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Formulation and Evaluation of Anti-bacterial and Anti-Inflammatory Mouth Wash Containing Cinnamomum Zeylanicum and Aloe Barbadensis

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ABSTRACT

Medicinal plants, plays a predominant role in curing and preventing diseases due to their antiviral and antimicrobial activity against human microorganism. Herbal Mouthwash are in high demand as compare to chemical mouthwash, because they act on mouth pathogen and microbes and reduce the pain instantly and are also has a no more side effects. The most commonly infectious diseases cause by a many pathogens and microbes are Dental carries and periodontal diseases at different stages of their life time. The aim of present work is to formulate and to evaluate itseffectiveness against microbes present in oral cavity. The four herb Azadirecta indica (neem), Mentha longifolia (mint), Syzgium aromaticum (clove) and Ocimum sanctum (tulsi) were selected for mouth wash and Prepared formulation was further evaluated for physical properties like pH, color and stability. The present mouthwash possesses a good antibacterial property. This preparation is stable in different temperature condition Present mouthwash is a liquid preparation which normally contains antibacterial and antiseptic agents. These solutions can be used to reduce the microbial growth and it reduces infection in the oral cavity.

Keywords: Soxhlet Extraction, Hydro Distillation, Perfume.

INTRODUCTION

Mouthwashes are solutions or liquids used to rinse the mouth for a number of purposes:(a) to remove or destroy bacteria (b) to act as astringent (c) to deodorize and (d) to have a therapeutic effect by relieving infection or preventing dental

caries. Mouthwashes also provide a safe, effective chemical means of reducing or eliminating plaque accumulation. In contradiction to one‘s thinking that mouthwashes are used only for refreshing the oral cavity or just for treatment of dental carries and cleansing the mouth, it has found much use in preventing and treating infection of mouth, treating inflammatory cases like ulcers and relieving the pain related to its etc. Aerosol mouthwash or mouth fresheners are recommended for breath freshening after eating, drinking or smoking and usually contain only flavoring agent, though they may contain antibacterial agents. Products used for freshening breath or cleaning teeth have been in existence for centuries. Water is the simplest mouthwash and aqueous saline is the least complex type of mouthwash. Many of the ancient societies including the Egyptians, Chinese, Greeks and Romans had recipe for such preparations. They used a variety of ingredients; from edible materials like fruit, honey or dried flowers to less appealing compounds such as ground lizard, minced mice or urine. These products were generally ineffective and in some cases were harmful to the sensitive enamel coat each tooth. While tooth cleaning preparation steadily improved over the years, it was not until the early 1800s when the modern toothpaste was developed that truly effective oral products became available. The first mouthwash was basically solution of grain alcohol and was likely developed accidentally during this era. One of the most famous brands, Listerine was developed during the 1800s and is still sold today. (1)

Functions and mechanism of mouthwash

Mouthwash can control the bad breath to a marked degree by one or more of the following mechanism like, Cleaning the teeth and tissue so that fermentative and putrefactive debris is mechanically removed, inhibiting the bacterial enzymatic activity in mouth so that the malodorous end product are not easily formed, using ingredients that modify or eliminate odorous substance by chemical reaction or physical adsorption, substituting pleasant odors for undesirable ones by a masking effect. But the major concern which leads to frequent use of mouthwashes is halitosis. Secondary reason for the use of mouthwash includes control of a plaque and gingivitis when used as an adjunct to mechanical means. The primary function of mouthwash is to freshen the mouth and Breathe by swishing/ swirling the

product in the mouth, followed by expectoration. This is achieved by a combination of three factors: the mechanical effect of rinsing debris from mouth, the effect of the flavor, the effect of any agent specifically added to deliver the required end result like antibacterial, astringent, etc.(2)

There is wide latitude in the choice of mouthwash components, depending on the characteristics and end purpose sought. Numerous chemical ingredients can be chosen for their antibacterial effect; the choice of flavor components can often be greatly varied; ingredients for special purposes, such as penetrants, astringents, therapeutic and preventive compounds and deodorants also are subject to great variation. [2]

MATERIALS AND METHODS

Methods

Selection of active

The analysis of Cinnamon and aloe plant parts contains cinnama ldehyde, cinnamic acid, cinnamyl acetate, cinnamyl alcohol, coumarin, eugenol, linalool, phenol, beta-caryophyllene, which provides a broad antimicrobial spectrum against different microorganisms and make it an interesting alternative to synthetic antimicrobials. Aloe vera is made up of water, amino acids, vitamins, lipids, sterols, tannins, and enzymes and also contains phenol, saponin, anthraquinones components, which have antiviral, antibacterial, and antifungal properties. Aloesin, formerly called aloeresin aloeresin A isoaloeresin D and aloeresin E [3,4]

Collection and Authentication

Herb authentication is a quality assurance process that ensures the correct plant species and plant parts are used as raw materials for herbal medicines. The proper authentication of herbal raw materials is critically important to the safety and efficacy of herbal medicines.

Cinnamomum Zeylanicum and Aloe Barbadensis were purchased from local market and authenticated in botanical department by botanist.

Extraction Method

Grinding Mill

A mill is a device that breaks solid materials into smaller pieces by grinding, crushing, or cutting. Such comminution is an important unit operation in

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many processes. There are many different types of mills and many types of materials processed in them.(5)

Soxhlet Extraction

Soxhlet extraction is a continuous solid / liquid extraction. A solid which contains the material to be extracted is placed in what is called a thimble. A thimble is made out of a material which will contain the solid but allow liquids to pass through. A lot like filter paper. The thimble containing the material is placed in the Soxhlet extractor. An organic solvent is then heated at reflux. As it boils its vapors rise up and are condensed by a condenser (5)

Selection of base

The main objective of the present study was to prepare a Mouthwash from Herbs Cinnamon and aloe base are used.

Table 1: Formulation of Mouthwash

Ingredients	Parts Used	Category	Qty%
Cinnamomum Zeylanicum	Bark	Anti-bacterial Anti-inflammatory	20
Aloe Barbadensis	Leaves	Anti-inflammatory Anti-bacterial	20

Table 2: Formulation Of Base

Ingredients	Category	Qty%
Mint Oil	Flavouring agent	5
Saccharin	Sweetning agent	5
PEG 40	Surfactan	6
Glycerol	co surfactant	4
Alcohol	Preservative	10
Distilled water	Vehicle	30

EXPERIMENTAL WORK

Soxhlet extraction

Soxhlet extraction has traditionally been used for a solid sample with limited solubility in a solvent in the presence of insoluble impurities. A porous thimble loaded with a solid sample is placed inside the main chamber of the Soxhlet extractor. By refluxing the solvent through the thimble using a condenser and a siphon side arm, the extraction cycle is typically repeated many times. Soxhlet extraction is a rugged, well-established technique and permits unattended extraction. However, it requires a long extraction time and the consumption of a large amount of solvent. Soxhlet extraction is a very useful tool for preparative purposes in which the analyte is concentrated from the matrix as a whole or separated from particular interfering substances. Sample preparation of environmental samples has been developed for decades using a wide variety of techniques. Solvent extraction of solid samples, which is commonly known as solid–liquid extraction (also referred to as leaching or Lixiviation in a more correct use of the physicochemical terminology), is one of the oldest methods for solid sample pretreatment.(6)

Fig. 1: Soxhletion Apparatus

Procedure

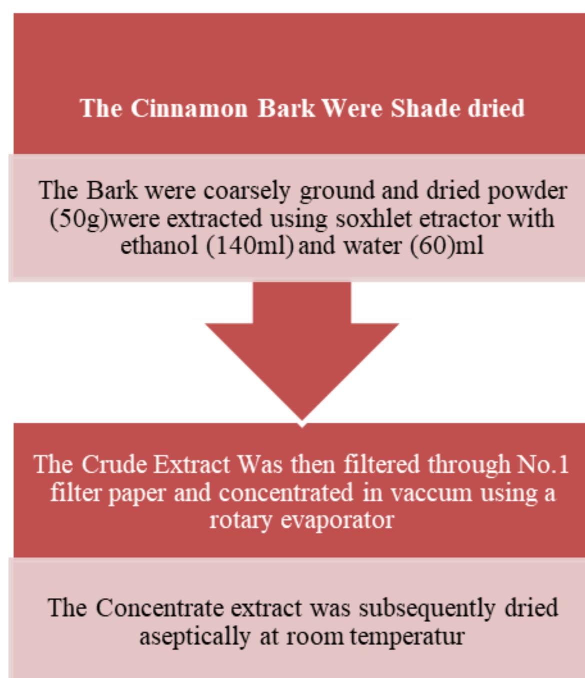

Fig. 2: Extraction of Cinnamon Oil From Bark

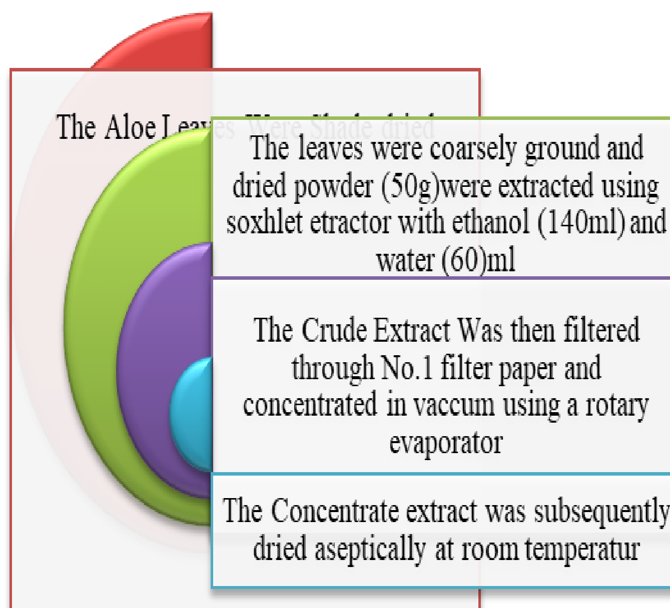

Fig. 3 : Extraction Of Aloe Barbadensis

Phytochemical screening (7)

The concentrated extracts of selected plant was subjected to different chemical tests for the detection of different phytoconstituents using standard methods

Test for saponins

Crude extract when mixed with 5ml distilled water in a test tube then it was shaken briskly. The formation of stable foam which indicate the presence of saponins.

Test for flavonoids

Crude extract when mixed with 10ml distilled water, 5ml of dilute ammonia solution were added to a portion of the aqueous filtrate solution then added 1ml concentrated sulphuric acid. Indication of yellow color shows the presence of flavanoids. [8]

Test for steroids

The crude extract of selected plant was dissolved in 0.5mL dichloromethane to prepare a dilute solution and then 0.5 mL of acetic anhydride was added followed by four drops of concentrated sulphuric acid. A blue-green colouration indicated the presence of steroids. [9,10]

Test for tannins

Curde extract of plant was mixed with small amount of water and heated on water bath. The mixture was filtered and ferric chloride was added drop by drop to the filtrate. A dark green appear which indicates the presence of tannins. [11,12]

Test for Alkaloids

Curde extract was dissolved with 2ml of 1% HCl and heated gently. Wagners and Mayers reagents were added to the mixture. Turbidity of the resulting precipitate was taken as confirmation for the presence of alkaloids.

Test for carbohydrate

Both Felhing A and Felhing B solution were mixed in equal volume. These reagent are added in crude extract and smoothly boiled. A brick red precipitate is appeared at the bottom of the test tube and indicate the presence of reducing sugar]13,14]

Table 3: Preliminary Phytochemical Analysis of Leaves

Phytochemical Constituents	Leaves Extract		
	Ethanol	Chloroform	Hexane
Alkaloids	+	+	-

Flavonoids	+	+	+
Terpenoids	+	+	-
Tannins	-	+	-
Saponins	+	-	-
Carbohydrate	+	+	-

+ = **indicates presence of phytochemicals, - = indicates absence of phytochemicals**

Table 4: Preliminary Phytochemical Analysis of Cinnamon Bark

Sr. No.	Chemical constituents	Cinnamon (Bark Extract)
1	Resin	-
2	Tannins	+
3	Anthraquinones	-
4	Trepenoids	+
5	Flavonoids	+
6	Alkaloids	-
7	Carbohydrates	+
8	Saponin	-

+ = **indicates presence of phytochemicals, - = indicates absence of phytochemicals**

Test for Antioxidant Activity of Extracts

Reducing Power method

Principle

This method is based on the principle of increase in the absorbance of the reaction mixture. Increase in the absorbance indicates increase in the antioxidant activity. In this, the antioxidant compound forms a coloured complex with potassium ferricyanide, trichloroacetic acid and ferric chloride, which is measured at 700nm.

Increase in absorbance of reaction mixture indicates the reducing power of the samples. [15,16]

Table 5: Determination of Antioxidant Activity of Extract

Sr. No.	Extract	Concentration (ug/ml) and absorbance	
1	**Cinnamon Oil**	**10**	**20**
		0.18	**0.96**

Microbial Assay

A microorganism occurs nearly anywhere in nature. They are carried by air currents from yhe earth's surface to the upper atmosphere. They occur most abundantly where they find food, moisture and temperature suitable for their growth and multiplication. They can be found on the surface of our bodies, in our mouth and on feet.

Material required

a) Instruments: autoclave, incubator

b) Medias: Nutrient agar

c) Reagent: Sterile distilled water

d) Apparatus: conical flask, test tube, measuring cylinder, Petri dishes, beaker, non-absorbent cotton plugs, inoculating needle, micropipette, burner.

All the apparatus was cleaned, dried and sterilized. The sterilization was done by carefully wrapping the apparatus in a paper and autoclaving in a hot air oven at 1600C for 1.5 to 2hrs.

Preparation of media

Weighed a medium in required quantity and added to a dry conical flask.

Added required quantity of freshly distilled water, a little at a time with constant agitation to prevent the formation of lumps.

Heated the flask in boiling water bath with intermittent stirring till the contents were completely dissolved.

Filled the test tubes using a funnel when medium is hot.

Plugged the test tubs of medium using non-absorbent cotton, covered them with paper and tie them together using a rubber band sterilized for 15 minutes at 1210C i.e. at 15 ibs p.s.i. pressure. [17,18]

Handling of culture media

1. the container was closed properly after use because the culture media were hygroscopic and forms lumps when exposed to high humidity or left open for a long time or if spatula wet.
2. Used the containers of proper size for preparation of media.
3. The media was melted using a water bath and media must be crystal clear.
4. Avoided overheating, incomplete mixing, prolonged sterilization, repeater melting and prolonged storage at high temperature.
5. Stored the prepared media in clean, cool and dry place to prevent drying Use the fresh medium a few days from date of preparation.

Aseptic transfer of microorganisms

Material required for aseptic transfer of microorganisms:

Sterile test tubes, inoculating needle, gas burners, disinfectant solution and pure culture of microorganisms.

Procedure

1. Wash the hand with disinfectant soap and apply disinfectant gel.
2. The working platform was cleaned with a disinfectant solution using a cotton swab the microorganisms present on the working platform.
3. The inoculating needle was heated to redness by holding vertical in the flame and the steel rod was heated on which the needle is mounted. This was necessary to destroy the microorganisms, which were presented on the inoculating needle.[19,20]
4. Holed the culture tube and media tube in left hand near flame and removed and holding the plugs with fingers of right hand. The plugs were not kept on the working platform. The tube was holed in approximately horizontal position and do not keep them open for a long period than necessary.
5. The mouth of test tube was pressed through flame. This destroyed the microorganisms on and near the mouth of the test tube. Flaming of the mouth also

creates outward convection currents, which decreases the chances of contamination.

6. A little growth of microorganisms was removed with sterilized and cooled loop transfer it on the fresh medium by moving the loop on medium surface in zigzag manner only once.
7. Sterilized the used loop in flame avoiding spurting.
8. Flamed the mouth of test tubes and plug them. The working platform and hand was cleaned as described earlier.
9. Incubated the inoculated tubes at 370C in an incubator and observed the growth after 24hrs for bacteria and 48hrs for fungi.

Observation

Growth of microorganisms on agar medium slope is observed as opaque mass of cell.

Preparation of petri dishes

1. The nutrient medium was melted in a hot water bath.
2. They were allowed to cool up to 45-
3. The melted nutrient agar was poured into petri dish, the dishes were immediately covered and were allowed to solidify.

Method used for evaluation of microbial activity of Cinnamon and Aloe

The well diffusion method: [21,22,23] **Naina Pattnaik et.al 2022**

- The Petri dishes prepared in the above step are used in this method.
- After the media solidified small well (3-4mm) bored on the media with the help of sterilized cork borer.
- Different concentration of extract prepared.
- These well were filled with different concentration of extract of Cinnamon and aloe incubated at 340C for 24hrs.
- Tetracycline solution used as standard.
- After 24 hrs. zone of inhibition was observed.
-

Table 6: Concentration Of Extract

Sr. No.	Concentrations	Extract
1.	1500mg	1.5ml
2.	2000mg	2ml

Observation

Following plate No.1&2 showing measurement of zone of inhibition when using well diffusion method for Propionibacterium acnes.

Result

As per the observation the zone of inhibition was found in well diffusion method against Staphylococcus aureus, Streptococcus pyogenes [24, 25, 26]

Evaluation Tests for Product

Thermal Stability

Formulation and development of a pharmaceutical product is not complete without proper stability analysis carried out on it to determine physical and chemical stability and thus safety of the product. A general methodology for predicting the stability is accelerated stability analysis which subjects the material to elevated temperatures. The stability studies were carried out as per ICH guidelines. Short term accelerated stability study was carried out for the period of 3 months for the formulation. The samples were stored at different storage conditions of temperatures such as 3-5oC, 250C RH=60% and 40oC±2% RH=75%. Samples Were withdrawn on monthly interval and analysed (50.) [27]

Table 7: Thermal Stability

Stability	1st week	2nd week	3rd week	4th week
A	Stable	Instable	Instable	Instable
B	Stable	Stable	Instable	Instable

C	Stable	Stable	Stable	Stable
D	Stable	Stable	Stable	Instable

Conclusion: From the above test it was observed that formulation C was stable throughout stability period, formulation A, B, and D shown instability at week 3 week 4.

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

Conclusion: pH of prepared Mouthwash is 6.5. [28]

Color and Odor: Physical parameters like odor and color was test by visual examination.

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.[29]

Stability Studies: The formulation and preparation of any product is incomplete without proper stability studies of the prepared 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 3-50 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.[30]

Preliminary antimicrobial activity of the pure oil and leave extract: The preliminary sensitivity of the aqueous leave extract against the test-organisms was assessed by the agar well diffusion method. Stock solution of the aqueous extract

can be prepared. Holes of diameter 4mm were aseptically bored with a sterile cork-borer on plates previously seeded with 0.1ml standardized inocula of the respective test organisms and then 0.2 mL of the graded concentration of the aqueous extract of active was introduced into them using separate plates for each organism. The assay plates were held at 4°Cfor 24 h for rapid diffusion of extract into the assay medium and suppression of immediate microbial growth and subsequently incubated at standard growth conditions for each test microorganism. After incubation, plates were observed for the presence or absence of growth inhibition zones.[31

Antimicrobial Study - Agar Diffusion Method: In vitro antibacterial activity was performed on isolated colonies of Streptococcus mutans. The Agar well diffusion technique was used for determining the zone of inhibition and minimum inhibitory concentrations (MIC). The strains of S. mutans were inoculated in prefabricated blood agar plate. Plates were dried and 3 wells were made with the help of 6 mm agar well cutter. 20µl, 40µl, 60µl of prepared mouthwash was loaded in all the respective wells. The agar plates were kept undisturbed to allow the passive diffusion of herbal mouth wash into the agar culture medium. Then the plates were incubated at 37°C for 24 hours. The zone of inhibition was calculated in mm.[32]

RESULT

The pH of the formulation was found to be 6.5. 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 studies are shown in the given Table [33]

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Table 8: Results of Stability Studies

Temperature	Evaluation parameter	Observation (months)			
		0	**1**	**2**	**3**
3 – 5°C	Visual Appearance	White to Yellowish	White to Yellowish	White to Yellowish	White to Yellowish
	Phase Separation	Nil	Nil	Nil	Nil
	Homogeneity	Good	Good	Good	Good
	pH	6.4	6.4	6.5	6.5
	Odor	No change	No change	No change	No change
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
	pH	6.4	6.4	6.5	6.5
	Odor	No change	No change	No change	No change
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
	pH	6.4	6.4	6.4	6.4
	Odor	No change	No change	No change	No change

A mouthwash containing Cinnamon and Aloe it is the best formulation is F2 now the stag mouthwash have been prepared long time it is freshness good antibacterial

in mouth bacterial disease and overcome and herbal mouthwash. It is stable in ph, color and odor for long time storage. Alcohol consumption as well as alcohol and tobacco use is 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 Cinnamon and Aloe other essential plant extracts have been found to reduce plaque and gingivitis when combined with daily brushing and flossing. They arise from a variety of sources that is breakdown of food, dental plaque and bacteria associated with oral disease.

It gives fresh breath and gives the good order and Cinnamon and Aloe plant gives in plant the good color of the preparation and it is also the clear the throat infection it is also the good antibacterial effect. Cinnamon in plant a good aromatic order and it constitute and Cinnamon and Aloe beneficial for teeth problem and also the anti-inflammatory agent.

The antibacterial activity was evaluated by agar diffusion method shown in the given Table . The result of zone of inhibition for S.mutans was found to be 25 mm for 60 µl, 20 mm for 40 µl, 16 mm for 20 µl respectively and 27 mm for 60 µl, 25 mm for 40 µl, 18 mm for 20µl respectively for S. salivarius. 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. The use of mouthwashes as cleaner can help mechanical methods to reduce plaques. Herbal formulations are safe to use as a gargle also as its systemic availability in traces does not cause any side effects.

Table 9: Result Of Agar Well Diffusion Antibacterial Assay

Formulation Batch			**F1**	**F2**	**F3**
S. No.	**Ingredient**	**Organism**	**Zone of inhibition (mm)**		
1.	Prepare formulation	S. mutans S.salivarius	16 18	25 27	20 25

Antibacterial activity

In vitro antibacterial activity was performed on isolated colonies of Streptococcus mutans. The Agar well diffusion technique was used for determining the zone of inhibition and minimum inhibitory concentrations (MIC). The strains of S. mutans were inoculated in prefabricated blood agar plate. Plates were dried and 3 wells were made with the help of 6 mm agar well cutter. 20µl, 40µl, 60µl of prepared mouthwash was loaded in all the respective wells. The agar plates were kept undisturbed to allow the passive diffusion of herbal mouth wash into the agar culture medium. Then the plates were incubated at 37°C for 24 hours. The zone of inhibition was calculated in mm. [34]

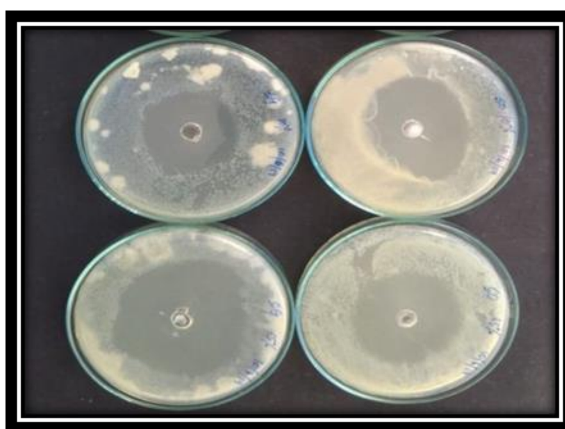

Fig. 4: Antibacterial Activity

Optimization of Oil Extraction

In order to optimize the extraction operating conditions for achieving maximum oil yield, the distillation was conducted at three unlike parameters conditions which were at different water to Raw material ratio (v/w)(8:1, 10:1, 12:1),different extraction period (30min, 60min, 90min, 120min, 150min) and different operating power (200W, 250W). [35;36]

Analysis of Sample

The extracted essential oils was dried over anhydrous sodium sulfate, weighed and stored in amber vials at 4 0c for the use of analysis. The amount of yield obtained from the extraction was analyzed to evaluate the performance of MAHD in Cinnamon extraction. As the result the yield of oil that obtained for every run was calculated by using [37]

$$\text{Yield of essential oil (\%)} = \frac{\text{amount of oil (c) obtained x100\%}}{\text{Amount of raw materials (c) used}}$$

The extracted essential oil samples were analyzed by using Gas Chromatography Mass Spectrometry (GC-MS) Agilent 6890 gas chromatography instrument coupled to an Agilent 5973 mass spectrometer and an Agilent Chem in order to identify their chemical constituents. This is an essential method to evaluate the quality of the oil samples. The following operating parameters were used for Cinnamon sample: capillary GC column HP-5MS 5% phenylmethyl siloxane \ (30 x 0.25 mm i.d. x 0.25 mm film thickness), a carrier gas Helium (flow rate 1.2 mL min-1) and a split-less injection mode. Injector temperature is 250°C, Oven temperature will be set initially at 50°C, and then will be raised to 250°C at a 10°C min-1 rate till the end of analysis. The eluted analytes detected using (5973 network) mass selective detector and Electron Impact ionization (EID) will be carried out at 70 eV .While, Cinnamon oil sample, the operating parameters of GC-MS was as followed: system operating in EI mode (70 eV) , equipped with a split/splitness injector (280 ºC, split ratio 1:20) , using DB -5 column (30 x 0.2 5 mm i.d x 0 . 2 5 mm) . The temperature program was 50ºC (5 min) rising to 300ºC. at rate of 5ºC/min. Injector and detector temperature was 280ºC. Helium was used as carrier gas at a flow rate 1 mL /min [38]

Effect of Extraction Time on Yield:

The yield of oil extracted from Cinnamon at different extraction time in a fix microwave power of 250W and water to raw material ratio of 8:1. From the graph, the amount of yield does not change significantly after 90 minutes. Most of the oil is extracted within 30 to 90 minutes with the yield of 0.61%, 0.80% and 0.85% respectively. Microwave assited hydrodistillation (MAHD) reach the highest yield of 0.85% w/w when extraction time was 90 minutes. However, further increase in extraction time resulted in no improvement in the extraction performance. Similar observations were also reported for MAHD of Satureja hortensis and Satureja Montana by. The extraction was fast at the beginning of the extraction but get slow gradually by time because when the raw material is exposed to the heat, the plant cell started to degrade and as a consequence the essential oil is released to

the environment. However prolong the extraction time caused over heat supplied to the plant material and this lead to the evaporation of the volatile component in the oil [38,39,40]

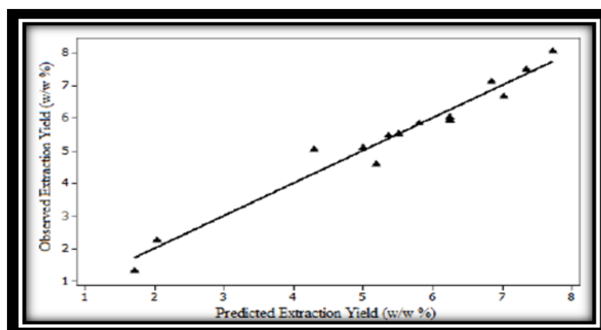

Fig. 5: Extraction Yield from Cinnamon and Aloe at Different Extraction Time by 250w In Raw Material To Water Ratio

The yield of oil extracted from Cinnamon and Aloe at different extraction time in a fix microwave power of 250W and water to raw material ratio of 8:1. From the graph, the amount of yield does not change significantly after 90 minutes. Most of the oil was extracted within 30 to 90 minutes with the yield of 0.75%, 1.29% and 1.37% respectively.

Determination of Cinnamon and Aloe Solubility and partition coefficient (Ko/w): The solubility of oils in n-hexane was 0.00407±0.25mg/ml, 0.00185±0.45mg/ml in methanol, 0.00084±0.67 mg/ml in PBS .The Partition coefficient (Ko/w) for oils was 3.6. From this value, it was shown that given drug comprised of about sufficient lipophilicity that is beneficial to develop the topical drug.

Soxhletion Method

Result obtained by is shown in Table below

Table 10: Weight of Oil With Respect To Time

Weight (g)	Time (mins)
0.30	250
0.40	490
0.50	730
0.55	980
0.75	1180

The oil produced by Distillation Method is 2.55g weight of oil per 130g of dry Cinnamon sample thereby producing 1.96% oil yield at 780C

Table 11: Result of Extraction

Method of extraction	% yield
Soxhletion Method	**1.96**

Calculation of Percentage Yield of Volatile Oil

Material Balance for Soxhletion Method of Cinnamon Bark

a) Weight of sliced l Cinnamon Bark= 100g
b) Quantity of Olive oil used= 600ml, Quantity of Ethanol used= 140ml
c) Weight of beaker= 97.86g
d) Weight ethanol and essential oil= 100.41g
e) The total weight= 2.55g
f) %yield = ME/MC x 100
   Where,
g) ME = Mass of Extract, MC = Mass of Cinnamon Sample
h) ME = 2.55g
i) MC = 100g
j) By substituting values
k) %yield = 2.55/100 x 100 = 2.55%
l) Therefore % yield= 2.55%

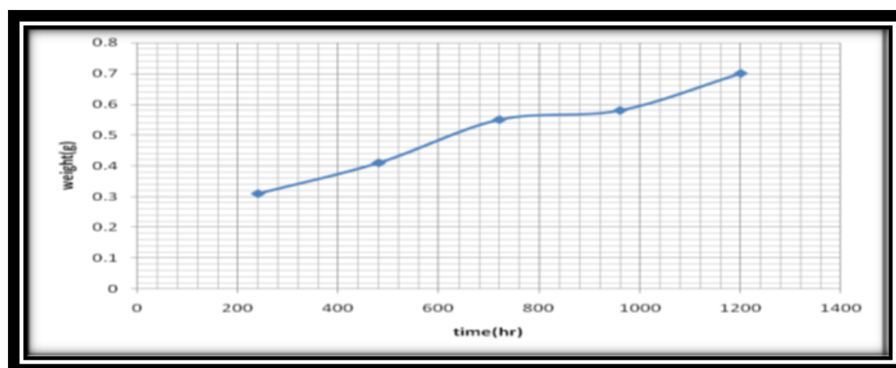

Fig. 6: Graph of the Weight (C) Of Essential Oil to the Time (Mins) For Extraction Method

Material Balance for Soxhletion Method of Aloe Leaves

a) Weight of Aloe Leaves= 100g

b) Quantity of Olive oil used= 600ml
c) Quantity of Ethanol used= 140ml
d) Weight of beaker= 97.86g
e) Weight ethanol and essential oil= 100.41g
f) The total weight= 2.55g
g) %yield = ME/MA x 100
h) ME = Mass of Extract, MA = Mass of Aloe Sample
i) ME = 2.55g
j) MA = 100g
k) By substituting values
l) %yield = 2.55/100 x 100 = 2.55%
m) Therefore % yield= 2.55%

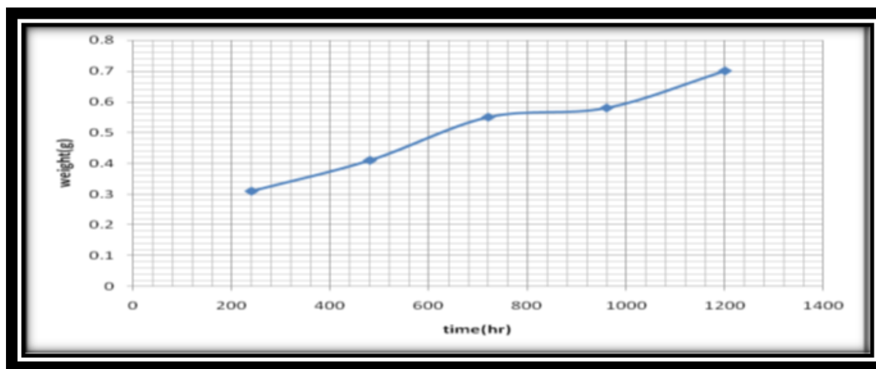

Fig. 7: Graph of the Weight (A) Of Essential Oil to the Time (Mins) For Extraction Method

CONCLUSION

The data presented in this study, it was concluded that the developed herbal mouthwash possess significant, therapeutically efficacious, suitable vehicle for drug delivery in low cost but definitely with high potential. Herbal Mouthwash preparations have potent action and minimal side effects when compared with that of the other marketed mouthwashes, hence there is need for increased usage of herbal preparations to avoid the adverse effects.

The present liquid herbal mouthwash can work in long way to help people to get rid of bad breath and many oral disorders. Besides we can be rest assured and take comfort in the fact that there aren't any unhealthy ingredients present in this

preparation. The physicochemical evaluation results confirm that the color and odor of present herbal formulation is acceptable with a pleasant odor and a better after effects. However this study was short-term study so long term studies are required with larger. The natural herbs used in present formulation have been medicinally proven to prevent the problem of oral hygiene and bad breath. Since years and decades, these herbs have been known for working wonders as reflected in many research findings. Person can easily rinse his mouth using this herbal mouthwash and stay clear of wide variety of oral health issues.Microbial dental plaque is a tenaciously adhering biofilm that grows intra-orally on hard and soft tissues. Bacterial colonies can accumulate on tooth surfaces and lead to periodontal disease. Gram-positive aerobic bacteria are early colonisers of dental plaque, followed by gram-negative anaerobic and fusiform bacteria. The primary goal of periodontal disease prevention is plaque reduction. Although tooth brushing is the most reliable source for mechanical plaque control, factors such as lack of manual dexterity, skill, or motivation hamper its effectiveness. Hence, chemical antiplaque agents in various formulations have been introduced to improve oral health. Rinsing with mouthwashes is easier to perform than tooth brushing, and it aids in controlling supragingival plaque and gingivitis along with mechanical plaque control.

The aim of this study was to prepare a polyherbal mouthwash and evaluate its antimicrobial and anti-inflammatory efficacy against commercially available herbal mouthwash. The objective was to signify whether the novel herbal combination (extracts of Cinnamomum Zeylanicum and Aloe Barbadensis could be a better alternative to commercially available herbal mouthwashes.

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CONFLICTS OF INTEREST

Authors have no conflicts of interest to declare.

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