Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2015; 7(3); 424-430

ISSN: 0975-4873

Research Article

Development and Evaluation of Polyherbal Tablet Triturate for Oral Hygene

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Available Online: 13th April, 2015

ABSTRACT

Herbal remedies as a source of medicine has been a primordial practice and important constituent of health care system in India. The natural phytochemicals have a long history of use for gum and tooth problems which consist of Anti-bacterial, Astringent, Immune strengthens activities. The exploration of botanicals used in traditional medicine, may lead to development of novel preventive or therapeutic strategies for oral health. The incidence of multidrug resistance in bacterial population has increased the importance of herbal medicines. In this study we have evaluated antibacterial activity of ethanolic extract of *Rubia cordifolia, Terminalia chebula, Hemidesmus indicus, Azadirachta indica, Ocimum tenuiflorum, Caryophyllus aromaticus, Cinnamomum zeylanicum, Quercus infectoria, Emblica officinalis, Terminalia chembula, Acacia Arabica* against mixed dental flora. Agar well diffusion method was used to determine antibacterial activity of the extracts against mixed dental flora. This polyherbal extracts inhibit growth of dental flora and can be used for dental health or oral hygiene. Tablet triturates were prepared from above extracts and it shows good antibacterial activity against mix dental flora. Hence the natural phytochemicals could offer an effective alternative to antibiotics and represent a promising approach in prevention and therapeutic strategies for dental caries and other dental infections.

KEYWORDS: Antibacterial Activity, Phytochemicals, Agar well diffusion method, Tablet Triturate, Dental Flora.

INTRODUCTION

Oral infections are one of the common health problems associated with majority of people around the globe. The prevalence of these infections is up to 90%in the school going children and even adults are also affected by same[1]. Oral hygiene refers to the cleaning of mouth by brushing and flossing to prevent oral infections and gum diseases. There are various methods to keep the teeth clean and make it hygienic.[2]The oral infections are caused by plaque forming bacteria and yeast which reside in the oral cavity such as *Actinomyces*, *Actinobacillus*, *Streptococcus* and *Candida* species[3]. Dental diseases impose both financial and social burdens as treatment is costly and both children and adults may miss time from school or work because of dental pain[8].

Oral hygiene refers to the act of keeping the teeth clean to prevent dental problems, most commonly dental cavities, gingivitis and bad breath. There are also oral pathologic conditions in which good oral hygiene is required for healing and regeneration of the oral tissues. These conditions included gingivitis, periodontitis and dental trauma such as subluxation, oral cysts and following wisdom tooth extraction[4-6]. Dental infections are caused by plaque forming bacteria's primarily the mutant streptococci(*Streptococcus mutant, Streptococcus Goldoni and S. sobrinus*)which metabolize sucrose to organic acids (mainly lactic acid) that dissolve the calcium phosphate in teeth, causing decalcification and eventual decay.[7] Some oral bacteria, Streptococcus Goldoni for example, cannot last for long periods without nutrients, while other types of bacteria can survive for weeks while in their prime, bio films can cause major damage to a person's teeth and gums. The remedies used are bisguanide-antiseptics, quaternary ammonium-antiseptics, phenol antiseptics, oxygenating agents, cetylpyridinium chloride, chlorhexidine, amine and metal ions. Apart from these agents fewer antibiotics like penicillin and cephalosporin, erythromycin, tetracycline and derivatives and metronidazole have also been used to treat oral disesses[16] but side effects due to antibiotics are gastric, hematological, neurological, dermatological, allergic and other disorders.A known adverse effect of fluoride over-usage is dental fluorosis. Enamel or dental fluorosis is a condition caused by 'excessive' intake of fluoride. Due to adverse effects of chemical based remedies the use of plants and plant based products emerged out as a best alternative. It is generally estimated that over 6000 plantain India are in use in traditional, folk and herbal medicine, representing about 75%of the medicinal needs of the third world countries.19 In India the twigs of Neem (*Azadiracta indica*), Babul (*Acacia arabica nilotica*) , Mango (*Mangifera indica*) , Guava (*Psidium guajava*) and roots of Pilu (Indian name of Salvado rapersica) are mainly used as tooth sticks. Due to a wide biodiversity in the Indian sub continent use of plant species for oral hygiene vary from state to state and region to region[20].

MATERIALS AND METHODS

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Plant Material

Plant material used in this study was collected from the local market of Pune, Maharashtra, India. It was authenticated at Agharkar Research Institute, Pune, India. Its authentication number is AHMA S/B – 065.

Preparation of aqueous extract

The plant components were washed under tap water and rinsed in distilled water. They were air dried under room temperature for 4 days and grounded into fine powder with a mechanical grinder Fig No.01. The powder was weighed into 5, 10, 25, and 50 g using a digital weighing machine and stored in air tight sterile containers. The powdered plant materials were deffated with ethyl alcohol and then subjected for extraction to obtain extracts of *Azadirachta indica* (Neem), *Hemidesmus indicus* (Anantmul), *Caryophyllus aromaticus* (Clove,Lavang), *Cinnaimomum zeylanicum* (Dalchini), *Ocimum sanctum* (tulsi), *Embilica officinalis* (Amla), *Terminali abelerica* (Behda), *Acacia Arabica* (Babhul). Thus obtained extract were filtered and concentrated on water bath to a thick paste and dried under vacuum.

Evaluation of Extracts

Characteristics of extracts

The ethanolic extracts of *Azadirachta indica, Hemidesmus indicus, Caryophyllus aromaticus, Cinnamomum zeylanicum, Ocimum sanctum, Embilica officinalis, Terminalia belerica, Acacia arabica* were evaluated for its physical state, colour, odour, taste.

Phytochemical screening of extracts[25]

The above ethanolic extracts of *Azadirachta indica, Hemidesmus indicus, Caryophyllus aromaticus, Cinnamomum zeylanicum, Ocimum sanctum, Embilica officinalis, Terminali abelerica, Acacia arabica* were subjected to preliminary phytochemical testing for the detection of major phyto constituents such as phenols, tannins, steroids, alkaloids, glycosides and flavonoids.

Tests for Alkaloids

Dragendorff's test

To the 1 ml of extract, add 1 ml of Dragendorff's reagent (potassium bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.

Mayer's test

To the 1 ml of extract, add 1 ml of Mayer's reagent (Potassium mercuric iodide solution). Whitish yellow or cream coloured precipitate indicates the presence of alkaloids.

Hager's test

To 1 ml of extract add 3ml of Hager's reagent (saturated aqueous solution of picric acid) yellow colour precipitate indicates the presence of alkaloids.

Wagner's test

To the 1 ml of extract add 2 ml of Wagner's reagent (iodine in potassium iodide) formation of reddish brown precipitate indicates the presence of alkaloids.

Tests for Glycosides

Legal's test

Dissolve the extract in pyridine and add sodium nitro prusside solution to make it alkaline. No formation of pink to red colour shows absence of glycosides.

Baljet's test

To 1ml of the test extract, add 1ml of sodium picrate solution and the yellow to orange colour reveals the presence of glycosides.

Keller-Killani test

1gm of powdered drug is extracted with 10ml of 70% alcohol for 2 minutes, filtered, add to the filtrate, 10ml of water and 0.5ml of strong solution of lead acetate and filtered and the filtrate is shaken with 5ml of chloroform. The chloroform layer was separated in a porcelain dish and removes the solvent by gentle evaporation. Dissolve the cooled residue in 3ml of glacial acetic acid containing 2 drops of 5% ferric chloride solution. Carefully transfer this solution to the surface of 2ml of concentrated sulphuric acid. A reddish brown layer forms at the junction of the two liquids and the upper layer slowly becomes bluish green, darkening with standing.

Borntrager's test

Add a few ml of dilute Sulphuric acid to 1ml of the extract solution. Boil, filter and extract the filtrate with chloroform. The chloroform layer is treated with 1ml of ammonia. The formation of red colour of the ammonical layer shows the presence of anthraquinone glycosides.

Modified Borntrager's test

To 5 ml extract, add 5 ml 5% FeCl3 and 5 ml dil. HCl. Heat for 5 min in boiling water bath. Cool and add benzene. Shake well. Separate organic layer, add equal volume dilute ammonia. No formation of pinkish red color of the ammonical layer shows the absence of glycosides.

Tests for Phenols and Tannins

Take the little quantity of test solution and mixed with basic lead acetate solution. Formation of white precipitates indicates the presence of tannins. To 1ml of the extract, add ferric chloride solution, formation of a dark blue or greenish black colour product shows the presence of tannins. To the test extract, add strong potassium dichromate solution, a yellow color precipitate indicates the presence of tannins and phenolic compounds. To the test extract, add dilute nitric acid solution, reddish to yellow colour indicates the presence of tannins.

Tests for Flavonoids

Table 1: Weight variation limits as per USP

Sr. No.	Average Weight of Tablet(mg)	Maximum %Difference
1	130 or less	± 10
2	130-324	± 7.5
3	More than 324	± 5

Shinoda's Test

The alcoholic extract is treated with magnesium foil and concentrated HCl give intense cherry red colour indicates the presence of flavonones or orange red color indicates the presence of flavonols. The extract is treated with sodium hydroxide; formation of yellow color indicates the presence of flavones. The extract is treated with concentrated H2SO4, formation of yellow or orange color indicates flavones.

Tests for Steroids

Salkowski test

Dissolve the extract in chloroform and add equal volume of conc. sulphuric acid. Formation of bluish red to cherry color in chloroform layer and green fluorescence in the

Fig. 1: Powdered Plant Materials

Fig. 2: Formulated Polyherbal Tablet Triturates

Table 2: Characteristics of Extracts

Sr. No.	Plant	Observations about Physical state		
		Colour	Odour	Taste
1.	Baheda	Gray	characteristic	Sweet
2.	Tulsi	Green	Aromatic	Slightly pungent
3.	Anantmul	Green	Aromatic	Bitter
4.	Lavang	Pale yellow	Persistant, characteristic	unpleasant
5.	Amla	Light yellow or brick red	None	Sore and astringent
6.	Neem	Green, brown	characteristic	Bitter
7.	Babhul	Brown-red	odourless	Bland and mucilaginous
8.	Dalchini	Yellowish-brown	Fragrant	Aromatic and sweet by warm sensation

Table 3:Phytochemical screening of extracts

Sr. No.	Test for Phytoconstituents	Plant extract							
		Baheda	Tulsi	Anantmul	Lavang	Amla	Neem	Babhul	Dalchinni
1	Phenols	+	+	+	+	+	+	+	+
2	Tannins	+	+	+	+	+	+	+	+
3	Steroids	+	+	+	-	+	+	+	+
4	Alkaloids	+	+	+	+	+	+	+	+
5	Glycosides	+	+	+	+	+	+	+	+
6	Flavonoids	+	+	+	+	+	+	+	+

acid layer represents the steroidal components in the tested extract.

Antibacterial Activity[26]

Microorganisms and Media

The test organisms used in this study is mixed dental flora. Nutrient Agar medium purchased from Hi-media.

Antibacterial Activity Testing

Sample Preparations

Tetracycline (10mg/ml) was used as a control Ethanol extracts of Baheda, Tulsi, Anantmul, Lavang, Amla, Neem, Babhul, Dalchinni were prepared.

Antibacterial Assay

Agar well-diffusion method was followed to determine the antimicrobial activity of herbal extract in which nutrient agar plates were seeded with 0.2 ml each of 24 hr broth culture of mix dental flora. Wells (8mm diameter and about 2 cm a part) were made in each of these plates using sterile cork borer. About 100 µl of different plant ethanol extracts were added sterile syringe into the wells and allowed to diffuse at room temperature for 24hrs. Control experiments comprising inoculums without plant extract were set up. The plates were incubated at 37°C for 18-24 hr and diameter of the inhibition zone (mm) was measured.Triplicates were maintained and the experiment was repeated thrice, for each replicates the readings were taken in three different fixed directions and the average values were recorded.

Pre-compression Micromeritic Properties of Tablet Powder Blend[27]

Angle of repose

The frictional force in a herbal powder can be measured by the angle of repose θ. It is defined as, the maximum angle possible between the surface of the pile of the powder and the horizontal plane. If more powder is added to the pile, it slides down the sides until the mutual friction of particles, producing a surface at an angle θ; it is in equilibrium with the gravitational force. Angle of repose has an indirect method of quantifying powder flow ability.

The angle of repose was determined by the fixed height cone method suggested by Newman. The blend of granules was poured through a funnel that can be raised vertically until a maximum cone height (h) was obtained. Radius of heap (r) was measured and the angle of repose was calculated using the following formula.

Bulk density

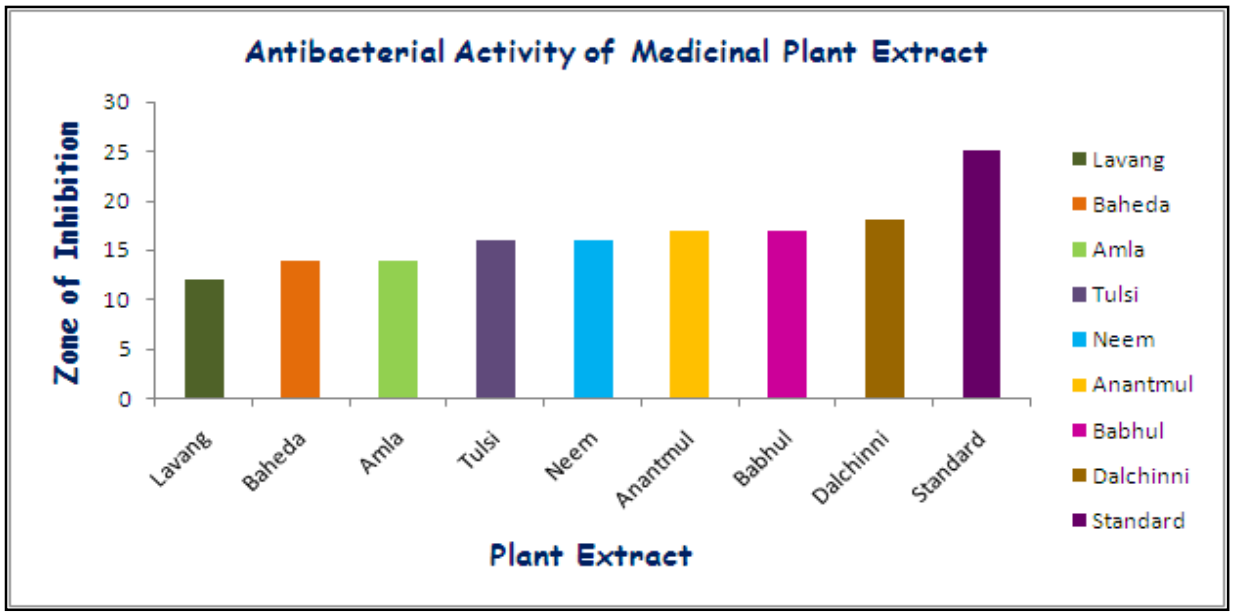

Fig. 3: Antibacterial Activity of Medicinal Plant Extract

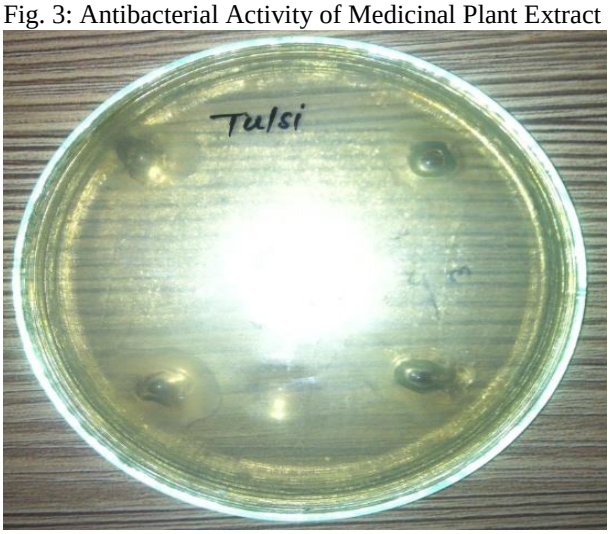

Fig. 4: Zone of inhibition of Ocimum Sanctum (Tulsi) against Dental flora

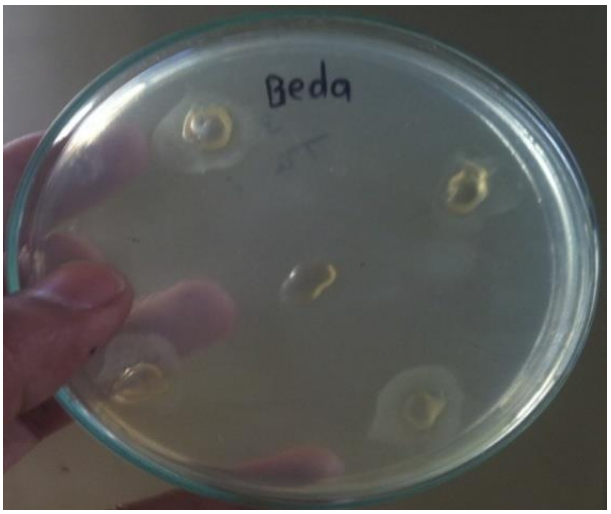

Fig. 5:Zone of inhibition of Terminalia Belerica (behda) against Dental flora

Bulk density, is defined as the mass of the powder divided by bulk volume and is expressed as gm/cm3 .The bulk density of a powder primarily depends on particle size distribution, particle shape and the tendency of particles to adhere together. The particles are packed in such a way so as to leave large gaps between their surfaces resulting in heavy powder of high bulk density. Bulk density was determined by pouring powder blend into a graduated cylinder. The bulk volume and weight of the powder was determined. The bulk density was calculated by using the following formula.

Tapped density

It is the ratio of total mass of the powder to the tapped volume of powder. The volume was measured by tapping the powder for fixed time. The maximum volume occupied in the cylinder and the weight was measured. The tapped density was calculated using the formula.

Compressibility Index (Carr's Index)

The simplest way for measurement of free flow of powder is compressibility index, indication of the ease with which a material can be induced to flow is given by compressibility index which was calculated as follows.

Hausner Ratio

Hausner ratio is an indirect method to predict powder flow properties.

Formulation of Poly herbal Tablet Triturates

After evaluation of pre compression parameter the powder blend was used for preparation of tablet triturates. The prepared ethanolic extracts of Azadirachta indica, Hemidesmus indicus, Caryophyllus aromaticus, Cinnamomum zeylanicum, Ocimum sanctum, Embilica officinalis, Terminali abelerica, Acacia arabica were weighed (1.4 gm) accurately, along with microcrystalline cellulose and starch powder. The entire ingredients are mixed. The poly vinyl pyrrolidone was weighed and dissolved in alcohol. This solution (5%) was added drop wise in powder blend of extract and microcrystalline cellulose till the damp mass prepared. The Rasberry syrup and Peppermint oil was added as sweeteners and flavouring agent. The above damp mass then filled in tablet triturates mold. These mold were kept in hot air oven for

Fig.6: Zone of inhibition of Hemidesmus Indicus (Anantmul) againts Dental flora

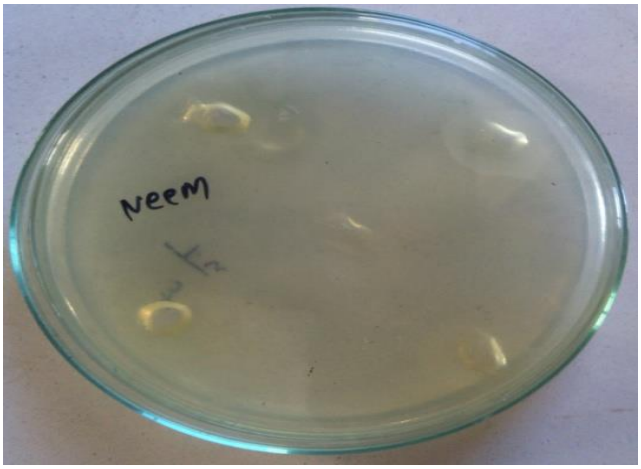

Fig.7: Zone of inhibition Azadirachta Indica (Neem) againts Dental flora

Fig.8: Zone of Inhibition of Embilica Officinalis (Amla) againts Dental flora

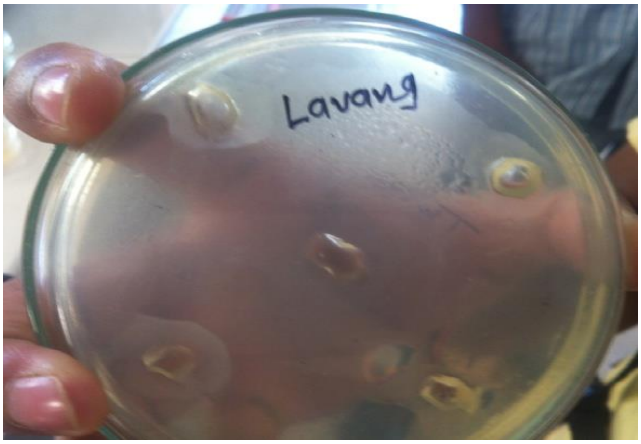

Fig. 9: Zone of Inhibition of Caryophyullus Aromaticus (lavang) against Dental flora

Fig. 10: Zone of Inhibition of Cinnamomum Zeylanicum (Dalchini) againts Dental flora

Fig. 11: Zone of Inhibition of Acacia Arabica (babhul) against Dental flora

drying at 100 C for 1 hour. The prepared tablet were evaluated and packed in container.

Evaluation of Tablet triturates[28]

Thickness

The thickness of a tablet is only dimensional variable related to the compression process. The thickness of the tablets was determined by the Digital VerniarCaliber instrument. Five tablets were used for the above test from each batch. Results were expressed in millimeter (mm). The tablet thickness was reported with Mean ± SD value.

Hardness test

Tablets require a certain amount of strength or hardness and resistance to friability to withstand mechanical shock of handling in manufacture, packing and shipping. To perform this test tablets were placed between two anvils, force to the anvils and the crushing strength that just causes the tablets to break was recorded. Monsanto hardness tester was used to measure the hardness of tablets. Three tablets from each batch were used for hardness studies and results were expressed in Mean ± SD kg/cm2.

Table 4: Pre-compression Parameter of Powder Blend

Test	Observation
Angle of repose	0.28
Bulk density	1.3
Tapped density	2.16
Carr's index	40
Hausner's ratio	1.6

Table 5: Different evaluation parameters of selected core tablets (S2 batch)

Test	Observation
Hardness	6
Friability	0.487%
Thickness	6.63
Weight variation	0.10

Friability Test

It was done in Roche Type Friabilator Apparatus, where the tablets were subjected to the combined effect of abrasion and shock by utilizing a plastic chamber that revolve at 25 rpm, dropping the tablets at a distance of six inches with each revolution. Normally, a preweighed sample of 20 tablets was placed in the friabilator, which is then operated for 100 revolutions. The tablets are then dusted and reweighed. Conventional compressed tablets that loss less than 0.5 to 1.0% of their weight are generally considered acceptable.

Weight Variation Test

Twenty tablets were selected at random, individually weighed in a single pan electronic balance and the average weight was calculated. The uniformity of the weight was calculated. The uniformity of the weight was determined according to USP specification. As per USP not more than two of individual weight should deviate from average weight by more than % limit and none deviate more than twice that percentage limit.

RESULT AND DICUSSIONS

Evaluation of extracts

Characteristics of ethanol extracts of Azadirachta indica (Neem), Hemidesmus indicus (Anantmul), Caryophyllus aromaticus (Clove,Lavang), Cinnamomum zeylanicum (Dalchini), Ocimum sanctum (tulsi), Embilica officinalis (Amla), Terminalia belerica (Behda), Acacia Arabica (babhul). The physical state, colour, odour, taste of all eight extracts are given in the table.

Phytochemical Screening of Extract

The ethanolic extracts of Azadirachta indica (Neem), Hemidesmus indicus (Anantmul), Caryophyllus aromaticus (Clove,Lavang), Cinnamomum zeylanicum (Dalchini), Ocimum sanctum (tulsi), Embilica officinalis (Amla), Terminalia belerica (Behda), Acacia Arabica (babhul). The following phyto constituents were found to present in the ethanolic extracts of Azadirachta indica (Neem), Hemidesmus indicus (Anantmul), Caryophyllus aromaticus (Clove,Lavang), Cinnamomum zeylanicum (Dalchini), Ocimum sanctum (tulsi), Embilica officinalis (Amla), Terminalia belerica (Behda), Acacia Arabica (babhul) respectively.

Antibacterial Activity of Extracts

The ethanolic extracts of Azadirachta indica (Neem), Hemidesmus indicus (Anantmul), Caryophyllus aromaticus (Clove,Lavang), Cinnamomum zeylanicum (Dalchini), Ocimum sanctum (tulsi), Embilica officinalis (Amla), Terminalia belerica (Behda), Acacia Arabica (babhul).were examined for antibacterial activities against dental flora.

From the results it was observed that the all extracts showed zone of inhibition. The zone of inhibition of all extract is less as compared to standard antibiotic Tetracycine as shown in Fig No.01 Zone of inhibition of ethanolic extracts against dental flora are shown in Fig.No 2 A, B,C, D, E,F,G,H respectively.

CONCLUSION

After the study of chemical based oral products and plant based products it is obvious that use of plant based or herbal products is more effective and safe. The use of chewing sticks, sponges etc. prove to be very effective means of preventing and controlling oral disorders. This study demonstrates that plant molecules and extracts could be developed into products which could be used by common man to treat oral infections. Use of fluoride based toothpaste has been found to associate with adverse effects and disadvantages of using hard brush which cause abrasion in adults. Plants have been found to be safer and effective in curing oral diseases. The use of plants for oral hygiene cannot be overemphasized because they have abilities to act on plaque, bacterial and inflammations as it has been mentioned in earlier. Even before the era of modern oral hygiene products, natural plant resources had been used by our ancestors for maintaining oral hygiene without side effects. Recent studies evaluating the effectiveness of these products have shown encouraging results. Use of natural plant products is not only safe but is also economically cheaper and moreover easily available. Researches may be directed to extract the active component from these plant extracts and incorporate them into oral hygiene products. But still standardization of these products in terms of preparation, dosage and manufacturing remains a concern. Screening of more such herbal resources with the indigenous knowledge behind the use and their sustainable utilization of such plant materials is important for the conservation of herbal resources for the mankind.

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