WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 5, 971-979. Research Article ISSN 2277– 7105

FORMULATION AND EVALUATION OF HERBAL MOUTHWASH

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Article Received on
01 March 2020,
Revised on 22 March 2020,
Accepted on 12 April 2020
DOI: 10.20959/wjpr20205-17200

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BRIEF INTRODUCTION

Medicinal plants, plays vital role in curing diseases due to their antimicrobial and antifungal activity against human pathogens through decades. Herbal Mouthwashes are in high demand, because they act on oral pathogens and relieve the pain instantly and are also less side effective. One of the most common infectious diseases encountered by many individuals are Dental carries and Periodontal diseases at different stages of their life time. Dental caries include the cavity formation, eruption of enamel, swollen gums, bleeding gums, formation of hollow black eruption on the surface of the teeth. In early days, Dental caries are high among Children and Adolescents, because they do not practice proper oral hygiene. Prenominal diseases affect the supporting tissues of teeth. Gingivitis, the mildest form of prenominal disease is generally caused by insufficient oral hygiene. Gingivitis is characterized by inflammation and bleeding of the gum. The main cause of gingivitis is plaque that forms on the surface of teeth and gums. Several antimicrobial chemical agents such as chlorhexidine metronidazole etc. have been used. However, these artificial drugs have unpleasant side effects, so researchers are trying to pay more attention to herbal drugs. Plants and plant's isolates demonstrates effects that are immune enhancing, anti-inflammatory, anticancer etc. Akarkara is used as an analgesic and anti-inflammatory and it is also a great anti- oxidant. It is beneficial for indigestion as it stimulates the secretion of saliva as well as digestive enzymes needed for digestion. The neem solutions are used in decreasing the inflammation of gums, to remove cancer and against dental cavities. Peppermint has an ability to inhibit biofilm formation in the oral cavity in addition to providing a therapeutic benefit in treating periodontitis, gingivitis, and halitosis. It also helps to fight bad breathe.

Guava leaves possess flavonoids which is used as an anti-oxidant in conditions of Xerostomia. Thereby, Guava leaves can also be included in the preparation of Herbal mouthwashes.

OBJECTIVE

To prepare Anti-bacterial Herbal Mouthwash from the aqueous extracts of 4 different powdered drugs namely *Anacyclus pyrethrum (Akarkara root), Azadirachta indica* (Neem), *Mentha piperita (Peppermint leaf), Psidium guajava (Guava)* that acts against the oral pathogens- *Staphylococcus aureus, Bacillus subtilis* and *Escherchia coli* and to check the Anti-microbial activity by using Agar well diffusion method and comparing it with the Chlorhexidine Mouthwash IP.

MATERIAL AND METHOD

Equipments: Sterile Petriplates, Testtubes, Conicalflask, Whattmann filterpaper, Incubator, Autoclave, Laminar air flow, Pippetting device, Hotair-oven.

Chlorhexidine Mouthwash- Rexidin 60ml- 0.2%w/v chlorhexidine gluconate

Extraction process

The Aqueous extract of each plant material was prepared by soaking the powdered plant parts in sterile distilled water and maintained in Incubator at 37°C for 72 h. The herbal extracts were filtered using Whatmann filter paper; marc was washed with 10 ml of sterile distilled water and pressed.

Antibacterial activity of Herbal leaves

The antibacterial activity of Aqueous extracts of *Anacyclus pyrethrum, Azadirachta indica* (Neem), *Mentha piperita*, and *Psidium guajava* was determined against the test organism- *Staphylococcus aureus, Bacillus subtilis and Eschrechia coli* by agar well diffusion method.

The extracts were taken in different dilutions and inoculated against test organisms in Nutrient agar and the sensitivity and resistance of the test organism against the formulated mouth wash were analysed. As by their effectiveness the dilution of the extracts were formulated.

Formulation of herbal Mouthwash

The herbal Mouthwash was prepared by the formula given in table 1. Salt solution was made by preparing 1% w/v solution of salt in sterile water. Then all the extracted ingredients are mixed in a fixed ratio.

Evaluation of herbal mouthwash

Color and Odour: Physical parameters like odour and color were examined by visual examination.

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

Test for microbial growth in formulated mouthwash: The formulated mouthwash 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.

Stability Studies: The formulation and preparation of any pharmaceutical product is incomplete without proper stability studies of the prepared product. This is done in order to determine the physical and chemical stability of the prepared product and thus determine the safety of the product. A general method for predicting the stability of any product is accelerated stability studies, where the product is subjected to elevated temperatures as per the ICH guidelines. A short term accelerated stability study was carried out for the period of 3 months for the prepared formulation. The samples were stored at under the following conditions of temperature as 3-5 0 C, 250 C RH=60%, 400 C ±2% RH= 75%. Finally the samples kept under accelerated study were withdrawn on monthly intervals and were analyzed.

Table 1: Formulation of herbal mouthwash.

S.No	Ingridients	Botanical name	Plant Part	Functions	Percentage
1	Neem	*Azadirachta indica*	Bark, stem	Antimicrobial	20%
2	Akarkara	*Anacyclus pyrethrum*	Root	analgesic and anti-inflammatory	20%
3	Gauva	*Psidium guajava*	Leaf	Anti-oxidant	20%
4	Peppermint	*Mentha piperita*	Leaf	inhibit biofilm formation, freshen breathe	20%
5	Liquorice	Glycyrrhiza glabara	Root	Sweetner, demulscent	10%
6	Salt	-	-	Osmolytic preservative	10%
7	Sodium benzoate	-	-	preservative	0.2%

RESULTS AND DISCUSSION

The pH of the formulation was found to be 6.1. As the skin is having an acidic pH around 5.5 this pH range of the formulation is suitable for oral disorders. The formulation was found to be free from heavy metals. The formulation was free from microbes as they have not produced any microbial growth when they got inoculated in the agar medium. This mouthwash is a purely herbal prepared without the addition of any kind of alcohol and any other additives as other products found in the market. The formulation was undertaken stability studies for physical and chemical change. No considerable variations in properties of the formulation were observed. The results of stability stability studies are shown in the given table 2.

Alcohol consumption as well as alcohol and tobacco use are known risk factors for head and neck cancers. It has always been the question of whether use of alcohol containing mouthwash increases the risk of cancer.

When used in mouthwashes antimicrobial ingredient like neem, akarkara and other essential plant extracts have been found to reduce plaque and gingivitis when combined with daily brushing and flossing. Volatile sulfur compounds are the major contributing factor to bad oral odour. They arise from a variety of sources that is breakdown of food, dental plaque and bacteria associated with oral disease.

The antibacterial acivity was evaluated by agar diffusion method for different concentrations of mouthwash. The result of zone of inhibition is provided in table no. 3, 4, 5, 6, 7 & 8. These

results showed that the herbal mouthwash has significant antibacterial activity and the present preparation is able to inhibit bacterial growth in oral cavity. The association of oral microbial load on oral diseases is well established.

Comparison of chlorhexidine mouthwash with formulated herbal mouthwash

The agar dilution method determined the inhibitory capacity of each mouthwash for each organism. According to Figure 1, Chlorhexidine was more effective as compared to the herbal mouthwash against standard strains of *Staphylococcus aureus, Escherchia coli and Bacillus subitilis.*

Table 2: Results of stability study of herbal mouthwash.

Temperature	**Evaluation Parameters**	**Observation (months)**			
		0	**1**	**2**	**3**
3 – 5°C	Visual Appearance	Light brown	Light brown	Light brown	Light brown
	Phase Separation	Nil	Nil	Nil	Nil
	Homogeneity	Good	Good	Good	Good
Room Temperature (25°C RH=60%)	Visual Appearance	Light brown	Light brown	Light brown	Light brown
	Phase Separation	Nil	Nil	Nil	Nil
	Homogeneity	Good	Good	Good	Good
40°C±2°C RH=75%	Visual Appearance	Light brown	Light brown	Light brown	Light brown
	Phase Separation	Nil	Nil	Nil	Nil
	Homogeneity	Good	Good	Good	Good

Table 3: Zone of inhibition in Neem Extracts.

S.no	**Oral Microbes**	**Dilution of the Extracts**	**Zone of Inhibition**	**Sensitivity**	**Resistance**
1.	*Staphylococcus aureus*	50 µl	7mm	+	-
		100 µl	10mm	+	-
		150 µl	15mm	+	-
2.	*Escherchia coli*	50 µl	24mm	+	-
		100 µl	17mm	+	-
		150 µl	21mm	+	-
3.	*Bacillus subitilis*	50 µl	5mm	-	+
		100 µl	8mm	-	+
		150 µl	10mm	-	+

Table 4: Zone of inhibition in Akarkara Extracts.

S.no	Oral microbes	Dilution of the Extracts	Zone of Inhibition	Sensitivity	Resistance
1.	*Staphylococcus aureus*	50 µl	2mm	+	-
		100 µl	3mm	+	-
		150 µl	6mm	+	-
2.	*Escherchia coli*	50 µl	1mm	+	-
		100 µl	2mm	+	-
		150 µl	4mm	+	-
3.	*Bacillus subitilis*	50 µl	1mm	-	+
		100 µl	2mm	-	+
		150 µl	2mm	-	+

Table 5: Zone of inhibition in Peppermint Extracts.

S.no	Oral microbes	Dilution of the extracts	Zone of Inhibition	Sensitivity	Resistance
1.	*Staphylococcus aureus*	50 µl	**15mm**	+	-
		100 µl	**17mm**	+	-
		150 µl	**18mm**	+	-
2.	*Escherchia coli*	50 µl	**9mm**	+	-
		100 µl	**10mm**	+	-
		150 µl	**10mm**	+	-
3.	*Bacillus subitilis*	50 µl	**4mm**	-	+
		100 µl	**5mm**	-	+
		150 µl	**7mm**	-	+

Table 6: Zone of inhibition in Guava leaf Extracts.

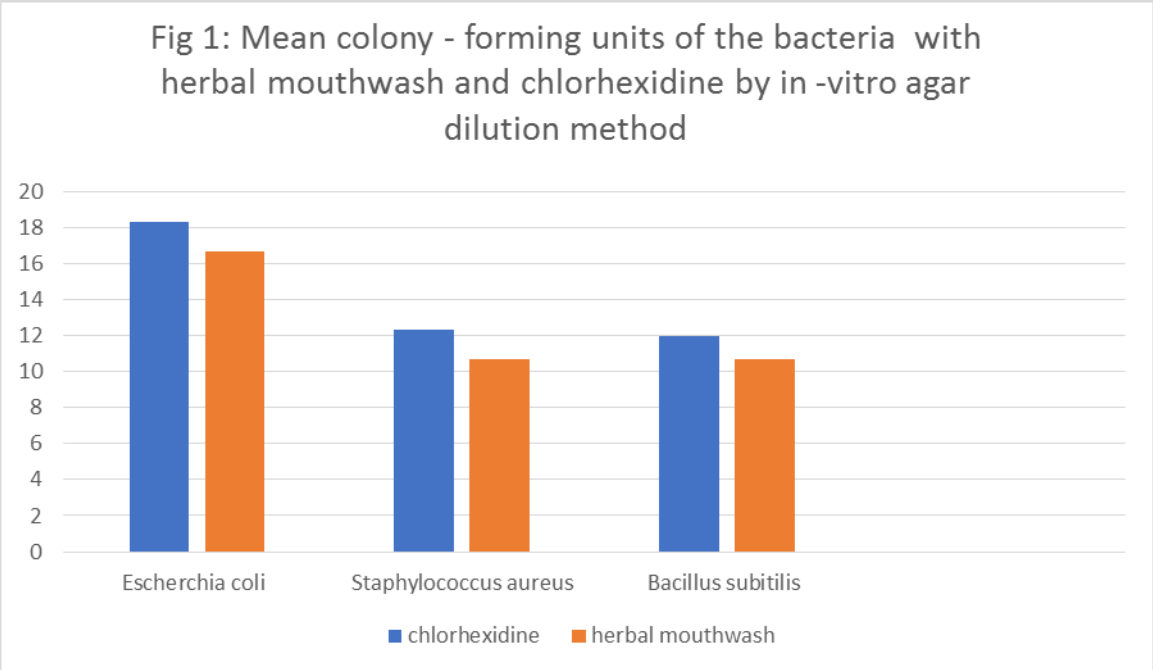
S.no	Oral microbes	Dilution of the Extracts	Zone of Inhibition	Sensitivity	Resistance
1.	*Staphylococcus aureus*	50 µl	**23mm**	+	-
		100 µl	**26mm**	+	-
		150 µl	**33mm**	+	-
2.	*Escherchia coli*	50 µl	**20mm**	+	-
		100 µl	**24mm**	+	-
		150 µl	**25mm**	+	-
3.	*Bacillus subitilis*	**50 µl**	**20mm**	+	-
		100 µl	**21mm**	+	-
		150 µl	**23mm**	+	-

Table 7: Zone of inhibition in formulated Herbal Mouthwash.

S.no	Oral microbes	Dilution of The Extracts	Zone of Inhibition	Sensitivity	Resistance
1.	*Staphylococcus aureus*	50 µl	9mm	+	-
		100 µl	10mm	+	-
		150 µl	13mm	+	-
2.	*Escherchia coli*	50 µl	13mm	+	-
		100 µl	18mm	+	-
		150 µl	19mm	+	-
3.	*Bacillus subitilis*	50 µl	9mm	+	-
		100 µl	11mm	+	-
		150 µl	12mm	+	-

Table 8: Zone of inhibition in Chlorhexidine Mouthwash IP.

S.no	Oral Microbes	Dilution Of the Extracts	Zone of Inhibition	Sensitivity	Resistance
1.	*Staphylococcus aureus*	50 µl	**11mm**	+	-
		100 µl	**12mm**	+	-
		150 µl	**14mm**	+	-
2.	*Escherchia coli*	50 µl	**15mm**	+	-
		100 µl	**20mm**	+	-
		150 µl	**20mm**	+	-
3.	*Bacillus subitilis*	50 µl	**10mm**	+	-
		100 µl	**12mm**	+	-
		150 µl	**14mm**	+	-



CONCLUSION

In the present study, Chlorhexidine showed higher levels of antimicrobial action against the selected bacterial species. However, the herbal mouthwash too was effective in these bacterial species in vitro method.

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