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# MODIFICATION AND EVALUATION OF MODIFIED FORMULATIONS OF ORAL HEALTH AND HYGIENE BY UTILIZATION OF THE PARTS OF PHYLLANTHUS RETICULATES

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UTICAL SCIENCES

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ARMAC

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one strain of the oral bacterial strain was used.

Keywords: Phyllanthus reticulatus, S. mutans, Anti-bacterial, UV spectroscopy Correspondence to Author: Dr. Sunita Gagare Professor, Shri D. D. Vispute College of Pharmacy and Research Center, New Panvel - 410206, Maharashtra, India.	<b>ABSTRACT:</b> <i>Phyllanthus reticulatus</i> Poir., of family Euphorbiaceae was an important therapeutic plant in the traditional medicine, and gaining favour in modern medicine as a plant with varied pharmacological properties. This sericeous shrub is also known as <i>Kirganelia reticulata</i> is widely distributed in India, Thailand, China, Africa and Malaysia. Mouthwash, gargles, mouth spray were formulations were modified using leaves, fruit and stem extract of the plant. The extract of <i>Phyllanthus reticulatus</i> Poir., were studied to have antibacterial, antimicrobial as well as anti-inflammatory activity. Various qualitative tests were performed to authenticate the plant and its property. Presences of alkaloids, phenolic compounds, tannins, coumarins in extract were indicated by the phytoconstituents and UV spectral tests.
E-mail: sunita.gagare19@gmail.com	The extract was found to be stable at acidic state. Fruit extract of <i>Phyllanthus reticulates</i> contain anthocyanin (Delphinidin) which ere detected by TLC and HPTLC. The modified formulations showed good sensitivity towards strains of <i>S. mutans</i> in comparison with marketed product containing Chlorhexidine. The accelerated study proves that the modified products are stable at room temperature. Hence a valuable insight of understanding the <i>Phyllanthus reticulatus</i> typical shrub and even the fruits were used in mouthwash, gargle and mouths pray formulation. Even though the results shows safety and efficacy of formulation, the study was short term so long term study is required with large scale of the formulations as the

**INTRODUCTION:** Oral health and diseases are one of outgrowing contents globally hence oral health care has become an integral part of routine in society. The goal of oral care is to avoid oral infectious disorders such the most prevalent bacterial growth in human buccal cavity, dental caries, oral cancer, periodontal inflammations, and gingivitis. Mouthwashes, toothpaste, gargles, mouth-sprays, mouth-gels and other oral care pharmaceutical and cosmeceutical items are used as oral hygiene products. Chemicals, flavoring's, and colours made synthetically makeup the majority of oral products.

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Even though such products have anti-plaque, antimicrobial, anti-inflammatory properties with appealing appearance but their prolonged use damage the DNA of the buccal cavity, causing corrosion of tissues, reduced cell elasticity, unable to naturally discard the seamer layer naturally, a reduction in taste functionality, dentin strength reduction, corrosive enamel, oral cancer and other problems, which is why herbal and poly-herbal oral products are gaining tremendous attention in the global market.

As the increasing significant of the herbal medicine in our daily life the herbal mouthwash, mouthspray, gargles could be utilize and be indigenous for the people to maintain oral health and hygiene. One of the most crucial and multi-purposed plant: Phyllanthus reticulatus. Poir, a sericeous shrub and is a member of the Euphorbiaceae family was an important therapeutic plant in the traditional

medicine, and gaining favour in modern medicine as a traditional counterpart for synthetic colorants future compounds with varied in and pharmacological properties. Phyllanthus reticulatus also known as *Kirganelia reticulata*, is a plant that is widely found in India (habituate near fences or waste grounds), Tropicalregion of Africa and Southeast Asia, China, Thailand and Malaysia. The sericeous shrub Phyllanthus reticulatus is known by common names Krishna-Kamboji, Panjuli, Madhukaper, Black-Honey plant, Pancoli, Karineli. The fruit extract produce the dye extract that are dye-sensitization. in the **Phyllanthus** used reticulatus shows numerous medicinal possessions and curative uses due to various chemical constituents and various curing aliments. Morphologically this deciduous shrub is around 8-10 feet tall in height. They are glabrous or pubescent with numerous branches of elliptical form, sagittate and alternate leaf arrangement (acute apex with dark green ventral side) about 2-5 cm long. On modest branches the pinkish flowers are arranged axillary. Ripe fruits are black purple, fleshy berries (0.8-3 cm) with five tepals and have 8-16 irregularly trigonous seeds arranged in axial placentation. Flowering occurs from end of summer to end of monsoon season (July-October), and fruiting happens often all year round <sup>1, 2, 4, 7, 13</sup>, 15, 32

Any part of this plant, which is affordable and environmentally friendly, can be use to treat variety of dental issues. The main objective of this article is to assess the potential of *Phyllanthus reticulatus* in terms of both historical and contemporary bioscientific research findings. Additionally, the plant contain several pharmacologically relevant components <sup>13, 15</sup> that have been proven in numerous pharmacological test. In order to highlight the plants potential usefulness, this article aims to bring together with information on its various evaluative features, traditional application, phytochemistry and ethnopharmacology <sup>13</sup>.

**METHODOLOGY:** The leaves stem and fruits of *Phyllanthus reticulatus* were collected nearby Karanjade, Panvel. A voucher specimen were authenticated by *Mr. Harshad M. Pandit, Ph.D.* (*botany*) at 302 Kaveri, Bhardawadi Road, Andheri (west) (authentication number sg p 012315901). The collected leaves, stem and fruit were cleansed

in water dissolved with rock salt and then flowing purified water. Fresh fruits were preserved for crude extraction. The leaves and stem were dried in shade. They were pulverized and stored for further investigation.

**Extraction** <sup>13</sup>: *Phyllanthus reticulatus* Fruits I- For the extraction, 25gm of riped *P. reticulatus* fruits were employed. The pulp of fruit was separated from the seeds and added to mortar and pestle. The pulp was triturated with gradual addition of 5ml distilled water to it. Trituration was constant until the smooth, thick, aqueous purplish black extract was obtained. The extract was filtered on the buchner funnel. The obtained residue was ovendried at 70°C for 45 minutes. The 12g dry powder of extract obtained was easily preserved and used as needed. The filtrate obtained was stored in beaker wrapped with the aluminium foil.

II 10 gm of *P. reticulatus* fruits weighed and the seeds were separated and pulp was collected in a beaker containing 100 ml of distilled water. This aqueous solution was mixed and heated on the hot plate magnetic stirrer electronic speed control up to 100 rpm and temperature  $100^{\circ}$ C for 45 minutes. The obtained solution was stored for further research work in beaker wrapped with aluminium foil.

*Phyllanthus reticulatus* Leaves: For the extraction, 5gm of *P. reticulatus* leaves [dried & powder] and 200 ml of distilled water were collected in a beaker. On the burner the solution was boiled till concentrated extract is obtained. The extract was filtered and filtrate was stored in the beaker with aluminium foil wrapped over it.

**Phyllanthus reticulatus Stem:** For the extraction, 15gm of *P. reticulatus* stem [dried & powder] and 200 ml of distilled water were collected in a beaker. On the burner the solution was boiled till concentrated extract is obtained. The extract was filtered and filtrate was stored in the beaker with aluminium foil wrapped over it.

# Qualitative Tests of Extract: Characterization:

**Phytochemical Screening**<sup>3, 31, 35</sup>: The extracts were subjected to phytochemical studies with standard procedure.

Chemical tests were employed to investigate the screening of various secondary metabolites such as alkaloids, carbohydrates, cardiac glycosides, amino acids, flavonoid, phenolic compounds, tannins, cholesterol, triterpenoids, quinones, anthocyanins *etc*.

**UV Spectroscopy** <sup>35</sup>: UV spectrophotometry was done using SHIMADZU UV 1800 double beam spectrophotometer. Sample analysis was conducted on room temperature. The spectrum was recorded of the diluted sample where water was used as blank. The absorbance peak was recorded at 514 nm for *P. reticulatus* fruits (D.F = 25), 360 nm for *P. reticulatus* stem (D.F = 100), 275nm for *P. reticulatus* leaves (D.F = 1000).

# Chromatography <sup>34, 35, 36</sup>:

**TLC:** The Thin Layer Chromatography was carried by loading the extracts on Merck TLC F254 silica gel plates using various mobile phases for detection and isolation of constituents present in the extracts. The sample of each extract was spotted 1cm from the bottom of silica gel plate. Constituents were profiled using a variety of solvent in varied combinations and ratios. Detecting reagent or UV detector was used to visualize the chromatogram. The Rf value were reported.

**HPTLC:** HPTLC was performed on the fruit extract of *P. reticulatus* with help of following steps Sample Preparation, Sample Application, Chromatography, Photo documentation.

**pH Determination:** The pH of the fresh extract of *P. reticulatus* fruits, *P. reticulatus* stem, *P. reticulatus* leaves were checked by using both pH paper as well as pH meter. pH meter is the widely used. It is highly accurate and needs calibration with buffer solution before its use. After calibration was done the pH was checked by dipping electrode in undiluted formulations. The results were noted.

# **Stability Studies**

**Thermal Stability:** For studying the thermal stability of the *P. reticulatus* [fruit, stem & leaves] extract the absorbance of aqueous extracts were measured on various temperature range 2°C, 6°C, 28°C, 45°C, 60 °C, 100°C & 120°C. The extract was kept at this temperature for 20 min and spectrum was taken. The absorbance peak recorded at 514 nm for *P. reticulatus* fruits (D.F = 50), 360

nm for *P. reticulatus* stem (D.F = 100), 275nm for *P. reticulatus* leaves (D.F = 1000).

**Preservative Stability:** pH stability checking of the *Phyllanthus reticulatus* fruit extracts was done. The 2ml of fruit extract was added to the 3 vials to respectively. 4ml HCl, 4ml NaOH, 4ml Water were added to vials. The vials were kept in fridge for 14 days. After 14 days the absorbance was reported. As dye is stable in acidic condition HCl, Citric acid, Oxalic acid are good preservatives. Citric acid is edible, cheap, green and easily available so it was selected to check the preservative stability. Different concentration of citric acid was added to dye and stored in fridge (6°C) for 3 months. After 3 months the extract was collected and absorbance at wavelength of 514 nm was reported.

# Formulations:

**Mouthwash:** Zinc chloride and zinc sulphate were dissolved in sufficient amount of hot distilled water. While constant stirring dilute HCl and Chloroform water were added gradually. Leaves and stem extract of *P. reticulatus* were added and stirred well. Lastly fruit extract was added, the batch was mixed thoroughly for 5 minutes. The solution was filtered with Whatman's filter paper. Finally the volume was adjusted with hot distilled water. The formulated mouthwash was stored in the glass beaker and the mouth of beaker was wrapped with aluminium foil.

# Gargle:

**Part A:** In a beaker sucrose and peppermint oil were mixed and in succession leaves, stem and fruit extract of *P. reticulatus* were added.

Part B: In other beaker citric acid was added.

**Part C:** Then the content B was added in content A and mixed thoroughly. The solution was filtered with Whatman's filter paper. Finally the volume was adjusted with hot distilled water. The formulated Gargle was stored in the glass beaker and the mouth of beaker was wrapped with aluminium foil.

### **Mouthspray:**

**Part A:** Leaves, stem and fruit extract of *P*. *reticulatus*, Clove extract, Fennel extract and Sodium saccharin were mixed uniformly in a beaker.

**Part B:** In other beaker Sodium benzoate and Sucralose were mixed uniformly.

**Part C:** The content from beaker A were added into beaker B and mixed thoroughly. The solution was filtered with Whatman's filter paper. Finally the volume was adjusted with hot distilled water. The formulated Mouthspray was stored in the glass beaker and the mouth of beaker was wrapped with aluminium foil.

### **Evaluation of Formulation:**

**Organoleptic Evaluation:** Evaluations of parameters like clarity, color, odor, taste done by simple touch and visual assessment.

**Visible Absorption Spectrum:** The spectrum was recorded of the diluted sample where water was used as blank. The absorbance peak was recorded at 360 nm for mouthwash and mouth spray, 274nm for gargle.

**pH Determination:** The pH was checked by calibrated pH meter via dipping electrode in undiluted formulations and also with pH paper. The results were compared and noted.

**Refractive Index:** For checking refractive index Abbe's refractometer was used. The results were compared to standard and noted in table.

Microbial Growth Test in Formulations: To check whether the prepared formulations is microbes free and have capacity for no microbial growth formation in it, the agar test was performed on them. 5.8 gm of agar powder was weighed and added in 100 ml of distilled water in a conical flask and mixed uniformly with sterile glass rod. Then the solution was sterilized in the autoclave at temperature 121°C for 1 hour. After 1 hour the solution was taken away from autoclave, poured in the 3 petriplates careful and unvarying manner. After the agar was solidified formulations were spread over it and each pertiplate were labeled. Finally petriplates were placed in the incubator and incubated at 45°C. The results were noted after 24 hours as well as after 48 hours.

**Thermal Stability:** Accelerated stability studies were carried out on formulated mouthwash, gargle and mouthspray to determine its physical and chemical stability thus safety of product. The sample of prepared formulation were collected in vials and stored under the following temperature ranges 2°C, 6°C, 28°C, 45°C & 60°C for 15 days. Finally the samples kept under accelerated study were withdrawn after 15 days, The absorbance peak was recorded at 360 nm for mouthwash and mouthspray, 274nm for gargle, analyzed and results were registered

In-vitro Studies <sup>13, 20, 25</sup>: In-vitro antibacterial activity was performed on isolated colonies of Streptococcus mutans. The Agar well diffusion technique was used for determining the zone inhibition and minimum inhibitory concentration (MIC). 5.8 gm of agar powder was weighed and added in 100ml of distilled water in a conical flask and mixed uniformly with sterile glass rod. Then the solution was sterilized in the autoclave at temperature 121°C for 1 hour. A saline solution was prepared [0.85gm NaCl in 100 ml of water] and a loop full of S. mutans and mix well. After sterilization on agar solution it was cooled till 45°C and saline solution was added, mixed and poured in the 3 petriplates careful and unvarying manner. The strains of S. mutans were inoculated in the agar plate. Plates were dried and 4 cavities were made in each plate with help of 6mm agar cutter. 30µl, 50µl, 70µl of prepared formulations was loaded in 3 respective cavity and 70µl of marketed formulation containing Chlorhexidine in one cavity. The agar plates were kept undisturbed to allow passive diffusion of formulations into the agar culture medium. Then the plates were incubated at 37 °C for 48 hours. After 48 hours the plates were taken away from autoclave and zone of inhibition was calculated in mm.

### **RESULTS:**

# **Qualitative Tests of Extract:**

**Phytochemical Screening:** The results have shown presence of alkaloids carbohydrates, flavonoids, phenolic compound, tannins, quinones, anthocyanins, coumarinsin fruit extract.

Alkaloids, amino acids, flavonoids, phenolic compound, tannins, triterpenoids, quinones, coumarins in stem extract. Alkaloids, cardiac glycosides, flavonoids, phenolic compound, tannins, triterpenoids, quinones, carboxylic acid, coumarins in leaves extract.

### **UV Spectroscopy:**



FIG. 2: UV SPECTROSCOPY OF LEAVES EXTRACT FIG. 3: UV SPECTROSCOPY OF STEM EXTRACT

### **Chromatography:**

### TABLE 1: RESULT OF HPTLC (FRUIT EXTRACT)

No.	Constituent	Rf	Components
1	Flavonoids	0.23, 0.5	Quercetin,
			Anthocyanin
2	Tannins	0.01	Methylellagic acid
3	Triterpenoids	0.75, 0.59	Lupeol, Betulinic acid
4	Coumarins	0.20, 0.79	Gallic acid, p-Couaric
			acid

When the Rf value were compared to standard limits and the components mentioned in **Table 1** were detected in the fruit extract. HPTLC 12 peaks were observed on 2 bands giving Rf value which were compared with standard limits. The component was found to be Delphinidin.

**pH Determination and Thermal stability:** The pH of the extracts was found in the weak acidic range and the extracts were stable in acidic condition.

As dye is stable in acidic condition HCl, Citric acid and Oxalic acid are good preservatives. Citric acid is edible, cheap, green and easily available so it was selected to check the preservative stability. The aqueous fruit extract was stable in acidic condition for 3 months. The dry powder of leaves and stem can be preserved for 5 months when kept in tightly closed container.

### **Thermal Stability:**



ABSORBANCE

# **Evaluation of Formulation: Organoleptic Evaluation:**

#### **TABLE 2: RESULT OF ORGANOLEPTIC EVALUATION**

Parameter	Mouth Wash	Mouthspray	Gargles
Clarity	Clear	Clear	Clear
Colour	Crimson	Ruby Red	Mahogany
Odour	Pleasant	Strong	Sweet
		Fragrant	

### Visible Absorption Spectrum:



#### TABLE 3: RESULT OF PH, REFRACTIVE INDEX, MICROBIAL GROWTH, THERMAL STABILITY

	Mouthwash	Gargles	Mouthspray
pH determination	In limits	In limits	In limits
Refractive index	In limits	In limits	In limits
Microbial growth	No growth seen in 48 hours	No growth seen in 48 hours	No growth seen in 48 hours
Thermal stability	Stable at 6 °C to 45 °C	Stable at 6 °C to 45 °C	Stable at 6 °C to 45 °C

#### In-vitro Studies:

### TABLE 4: RESULT OF IN-VITRO STUDIES

Formulations	Concentration of Formulation			
	P. reticulatus			Marketed
	30 µl	50 µl	70 µl	70 µl
Mouthwash	14mm	20mm	24mm	20 mm
Mouthspray	-	19mm	23mm	18mm
Gargle	10mm	16mm	25mm	21mm

The antibacterial property of the formulations was more than the marketed products as the zone



FIG. 8: ZONE INHIBITION OF MOUTHWASH

inhibition was observed to be large then the marketed product containing Chlorhexidine.



FIG. 9: ZONE INHIBITION OF MOUTH SPRAY

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**DISCUSSION:** The phytoconstituents like alkaloids, flavonoid, anthocyanin, tannins, triterpenoids, coumarins were detected. The fruit was found to be stable in the acidic condition, leaves and stem were found to be stable in acidic



FIG. 10: ZONE INHIBITION OF GARGLES

The mouthwash, gargle and mouthspray with natural additives formulated and evaluated on specific parameters successfully. From the above results the formulations were found to clear optically no suspended particle were seen with appealing colour, pleasant and agreeable odour. They have agreeable and sweet taste with soothing and astringency feel in the mouth. The pH and the refractive index of the formulations were found to be within the limits. No microbial growth was seen in any of the formulations. Accelerated stability study showed that physically and chemically the formulations are found to be stable within temperature ranging from 6 °C to 45 °C.

*In-vitro* studies showed that the antibacterial property of the formulations were more than the marketed products as the zone inhibition was more. The results of physicochemical examination show that the colour and odor of current herbal formulation are agreeable with pleasant odor and better after effects.

The pH of the mouthwash, mouthspray and gargle was in the range to be used and rinse the mouth and throat. The formulation was microbes free which determines its safety to use. The zone inhibition data also revealed that these formulations are effective plaque inhibitor, oral infections, foul breath, bacterial inflammations *etc* then the marketed synthetic products and ease of use. The zone inhibition test indicates that *Phyllanthus reticulatus* fruits and leaves have high anti bacterial property then the marketed products which is major

and neural condition. From the results the extract was found to be stable at the temperature of  $6^{\circ}$ C to  $60^{\circ}$ Cand unstable at very high and very low temperature. Citric acid has been shown to be an effective preservative for fruit extract.



FIG. 11: GRAPH OF ZONE COMPARISON

finding from the future herbal product advantages and markets globally.

**CONCLUSION:** The findings clearly indicate that these formulations are highly anti-bacterial, desensitizer and posse's anti-inflammatory properties which helps inhibits induced germs. These formulations are appropriate for human use as they qualify quality control and stability evaluations. The *Phyllanthus reticulatus* have been proven to work miracles for years and decades, as evidence by several research findings.

These formulations can be easily used for rinse mouth bacteria's. As a result they can be used in conjunction with synthetic and mechanical therapies to treat the oral disorders. The current report has a significant impact on the development of effective and low cost interventions for low income areas. Hence a valuable insight of understanding the *Phyllanthus reticulatus* typical shrub and even the fruits were used in mouthwash, gargle and mouthspray formulation.

Even though the results shows safety and efficacy of formulation, the study was short term so long term study is required with large scale of the formulations as the one strain of the oral bacterial strain was used. The formulated liquid herbal mouthwash, gargle and mouth spray can go long way toward helping people to get rid of varied oral health problems. Apart from that, we can be rest secure and take comfort in knowledge that there are no unhealthy substances or chemical in the formulation.

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### **CONFLICTS OF INTEREST:** Nil

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