extract

S.No	Diameter of zone of inhibition in mm							
	Escherichia coli				Staphylococcus aureus			
	100µg	200µg	400µg	Standard	100µg	200µg	400µg	Standard
1	-	-	-	22.0 mm	-	-	-	20.0mm

Table No.4: Result of the antibacterial study

S.No	Diameter of zone of inhibition in mm					
	Escherichia coli			Staphylococcus aureus		
	600µg	800µg	Standard	600µg	800µg	Standard
1	13.0mm	14.0mm	21.5mm	11.5mm	13.0mm	20.0mm

Table No.5: Result of MIC

S.No	Concentrations	550µg/ml	560µg/ml	570µg/ml	580µg/ml	590µg/ml
1	E.coli	–	+	+	+	+
2	S.aureous	–	–	+	+	+

– = no turbidity +=turbidity

Table No.6: Anti-inflammatory study of drug extract

S.No	Sample	Concentration (µg)	Absorbance	% inhibiton
1	Control	-	0.812	-
2	Standard	6.25	0.662	12.08499336
		12.5	0.638	15.27224436
		25	0.554	26.42762284
		50	0.411	45.41832669
		100	0.285	62.15139442
3	Drug Extract	6.25	0.716	6.159895151
		12.5	0.655	14.15465269
		25	0.592	22.41153342
		50	0.473	38.0078637
		100	0.349	54.25950197

IC50 value of the standard-71.45µg

IC50 value of the drug extract–85.48µg

Table No.7: FT-IR characterization of drug extract and base

S.No	Functional group	Observed peaks (cm-1)
1	OH-group	3748.83
2	Carbonyl group	1628.4
3	Unsaturated fatty acid	2938.5
4	Aromatic compound	1459
5	Carboxylic acid	2754
6	Alkane compound	2938

Table No.8: Result showing the zone of inhibition of prepared mouthwash

S.No	Diameter of zone of inhibition in mm									
	Escherichia coli					Staphylococcus aureus				
	F1	F2	F3	Standard	Blank	F1	F2	F3	Standard	Blank
1	15.5mm	16.0mm	17.0mm	19.0mm	4.5mm	14.0mm	15.0mm	16.5mm	18.5mm	4.0mm

Figure No.1: Ziziphus mauritiana tree

Figure No.2: Ziziphus mauritiana stem bark

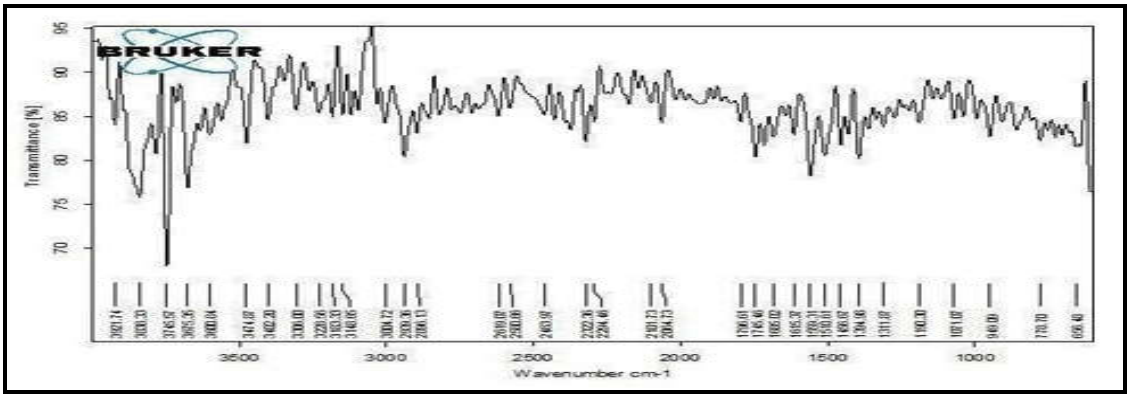

Figure No.3: FT-IR spectrum of drug extract

Figure No.4: Zone of inhibition of drug extract

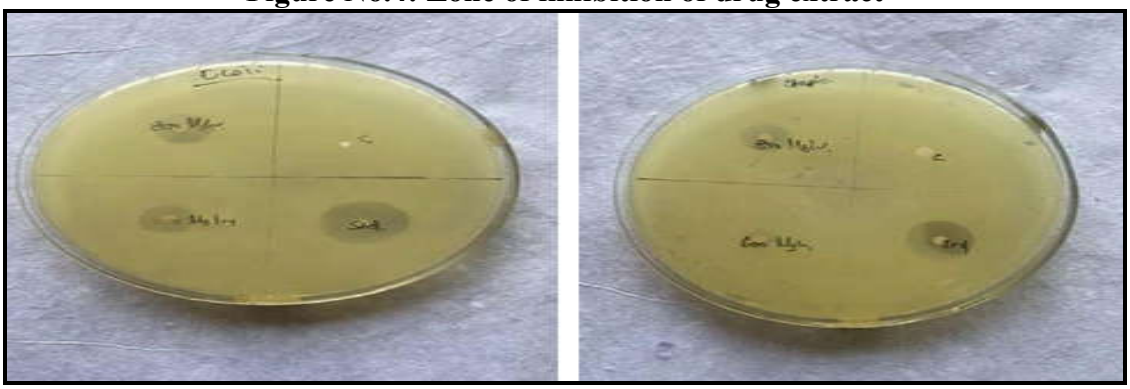

Figure No.5: Antimicrobial activity of drug extract

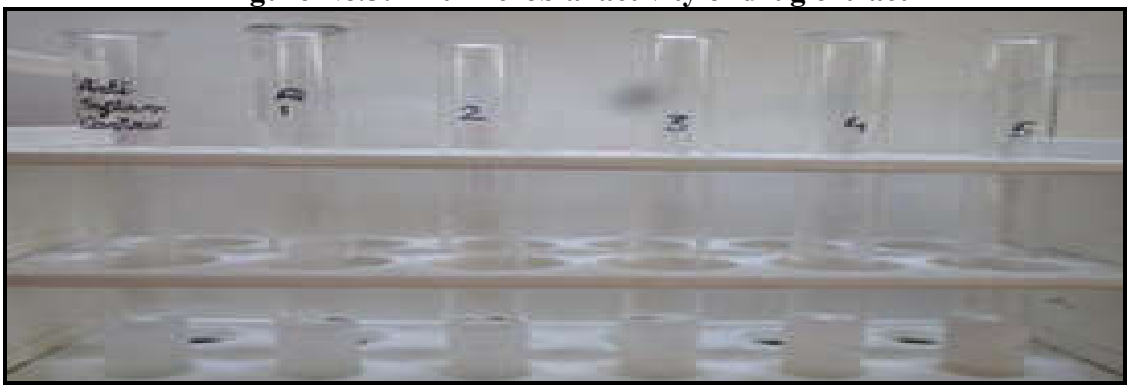

Figure No.6: Anti-inflammatory activity of drug extract

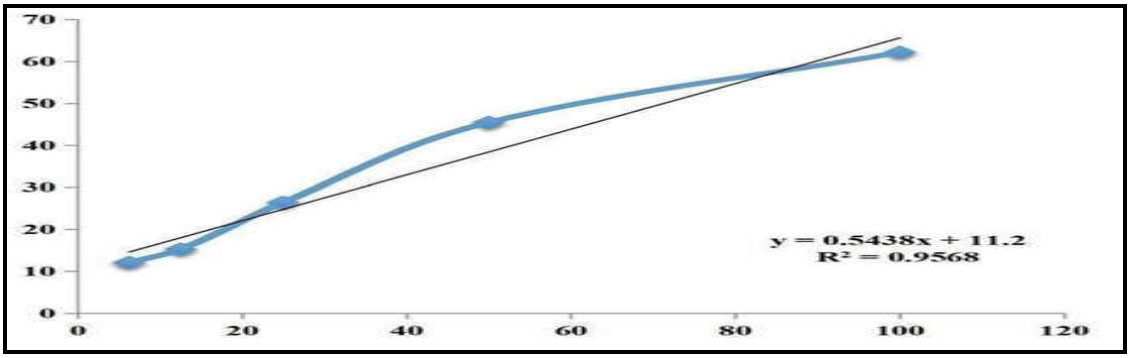

Figure No.7: Graphical representation of the standard (IC50 value-71.45µg)

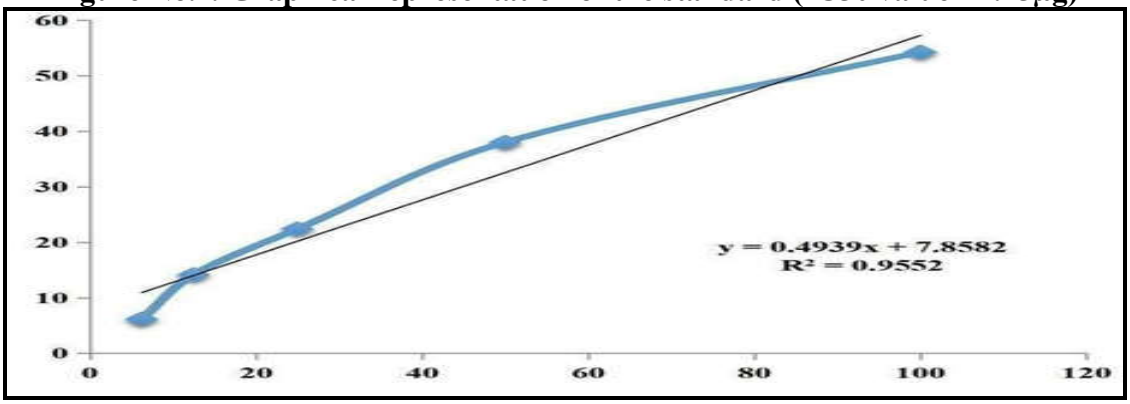

Figure No.8: Graphical representation of the drug extract (IC50 value-85.48µg)

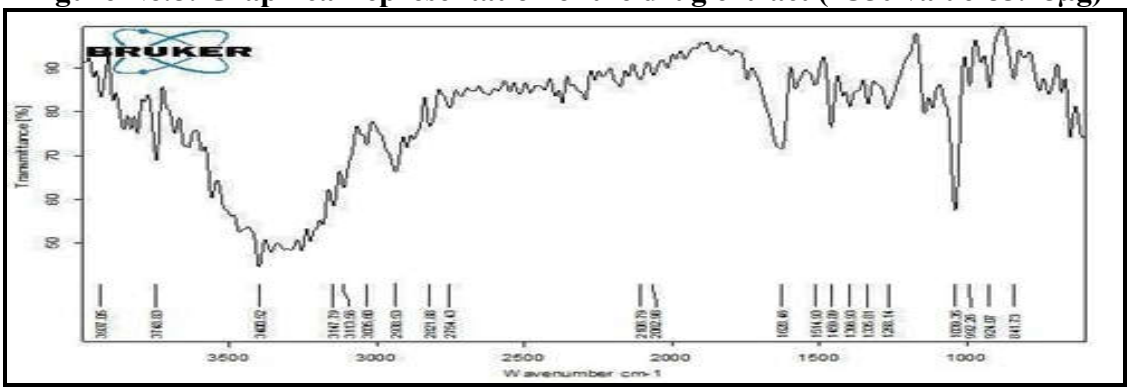

Figure No.9: FT-IR spectrum of Drug extract and base

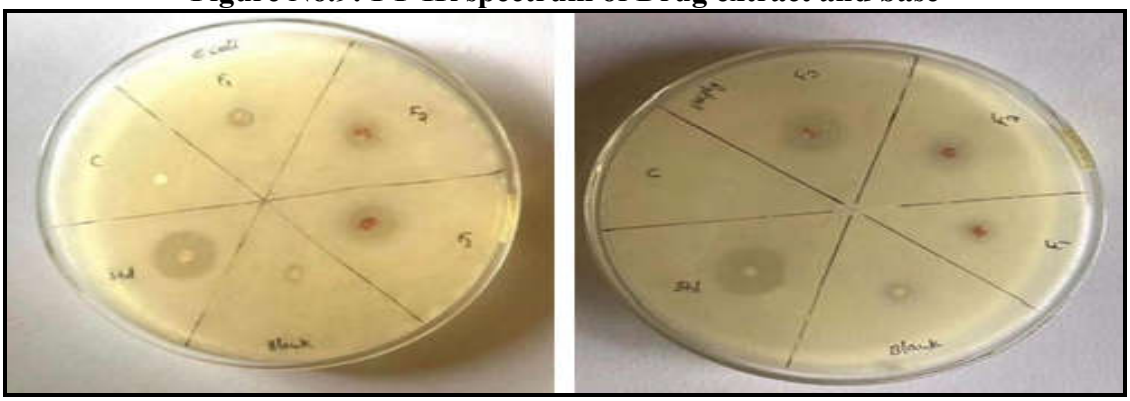

Figure No.10: Antimicrobial activity of prepared formulation using clove oil as standard

Figure No.11: Test for microbial growth in the prepared formulation

CONCLUSION

Medicinal plants play a vital role in the treatment of mouth ulcer. The herbal medicine is the best choice for the treatment of mouth ulcer due to the presence of chemical constituents which are naturally available and with their great uses and healing effects. Canker sore or mouth ulcer is an ulcer that occurs on the mucous membrane of the oral cavity. Mouth ulcers are very common, occurring in association with many diseases and by many different mechanisms.

The present study aimed at the formulation of *Ziziphus mauritiana* and evaluation of the same for antibacterial and anti-inflammatory activity. Extractions of *Ziziphus mauritiana* were done by soxhlet extraction. Aqueous extract of stem bark was yielded and pre-formulation study were performed.

Herbal mouthwash preparation of aqueous extract of *Ziziphus mauritiana* were prepared using clove oil, saccharine, PEG40, glycerol, sodium bicarbonate and purified water, it yielded formulation of satisfactory physicochemical appearance. Study for the antibacterial effect of the extract and the finished herbal mouthwash were performed by disc diffusion method using the microorganisms *E.coli* and *S. aureus*. Study for the anti inflammatory effect of the extract is also studied by the inhibition of protein denaturation.

The phytochemical screening of the stem bark of *Ziziphus mauritiana* obtained from various research works claimed that it contains different types of secondary metabolites such as alkaloids, flavonoids, glycosides, phenol, lignins, saponins, sterols and tannins were presented. *Ziziphus mauritiana* Lam. bark is very effective compared to other part because most parts of secondary metabolites are present in it.

Herbal mouthwash containing 3 different concentrations of aqueous extract of *Ziziphus mauritiana* were prepared. Various evaluation studies like pH, physical appearance, antimicrobial study and the presence of microbial growth were evaluated. Formulation F3 shows better activity throughout the antimicrobial study compared to others. It shows good activity against both gram + ve and gram-ve bacteria. Stability studies were also carried out and there is no stability issue found. It can be successfully used for the treatment of canker sore. It contains bioactive components with strong antimicrobial effects. Although, mechanism of antimicrobial action of this extract is not yet known.

As perour research work, it reveals that drug extract of *Ziziphus mauritiana* posses a good antibacterial and anti-inflammatory activity and the anti-inflammatory activity of plants may due to the presence of active compounds such as flavonoids and triterpenoid. And their aqueous extract was formulated with good physicochemical parameter. The studies reveal that *Ziziphus mauritiana* mouthwash is a promising dosage form for treating canker sore. With Further studies they can be formulated in the market.

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CONFLICT OF INTEREST

We declare that we have no conflict of Interest.

REFERNCES

1. Mortazavi H, Safi Y, Baharvand M, Rahmani S. Diagnostic features of common oral ulcerative lesions: An updated decision tree, *Int J De,* 2016, Article ID: 7278925, 2016, 14.
2. Natah S S *et al*. Recurrent aphthous ulcer today: A review of the growing knowledge, *Int J Oral Maxi Surg,* 33(3), 2004, 221-234.
3. Crispian Scully. Clinical practice: Aphthous ulceration, *Nengl J Med,* 355(2), 2006, 165-172.
4. Bruce A J, Rogers R S. Acuteoral ulcers, *Ermatol Clin,* 21(1), 2003, 1-15.
5. Smith J E. A brief history of herbal medicine, *Inspired Times,* 4, 2010.
6. Bhavna Jha Kukreja, Vidya Dodwad. Herbal Mouthwashes- A gift of nature, *International Journal of Pharma and Bio Scienes,* 3(2), 2012, 46-52.
7. Sarfaraz K. Niazi. Handbook of pre-formulation, *CRC Press,* 1st Edition, 2004-2005, 574.
8. Mirza Azim Beg, Farooq S. *In vitro* antibacterial and anticancer activity of *Ziziphus*, *Jour of Med Pla Stu,* 4(5), 2016, 230-233.
9. Sakat S, Juvekar A R, Gambhire M N. *In vitro* antioxidant and anti-inflammatory activity of methanol extract of oxaliscorniculata linn, *Inter Jour of Pha and Pharma Sci,* 2(1), 2010, 146-155.
10. Mizushima Y and Kobayashi M. Interaction of anti-inflammatory drugs with serm proteins, especially with some biologically active proteins, *Journal of Pharma Pharmacol,* 20(3), 1968, 169-173.
11. Saxena R S, Gupta B, Saxena K K, Singh R, Prasad D N. Study of anti-inflammatory activity of the leaves of *Ziziphus Mauritiana* linn: An Indian medicinal plant, *Journal of Ethnopharmacology,* 11(3), 1987, 319-330.
12. Kokate C K, Purohit A P, Gokhale S B. Carbohydrate and derived Products, drugs containing glycosides, drugs containing tannins, lipids andprotein alkaloids, *Text Book of Pharmacognosy,* 7th Edition, 2001, 133-166, 167-254, 255-269, 272-310, 428-523.
13. Geetha R V, Anitha Roy. *In vitro* evaluation of anti bacterial activity of on oral microbes, *Int. J. Drug Dev and Res,* 4(4), 2012, 161-165.
14. Kachan Upadhye, Kirti Charde, Gauri Dixit, Suparna Bakhle. Formualtion and evaluation of herbal gel for management of mouth ulcers, *Indian Journal of Pharmacy and Pharmacognosy,* 8(3), 2021, 226-230.
15. Note for guidance on stability testing: Stability testing of new drug substances and products (CPMP/ICH/2736/99).
16. Devyani Nigam. Formulation and evaluation of herbal Mouthwash again storal infectious disease, *International Journal of Pharmacy and Life Sciences,* 11(7), 2020, 6746-6750.

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