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Design and Development of Value Added Herbal Delivery Systems for Topical Treatment of Periodontal Diseases-Tooth Paste

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ABSTRACT

Arimedadi oil recommended for oil pulling therapy/procedure in *Sushruta Samhita, Bhaisajyaratnavali* and *Charaka Samhita*. Indicated as prophylactic oral care product as well as for local treatment of almost all *dantarogas* including stomatitis, glossitis, apthous ulcers, dental caries, pyorrhea, gingivitis, stain removal and hyperemia of gums. The purpose of this study was to design and development of value added herbal delivery system for topical treatment of periodontal diseases. In the present study, an attempt was made to formulate toothpaste by liquid phase process. Toothpaste of *Arimedadi* oil to achieve the high foaming power, penetrability, antimicrobial and anti-inflammatory efficacy at much lower concentrations than the neat oil. The batches of toothpaste of *Arimedadi*oil were prepared based on the high PG, high GLY, low PG and low GLY The toothpaste of *Arimedadi* oil containing high concentration of glycerine was superior in spreadability, foaming power, pH, washability and content of oil. Its antimicrobial efficacy (15mm) against S. *mutans* was better than the reference herbal toothpaste (12 mm). The formulation also remained stable and revealed no significant changes in any of its physical, physicochemical and functional characteristics after storage for 30 days.

INTRODUCTION

The term ‘periodontal diseases’ encompasses a wide variety of inflammatory conditions of the gingiva (or gums, the soft tissue surrounding the teeth), bone and ligament (the connective tissue collagen fibres that anchor a tooth to alveolar bone) supporting the teeth [1]. Periodontal disease begins with gingivitis, the localized inflammation of the gingiva that is initiated by bacteria in the dental plaque (Fig.1). It is the most common oral condition of human population. Periodontal diseases are responsible for 3.5 million years lived with disability. Compared with developed countries, developing nations have higher prevalence of calculus and bleeding on probing among adolescents. The proportion of adolescents with calculus deposits ranged from 35% to 70% in developing countries while it ranged from 4% to 34% in developed nations [2]. Equivalently, 14-47% of adult populations in developed countries had calculus deposits compared with 36-63% of adults in developing nations. Overall, periodontal disease affects about 20-50% of the population worldwide [2, 3].

The global cost of lost productivity from severe periodontitis alone has been estimated to be 54 billion USD/year, while the total economic impact of periodontal diseases accounts for a major component of the 442 billion USD, direct and indirect cost of oral diseases incurred in 2010. The prevalence of periodontitis increases with age, and the incidence rises steeply in adults aged 30–40 years. There are approximately 800 species of bacteria identified in the oral cavity and it is hypothesized that complex interaction of bacterial infection and host response, modified by behavioural factors such as smoking, diabetes mellitus, RA, stress, cardiovascular diseases, obesity, pregnancy, drug induces diseases can result in periodontal disease. Dental care preparations are available as medicated toothpowders, dental creams, mouthwashes, toothpaste and gels.

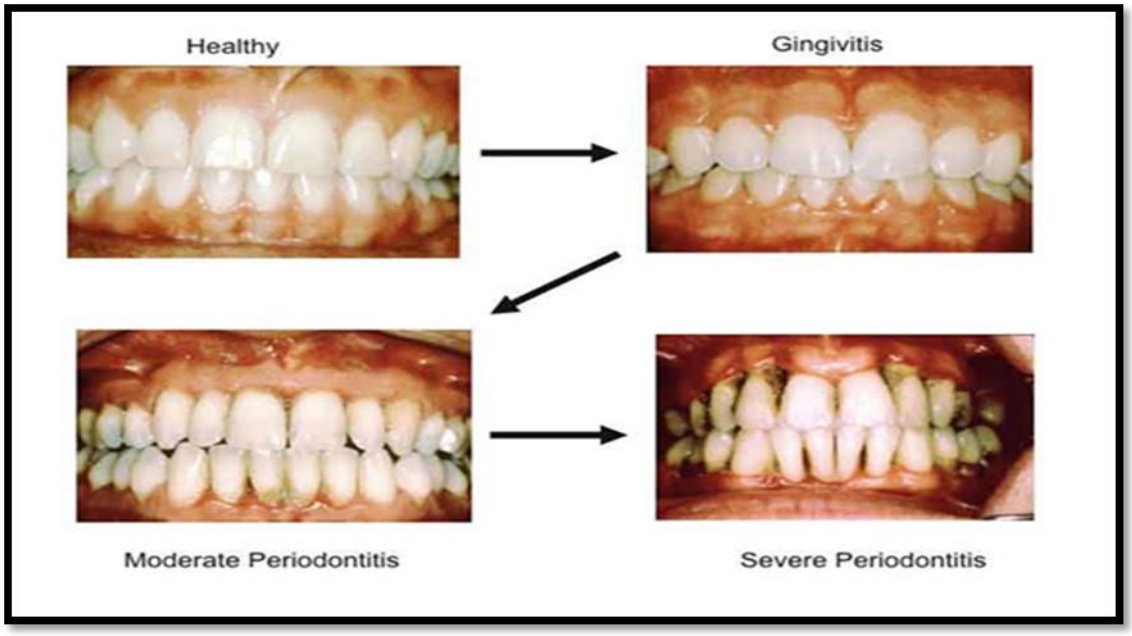

Figure No. 1: Stages of Periodontal disease

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In synthetic toothpaste, fluoride is the important factor of the paste. Less amount of fluoride gives protection to teeth from sacrilege and strengthens enamel. The increase in the amount of fluoride affects the health of teeth and bones and also causes Alzheimer's disease [2, 3]. The detergents such as sodium lauryl phosphate add for spumes in pastes causes problems in mouth. The polishing agents in pastes may cause to acidulous of teeth. It causes because of the destruction of enamel. Flavoured toothpaste doesn't protect against plaque, food tastes really bad after brushing the teeth.

Arimedadi (Irimedadi) Thailam:

A polyherbo-mineral, medicated oil indicated for gargling, the procedure famously known as "oil-pulling" or "*Gandoosha*in Ayurved. As per *Sharangdhar Samhita*, this oil is a specific remedy to treat dental and gum problems resulting from bad oral hygiene. It has been strongly recommended to alleviate bleeding gums gingivitis, toothache and other dental diseases. Prophylactically it is recommended to improve strength of teeth and sense organs.

Typical composition includes extracts of many herbo-mineral ingredients in sesame oil base. (Table No.1).

Table No. 1: Herbs and mineral components of *Arimedadi* oil formulation

Source 1		Source 2	
Name of the ingredient (*Ayurved*)	**English name and biological source**	**Name of the ingredient (*Ayurved*)**	**English name and biological source**
Agaru	*Aquilaria agallocha*	Agaru	*Aquilaria agallocha*
Ashwattha	*Ficus religiosa*	*Bilvapatra*	*Aegle marmelos*
Bilvapatra	*Aegle marmelos*	*Brihati*	*Solanum indicum*
Brihati	*Solanum indicum*	*Daruharidra*	*Berberis aristata*
Daruharidra	*Berberis aristata*	*Dhataki*	*Woodfordia fruticosa*
Dhataki	*Woodfordia fruticosa*	*Gairika*	Red ochre
Gairika	Red ochre	*Gayatri*	*Acacia catechu*
Gayatri	*Acacia catechu*	*Irimedatwak/ Kshirivrikshatwak*	*Acacia leucophloea/farnesiana*
Jati	*Myristica fragrans*	Jati	*Myristica fragrans*
Jaya	*Abutilon theophrasti*	*Jaya*	*Abutilon theophrasti*
Karpoora	*Camphor – Cinnamomum camphora*	*Karpoora*	Camphor, *Cinnamomum camphora*
Katphala	*Myrica nagi/ Myrica esculenta*	*Katphala*	*Myrica nagi*
Kshirivrikshatwak/ IrimedaTwak	*Acacia leucophloea /farnesiana*	*Kumkuma*	*Crocus sativus*
Kumkuma	*Crocus sativus*	*Laksha*	*Laccifer lacca*
Laksha	*Laccifer lacca*	*Lodhra*	*Symplocos racemosa*
Lodhra	*Symplocosracemosa*	*Madana*	*Randia dumetorum*
Madana	*Randia dumetorum*	*Mamsi*	*Nardostachys jatamansi*

Mamsi	*Nardostachys jatamansi*	Manjishta	*Rubia cordifolia*
Manjishta	*Rubia cordifolia*	*Mishi*	*Anethum sowa*
Mishi	*Anethum sowa*	*Mrinala*	*Cymbopogon jwarancusa*
Mrinala	*Cymbopogon jwarancusa*	*Musta*	*Cyperus rotundus*
Musta	*Cyperus rotundus*	*Padmakesara*	*Nelumbo nucifera*
Nyagrodha	*Ficus bengalensis*	*Palasha*	*Butea monosperma*
Padmakesara	*Nelumbo nucifera*	*Pradhakaleya*	*Coscinium fenestratum*
Palasha	*Butea monosperma*	*Priyangu*	*Callicarpa macrophylla*
Plaksha	*Ficus lacor*	*Pushkara*	*Inula raceomsa*
Pradhakaleya	*Coscinium fenestratum*	*Rajani*	Turmeric, *Curcuma longa*
Priyangu	*Callicarpa macrophylla*	*Raktachandana*	*Pterocarpus santalinus*
Pushkara	*Inula raceomsa*	*Samanga, manjishta*	*Rubia cordifolia*
Rajani / Turmeric	*Curcuma longa*	*Shaileya / AsphaltumSarala*	*Pinus roxburghi*
RaktaChandana	*Pterocarpus santalinus*	*Shvetachandana*	*Santalum album*
Shaileya /AsphaltumSarala	*Pinus roxburghi*	*Sprikka*	*Frlphiniumzalil*
ShvetaChandana	*Santalum album*	*Suradruma*	*Cedrus deodara*
Sprikka	*Frlphiniumzalil*	*Takkola*	*Illicium verum*
Suradruma	*Cedrus deodara*	*Tejani*	*Clematis triloba*
Takkola	*Illicium verum*	*Tilataila*	Sesame oil, oil from seeds of *Sesamum inidicum*
Tejani	*Clematis triloba*	*Trijatha*	*Cinnamon, cardamom and Cinnamomum tamala*
Tila Taila	Sesame oil –	*Vaidedi*	*Piper longum*

	Sesamum indicum		
Trijatha	Cinnamon, cardamom and *Cinnamomum tamala*	*Vyaghri*	*Solanum xanthocarpum*
Udumbara	*Ficus racemosa*		

MATERIALS AND METHODS

Chemicals

All the chemicals used were of analytical grade and obtained from SD fine chemical Ltd, Mumbai, India.

Drug

Arimedadi oil were purchased from Nagarjuna Pharmaceuticals Pvt. Ltd, Pune, India.

Preparation of medicated toothpaste

Medicated toothpaste was prepared using Arimedadi oil (1%), calcium carbonate (45%), Sodium lauryl sulphate (2%), sodium carboxyl methylcellulose (2%), glycerine (40%), sorbitol (0.25%), peppermint oil (1.5%), and methyl/propylparaben (2%) by heated liquid phase process. Arimedadi oil possesses the antibacterial, anti-microbial and anti-inflammatory activity. Calcium carbonate as an abrasive and polishing agent. Sodium lauryl sulphate used gives foaming, glycerine as humectants. Sorbitol as sweetening agents and plasticizer. Peppermint oil used as flavouring agent. Methyl/propylparaben used as preservative.

Evaluation Studies

Organoleptic assessment

***Appearance*:**

2 gm. of toothpaste sample was placed in watch glass and was observed visually for homogeneity, consistency with unaided eyes as well as with magnifying glass.

***Colour*:**

2 gm of toothpaste was placed in watch glass and was observed for colour (if any) /uniformity of colour by subjective perception, with unaided eyes as well as with magnifying glass.

***Odour*:**

2 gm of toothpaste was placed in watch glass and its odour was sensed by subjective perception with unaided eyes as well as with magnifying glass.

Taste/flavour:

2-3 gm. of toothpaste was placed on the tip of tongue and the taste was sensed by subjective olfactory perception and by perception of panel of tastes.

***Texture*:**

2 gm of toothpaste was placed in the palm and texture was sensed by touch using middle figure and thumb to feel palpably gritty particles.

Presence of palpably hard and sharp edged abrasive particles:

According to *Mangilal T,*10 gm. of paste sample was extruded horizontally from the collapsible tube over a length of about 15 to 20 cm on a square shape butter paper sheet (20X20 cm). The extruded mass of paste was pressed along the entire length with top phalange of the middle finger along the entire length of extruded mass and presence of palpably hard and sharp gritty particles was felt by touch. [5,6]

Functional Characteristic

***Spreadability*:**

According to *Mangilal T,*1 gm. of sample of each type of paste formulation was weighed and placed at the centre of a clean, smooth glass plate (10X10 cm) and another glass plate of the same dimensions was placed over it carefully. A weight of 2 kg was placed over the assembly of the glass plates at the centre. After 30 minutes, the corresponding spreading of the glob of paste sample was noted in terms the diameter (cm) as well as area (cm^2). For this, a PVC sheet with concentric circles drawn at 4cm was placed underneath the lower slide to

measure the spreadability. The procedure was repeated three times and the averages value were reported. [5,6]

Foaming power:

According to *Mangilal T,* A suspension of each of the paste formulation was prepared using accurately weighed 5 g of toothpaste. In a glass beaker (100 ml), the mass of paste was diluted with 10 ml of water and was left undisturbed (after covering with glass plate) for 30 minutes. The suspension was heated gently with constant stirring with glass rod so as to dissolve surfactant if any. The homogeneous suspension was then transferred carefully into 250 ml measuring cylinder. The final volume of suspension was made to 50 ml with more quantity of water (V1). The contents in the cylinder were observed for generation of foam after manual shaking (12 times, up-down) and leaving to stand for 5 minutes. The final volume of suspension (V2) as well as the volume and quality of foam produced was noted carefully. The results were expressed and compared in terms of foaming power using following formula**.**[5,6]

(Eq.No: 1) Foaming power = V2 – V1

Where,

V2 - Volume (ml) of foam with water

V1 - Volume in ml of water only

Presence of grit:

According to *Mangilal T,* Accurately weighed 5.00 g quantity of paste sample (M) was transferred into 100 ml beaker and was diluted with 50 ml of water, added gradually in small portions at a time and the resulting suspension was left undisturbed over 30 min (except occasional stirring with glass rod for a few times) to ensure complete dispersion of the paste. The dispersion was passed through a standard sieve (S.No.75#). Subsequently, the sieve was rinsed under running tap water until almost all the matter passed through the mesh**.** The residue (if any) left on the mesh was collected and transferred into a previously dried and weighed (WC1) porcelain crucible. The residue was dried in an oven at 105 +_ 2 ºC. The crucible was weighed again after cooling (WC2), from this, weight of only gritty residue

(M1) was calculated amount of gritty particles was calculated using the following formula [5].

$$\text{Percentage by mass} = M1/M \times 100$$

Where M1 - Mass in g of residue retained on sieve

Determination of pH:

Accurately weighed 1 gm of sample of each paste formulation was transferred into 100 ml beaker and was diluted with 10 ml of freshly boiled and cooled water. It was stirred well to form a uniform dispersion and the pH was determined in triplicate within 5 min using digital pH meter (Toshkonh Industries. Ltd) and the average value have been noted [5, 6].

Determination of moisture content:

Accurately weighed 5 g of sample of toothpaste was transferred into a dry, clean porcelain dish that was weighed previously. The contents of the porcelain dish were heated in hot air oven (105^0C+_ 1 ^{O}C) and then cooled to room temperature and weighed to the constant weight. From this, the weight of residue only was calculated and the value was substituted into following equation [5, 6].

$$\% \text{ by mass} = 100\ M1 / M$$

M1 - loss of mass (in grams) on drying

M - Mass (in grams) of the material taken for the test.

Minimum inhibitory concentration (MIC): [4, 6]

1. Various concentration *Arimedadi* oil (0.325µg/ml to 20µg/ml) was prepared in ACE.

2. Two fold dilution of this solution was prepared in Mueller Hinton (MH) broth as described below:

a. A set of 10 sterile tubes was labelled as 2 through 8 while the first one (1st) was labelled as antibiotic control (A.C.) and the last one (10 th) was labelled as growth control (G.C.).

b. To each of these tubes, 2 ml of MH broth was added.

c. Subsequently, 2 ml of antibiotic solution was added into test tube No 1 designated as A.C.

d. After adequate mixing, 2 ml of contents of tube 1 were transferred to tube number 2 using sterile micropipette and tips.

e. Similarly, 2 ml was transferred from tube number 2 to tube number 3 using a separate micropipette.

The procedure was repeated through the subsequent.

f. After adequate mixing, 2 ml of contents of tube 1 were transferred to tube number 2 using sterile micropipette and tips.

g. Similarly, 2 ml was transferred from tube number 2 to tube number 3 using a separate micropipette.

h. The procedure was repeated through the subsequent tubes up to tube number 8 in the series, using fresh sterile pipette for each dilution.

i. The 2 ml contents from tube number 8 were discarded.

j. The last tube (10th) received no antimicrobial agent (G.C).

Each of the 10 tubes, except the antibiotic control was inoculated with 2 ml of the culture of *S. mutans*.

3. The final concentration of antimicrobial agent in each of the test tubes was half of the initial dilution because of the addition of an equal concentration of inoculums in MH broth.

4. The tubes were incubated at 37^{o}C for 24 hrs and were examined for growth visually (cloudy) and observations were recorded as positive growth (+) or negative or no growth as (-).

5. The MIC of test antibiotic (*Irimedadi* oil), that was bacteriostatic for the bacterium,

6. For determination of MBC, the concentration which was bactericidal was then found by sub-cultured the contents of selective tubes into a series of MH broth, which did not contain any antibiotic and started from last two non- visible tube to the 1st two visible tube (direction

tube no 1 to tube no. 8). Then was inoculated Petri plate by 0.1 sterile micropipettes and separate 0.1 ml sterile tips in drop method.

7. The plates were incubated at 37^{O}C for 24 hrs.

Stability study:

The stability study was performed as per ICH guidelines. The formulated paste was filled in collapsible tube and stored at different temperature and humidity conditions, 25ºC± 2ºC / 60% ± 5%, RH, 30º C ± 2ºC / 65% ± 5% RH, 40ºC ± 2ºC / 75% ±5% RH for the period of a months and studied for appearance, pH and spreadability.[7,8]

Comparison:

The formulated herbal toothpaste was compared with marketed preparation follows antibacterial activity, spreadability, foamability, pH determination, % moisture content.

RESULTS AND DISCUSSION

The herbal toothpaste formulation was prepared from Arimedadi oil and synthetic ingredient. The formulated toothpaste is white in colour and showed the good homogeneity with absence of lumps and good antimicrobial activity against *S. mutans*. The aim of current research is to formulate toothpaste utilizing Arimedadi oil. The toothpaste formulations of *Arimedadi* oil containing high (40% w/w) and low (30% w/w) concentration of PG as well those containing GLY, matched all the physical, physic-chemical and functional criteria for acceptance with those of commercial sample of leading brand of herbal toothpaste, Hence the lab made herbal toothpaste was found to be of good quality.

UV-spectrophotometric characteristics of *Arimedadi* oil:

i. λmax of Arimedadi in ACE:(*Shimadzu)*

The λ max of *Arimedadi* oil in ACE was found to be 291.

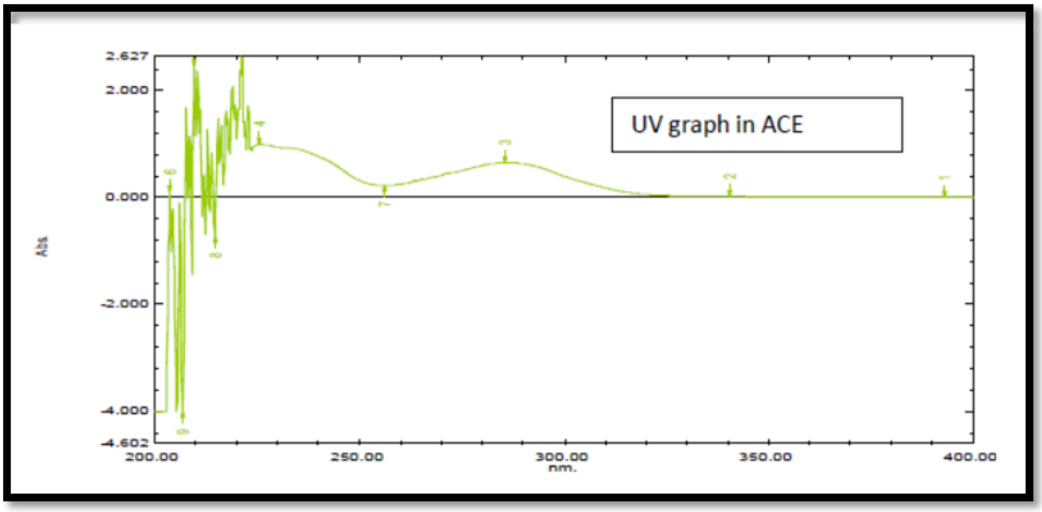


Figure No. 2: UV Spectrum of *Arimedadi* oil in ACE.

ii. Calibration curve of Arimedadi oil in ACE*: *(Shimadzu)

The calibration curve of *Arimedadi* oil in ACE was found to be linear over the range of 10-60 µg/ml. Hence, the calibration curve of *Arimedadi oil* followed Beer's-Lambert law over this range.

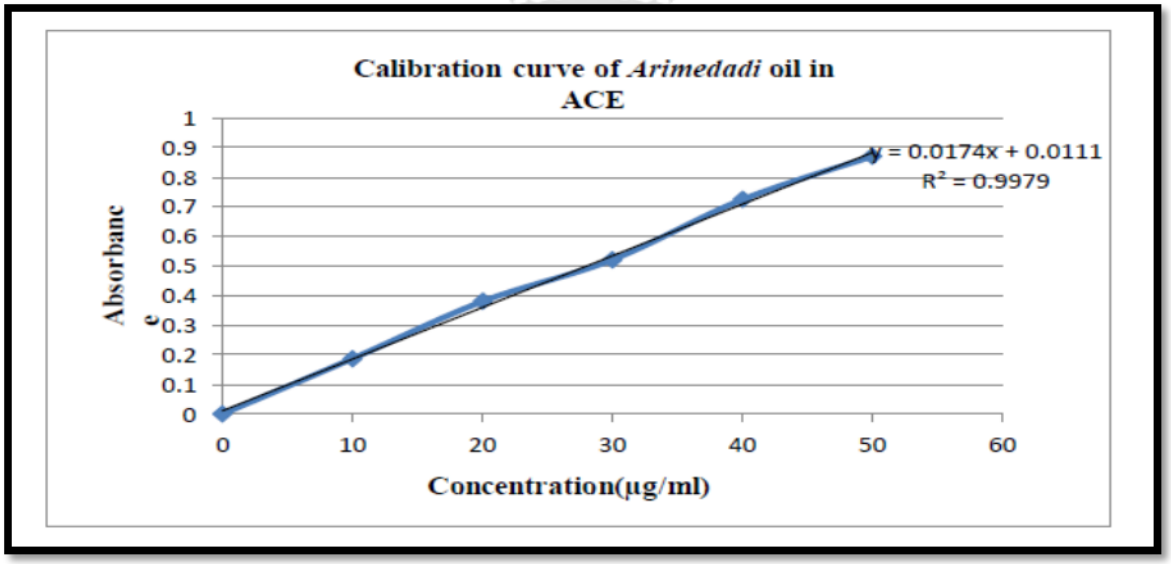


Figure No. 3: Calibration curve of *Arimedadi* oil in ACE.

Infra-red spectral characteristics of Arimedadi oil:

The IR spectrum revealed presence of peaks associated with major functional groups in the structure *Arimedadi* oil (Fig. 4).

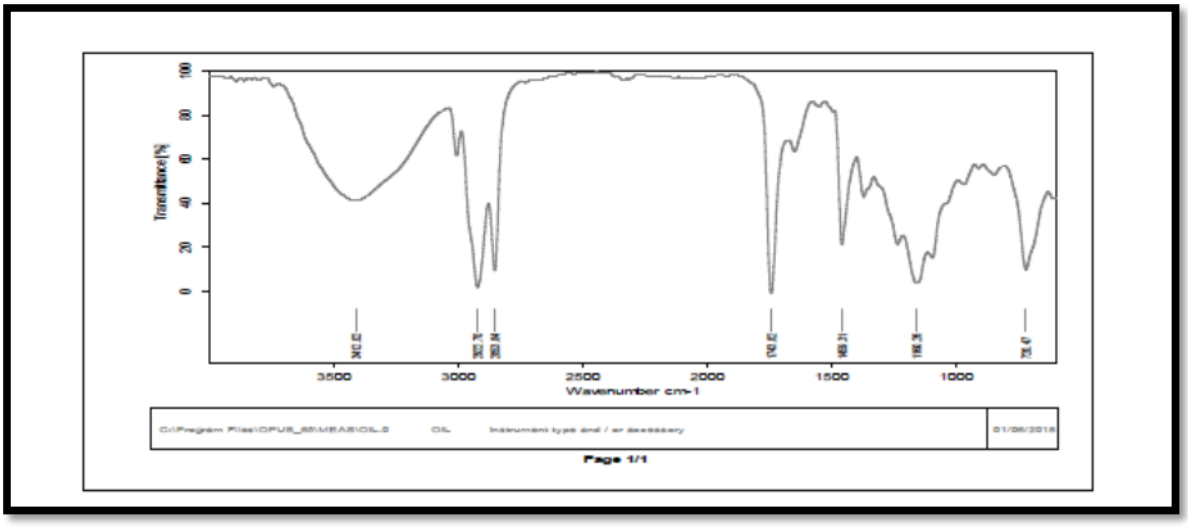
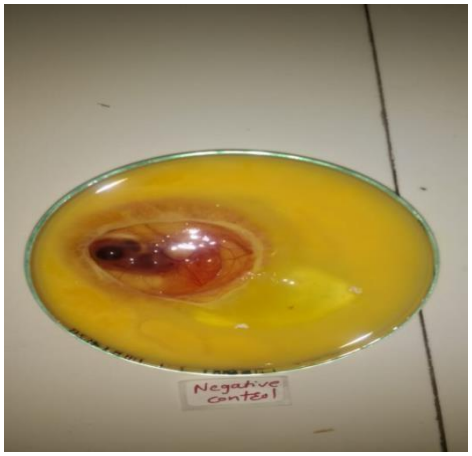
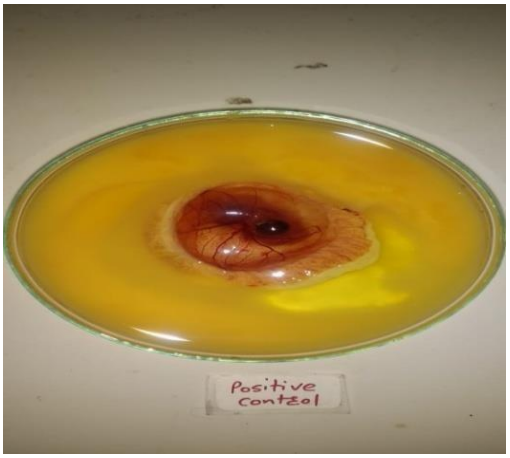


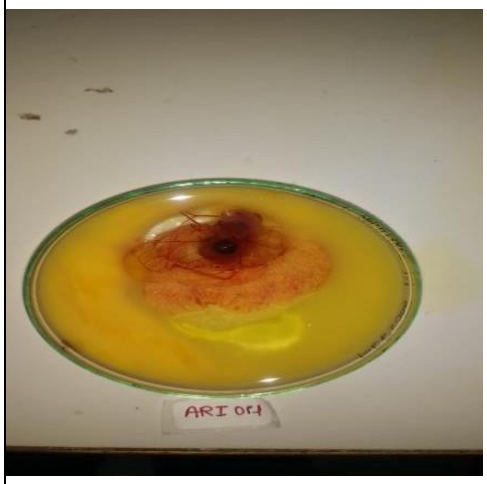
Figure No. 4: IR spectrum of *Arimedadi* oil

Dermal irritancy potential of *Arimedadi* oil:

Hen's eggs injected with Sodium Chloride Injection I.P. (- ve control) as well as those injected with selected concentration (0.4ml and 0.8 ml) of *Arimedadi* oil sample demonstrated establishment and retention of dense vascular network around the ChorioAllantoic membrane (CAM) of embryo (photo.5: a, b c d).In contrast, however, the incubated eggs treated with positive irritant (1%w/v, SLS) possessed only sparse vascular network which was subsequently damaged after 8 days of incubation.

	
Photograph No.8.3a:-ve control 0.9w/v sodium chloride (NaCl Injection I.P.)	**Photograph No.8.3b.:+ve control** (1%w/v SLS solution)

Photograph No.8.3c: Test sample of *Arimedadi* oil 0.4 ml (lower



Photograph No.8.3d.: Test sample of *Arimedadi* oil 0.8ml (higher

Figure No. 5: Dermal irritancy test (HET-CAM)

Table No. 2: HET-CAM irritation and anti-irritation scores for *Arimedadi* oil:

Photograph	Chemicals used	Assessment
A	0.9 % Nacl solution (negative control)	Non-irritant
B	1% SLS solution (positive control)	Strong irritation
C	0.4 ml Test sample (lower concentration)	Practically no irritation
d	0.8 ml Test sample (higher concentration)	Practically no irritation

Table 3: Physical examination of toothpaste

Sr. No.	Parameters	Medicated TPGLYH	Marketed formulation
1	**Appearance**	Homogeneous	Homogeneous
2	**Color**	White	Dull, chocolate like
3	**Odour**	Mild, Mint like	Mild, mint like.
4	**Taste/flavor**	Mint	Sweet, aromatic
5	**Texture**	Smooth	Sticky, Smooth.

Table No. 4: Evaluation results of toothpaste

Sr. No.	Parameters	Medicated TPGLYH	Marketed formulation
1.	**Hard and sharp abrasive**	Absent	Absent
2.	**Spreadability (cm)**	3.7±0.5	3.4±0.3
3.	**Foaming power(ml)**	77	75
4.	**Grittiness (%by mass)**	0.2	0.3
5.	**pH**	8.7±0.6	8.5±0.5
6.	**Moisture contents%**	1.9	1.4

Content of *Arimedadi* oil

The % contents of *Arimedadi* oil in experimental toothpastes contains different types a concentration of humectants indicates greater than 96% of oil suggest uniform dispersion of oil within the paste additives.

Table 5: Contents of *Arimedadi* oil in toothpaste.

Sr. No.	Formulation code	Content (%) of *Arimedadi* oil
1.	**TPGLYH**	98.3

Efficacy of medicated Toothpaste against *S. mutans*:

Experimental toothpaste formulation of *Arimedadi* oil for anti-microbial efficacy study and it was found to be more effective in inhibiting the growth of the *S .mutans*.

Table No. 6: Antimicrobial efficacy (expressed as zone of inhibition of S.*mutans*) of toothpaste of *Arimedadi* oil.

Sr. No.	Formulation Name	Zone of inhibition(mm)
1.	Toothpaste (TPGLYH)	15
2.	Marketed formulation	12

The experimental toothpaste formulation of *Arimedadi* oil was found to be more effective against *S. mutans* than the leading brand of herbal toothpaste. Arimedadi oil product greater zone of inhibition than that of the reference product.

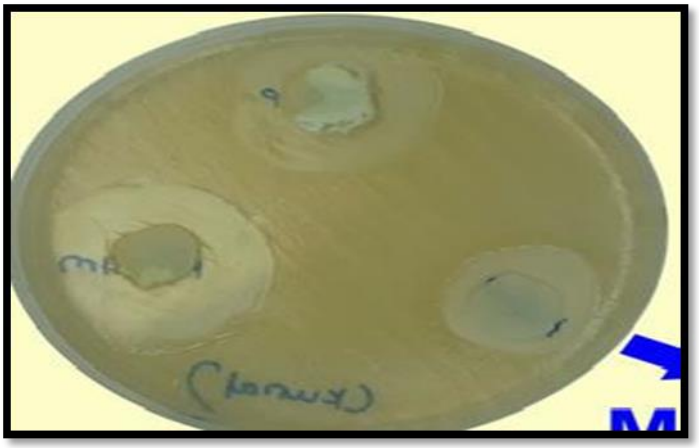


Figure No. 6: Comparative antibacterial efficacy of experimental toothpaste containing *Arimedadi* oil and marketed herbal toothpaste sample.

Stability:

Table No. 7: Stability data of toothpaste of *Arimedadi* oil (TPGLYH)

Sr. No.	Characteristics	Observations		
		0 Day	**7 day**	**15 day**
1	**Appearance**	Homogeneous	Homogeneous	Homogeneous
2	**Colour**	White	White	White
3	**Odour**	Mild, Mint like	Mild, Mint like	Mild, Mint like
4	**Test**	Mint	Mint	Mint
5	**Texture**	Smooth	Smooth	Smooth
6	**Content of *Arimedadi* oil (%)**	98.3	97.9	97.1
7	**Compatibility with primary packaging**	No probable losses of any of the organoleptic characteristics as well as contents of Arimedadi oil were noted. Moreover, the primary package did not indicate any surface coating defects such as cracking formation of pinhole –the extrude contents of paste were free from any Contaminating metal particles originated as tube extraneous source.		

CONCLUSION

This research concluded that Herbal toothpaste is more acceptable in dental research and they are safer with minimum side effect than synthetic preparation. The formulated medicated toothpaste capable to the tooth and oral hygiene and show the anti-microbial activity against pathogen (*S. mutans*). The formulation compared with marketed preparation (Dantkanti herbal toothpaste) therefore it shows the equal patronizing and engrossing passion over the marketed formulation. Oral hygiene can be maintained in a reliable, safe and inexpensive way by using Arimedadi oil toothpaste.

ACKNOWLEDGEMENT

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