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A Preliminary Standardisation and a Phase 2 Efficacy Trial on Healthy Volunteers of a Polyherbal Formulation Against Hiora-K Herbal Mouthwash

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ABSTRACT

Oral hygiene plays an important role in a man's health. Many bacteria are residing in the oral cavity. When the healthy favourable condition of the oral cavity changes, these are producing diseases. Many types of oral toiletries are available in the market for maintaining the oral health. But the increasing prevalence of the oral diseases indicates its insufficiency. And this chemical compounds also poses threat to the human health. Hence, a cost effective simple palatable fast acting herbal substitute having antimicrobial activity is needed. *Susrutācārya* mentioned a *pratisārana* yoga containing *Dhātaki, Pathya, Jambu* along with honey in the context of "*Dantakāṣthagata viṣa*.", here it was used as *kabala*. **Methods:** Preliminary standardization of the drug by determination of physiochemical parameters,phytochemical screening and HPTLC Profiling were done. An RCT to assess the antimicrobial activity clinically in 50 healthy volunteers by counting the number of colonies before and after in comparison with Hiora-K Herbal mouthwash with OHAT score one and above 1.The observations are statistically analysed using dependent sample 't' test. **Result:** Clinically this formulation showed highly significant antimicrobial activity against Hiora-K herbal mouthwash. *Conclusion:* Dhātakyādi agada is a safe and cost effective antimicrobial drug. The presence of tannins, alkaloids, flavonoids, alkaloids, glycosides, steroids, phenols, saponins etc may contribute to this action. It process molecular, cellular and even DNA level action. It is evident that this agada is bacteriostatic and bactericidal in properties It also has *kṛimihara, vṛanahara* and *viṣaharafproperty*.

Keywords: Oral health, *Dhatakyadi agada*, Antimicrobial activity.

INTRODUCTION

Health is only the reflection of one's healthy habits and food. Healthy habits are intricately linked to micro and macro environment created in the digestive tract. Everything that passes through the alimentary canal influences the local micro flora and its patency as well as the remote tissues and organs, their functions and interactions. Oral cavity being the gateway to human body and gut has a primary role to play in maintaining the health of an individual [1]. The specificity in the pH and the complex interactions between the microbial flora inhabiting the mouth provides sustained protection from microbial invasion through this route [2]. Constant contact with food and the complex anatomical peculiarities of oral cavity provides a permanent threat from pathogenic microbes invading deeper into the body producing mortality and morbidity. Thus the hygiene of oral cavity became an important criterion in public health [3]. The arrays of products available in the market for promoting oral hygiene are also finding an attractive market worldwide. The billion dollar industry is now posing threat to human lives as many toxic chemicals are entering into toiletry products for luring customers by providing unrealistic claims. This poses multitude of problems including soft tissue injuries, flushing out favorable microbes and developing multidrug resistant pathogens and many more [4, 5]. Search for a more natural cure for this situation had brought us to the time tested remedies treasured in traditional health care systems like *Ayurveda*. Our search has unearthed a polyherbal formulation comprising of *Woodfordia fruiticosa, Terminalia chebula and Syzygium cumini* used as a hot infusion for gargling the mouth. The formulation has been proved to be effective in in-vitro studies against oral microbes like Escherichia coli, Staphylococcus aureus, streptococcus mutans, Klebsiella pneumoneae and Proteus mirabilis [6]. A comparative trial was conducted on volunteers with the poly-herbal formulation *Dhatakyadi agada* (DA) against Hi-Ora herbal mouth wash (HK) which is a leading brand of mouthwash proved its efficacy in a clinical trial. This study provides a natural mouthwash that can substitute alcoholic and heavily chemical laden chemical mouthwashes available in the market.

MATERIALS AND METHODS

Drug

The fresh drugs were purchased from Arya Vaidya Sala Kottakkal and the plants were authenticated from

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Department of Agadatantra, Vaidyaratnam P.S Varier Ayurveda College, Kottakkal. The plants were properly washed and all the foreign matter was removed, and it was pulverised and sieved through a mesh of size 40. All the three drugs were mixed in 1:1:1 proportion and was stored in an airtight glass container till the beginning of the study.

Extraction

The powdered plant materials were extracted with water (AE), Acetone (AC), Ethyl acetate (EA) and Chloroform (CHF) using continuous hot percolation method in a Soxhlet extractor with 250 ml of solvents for 25 grams of drug powder. The extraction was carried for a period of eight hours. The extract was later filtered through a filter paper (Whatman No. 40) and concentrated under reduced temperature and pressure in a flash evaporator at 40^0C. The extract was further evaporated under a continuous flow of hot air in an inert atmosphere containing nitrogen till last trace of solvents were removed and the dried extract powder was stored in an air tight container and freezed at -20^0C till the beginning of the experiments [7].

Physico-Chemical Parameters

Physico-chemical characteristics were determined as part of a preliminary standardization of the formulation. The parameters estimated in the present study were; loss on drying, total ash, water insoluble ash, sulphated ash, alcohol soluble extractives, water soluble extractives, Crude fiber content, Swelling index and p^H as per the standard procedures laid down in the Pharmacopoeia of India [8, 9].

Fluorescence Analysis [10]

Fluorescence exhibited by crude drugs owes to its unique chemical constituents present and forms a standard for the drug. The drug powder was treated with various reagents and solvents and viewed in visible, short UV (254 nm), mid UV (302 nm) and Long UV (356 nm) rays and the fluorescence were noted.

Phytochemical Evaluation [11]

Secondary metabolites like alkaloids, carbohydrates, glycosides, saponins, phytosterols, fixed oils & fat, Resins, Phenols, tannins, flavonoids, proteins & amino acids were screened as per accepted qualitative methods.

HPTLC Profiling [12]

Sample preparation and application

DA at a concentration of 5 mg/ml was prepared with HPTLC grade methanol and filtered through Whatman filter paper No.1. Pre-coated aluminium TLC plates containing silica gel 60 F254 (Merck, 1.05554.0007) were spotted with 15 uL of the sample using Linomat 5 sample applicator set a speed of 150 nL/ sec with a band length of 5 mm.

Developing solvent system

The mobile phase giving satisfactory resolution and maximum number of spots was fixed after several trials and the solvent system consisting of Toluene: Ethyl acetate: Formic acid: Methanol:: 7:5:1:0.5 was used for the present study.

Development of Chromatogram

The plate was run in a twin trough chamber of 10 x 10 of Camag make with the solvent system of Toluene: Ethyl acetate: Formic acid: Methanol:: 7:5:1:0.5 for 20 minutes up to a distance of 80 mm.

Scanning and detection of spots

The air dried plates were derivatized using 10% sulphuric acid reagent and visualized at 254, 366 and 400-600 nm wavelengths. The plate was scanned using CAMAG HPTLC Scanner 3 in absorbance mode and the Rf values and fingerprint data were recorded.

Preparation of Drug

The mode of preparation was hot infusion as detailed in Ayurvedic Formulary of India. 8gm of DA powder was boiled in 75 ml water for two minutes and strained through a two layered muslin cloth. The infusion was mixed with 5 ml of honey after the mixture cools down to room temperature. The participants were advised to use 15 ml of the mixture for 30 seconds twice daily after breakfast and dinner. There were no specific food restrictions and the subjects were allowed to follow their conventional brushing methods.

Study population

A total of 50 volunteers participated in the study. Informed consent was obtained form each participant after briefing about the objectives of the study. Ethical clearance was obtained from the Institutional Ethics Committee of Vaidyaratnam P.S Varier Ayurveda College Kottakkal (IEC/Doc/2/2016 dtd 18/04/2016) before the beginning of the study. All participants with an Oral Health Assessment Tool (OHAT) score one or above and between 18 – 70 years of age were included in the study. Vulnerable groups like pregnant ladies, children and those who were undergoing systemic antibiotics, steroids, and hormone replacement therapy were excluded from the study.

Plan of study

Volunteers were randomly segregated to Trial group and control group using a Random Number Table. The control group was instructed to use 15ml Hiora –K herbal mouthwash (Himalaya healthcare Pvt. Ltd., batch number: 18501311) for 30 sec for 7 days and trial group used the DA 15 ml for 30 sec for 7 days. The samples were collected into sterile normal saline using a sterile cotton swab on day 1 and 7, 1 hour after breakfast. They were advised not to take any food or liquid in between. The samples were transferred aseptically on to nutrient agar culture plates and incubated for 24 hours at 37^0C. Colony count was determined on a Digital Colony counter (Labtronics, India and Model No.37). Each determination was repeated thrice and the mean value was taken as the final score.

A pilot study was conducted on five volunteers before the beginning for the study. Since the bacterial concentration was very high, the number of colonies could not be determined initially. So the normal saline was serially diluted and a 10^{-2} dilution was fixed as the concentration that gave manageable number of bacterial colonies. 0.1 ml of the saline solution was aseptically transferred to a nutrient agar plate and evenly spread over the plate using a sterile spreader. The plates were incubated at 37^0C for 24 hours before counting the colonies using a digital colony counter [13].

Assessment

Assessment was done by computing the mean of three replications of each sample and were expressed as mean $\pm$ SD. The efficacy of the treatment was assessed using independent sample‘t’ test and dependent sample ‘t’ tests. 5% was fixed as the minimum level of significance. Every sample was autoclaved before safe disposal.

RESULTS

Physico-Chemical Parameters

The physico-chemical parameters of *DA* were recorded as the mean and standard deviation of three repetitions as shown in Table 1.

Fluorescence Analysis

The fluorescence characteristics of powder of *DA* on treating with various reagents and solvents recorded in three wavelengths is shown in Table 2.

Phytochemical screening

The extracts were qualitatively assessed for the presence of major secondary metabolites. The results of the analysis are shown in Table 3.

HPTLC Profiling of *DAME*

The HPTLC profile of DAME showed best separation with Toluene: Ethyl acetate: Formic acid: Methanol (7:5:1:0.5) as solvent system. After scanning and visualizing the plates in absorbance mode at 254 nm, 366 nm and visible light range and after derivatization with 10% sulphuric acid showed 8 peaks in 254 nm and 12 peaks in 366 nm (table:4 Fig:1-7).

Oral microbial growth prevention study

While comparing the oral microbial load before and after treatment with the corresponding drugs using dependent sample 't' test, there was a significant reduction in oral microbial load as shown by decreased number of Colony Forming Units (CFU's) which was statistically significant at $p<0.001$ in DA group and insignificant result with $p>0.05$ was observed in HK group. On comparing both groups using independent sample't' test, DA group possessed statistically significant results with $p<0.01$ than HK groups.

TABLES AND FIGURES

Table 1: Physicochemical parameters of *DA*

Sl No.	Test	Mean ± SD
1.	Total ash	0.142 ± 0.08
2.	Acid insoluble ash	0.035 ± 0.02
3.	Sulphated ash	21.4 ± 0.07
4.	Alcohol soluble extractive	26.3 ± 0.01
5.	Water soluble extractive	26.3 ± 0.07
8.	Crude fibre content	19.9 %
9.	Foaming index	<100
10.	P^H	3.7 ± 1.6 at 29^0C
11.	Swelling index	0.734

Table 2: Fluorescence analysis of *DA*

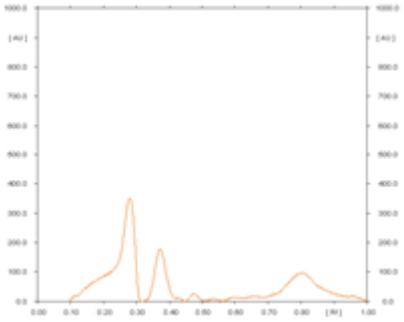
SlNo	Solvent	Visible Light	UV Short (254 nm)	UV Long (365 nm)	UV Medium (302 nm)
1	Powder alone	Dark brown	Black	Greenish brown	Creamy Brown
2	P + Distilled water	Black	Black	Greenish brown	Brown
3.	P + Conc.H_2SO_4	Black	Black	Greenish black	Reddish black
4.	P + Conc.HNO_3	Black	Black	Greenish brown	Brick red
5.	P + 50%HNO_3	Black	Black	Greenish black	Reddish Brown
6.	P + 1N H_2SO_4	Black	Black	Greenish brown	Creamy Brown
7.	P + 1N HNO_3	Black	Black	Greenish black	Reddish Brown
8.	P + 1N HCl	Black	Black	Greenish black	Creamy Brown
9.	P + 1N NaOH	Black	Black	Greenish brown	Orange Brown
10.	P+ NaOH in MeOH	Black	Black	Greenish brown	Yellow Brown
11.	P + 5% $FeCl_3$	Black	Black	Dark brown	Bluish green
12.	P + 10% Iodine	Black	Black	Greenish brown	Greenish brown
13.	P + Conc. HCl	Black	Black	Greenish brown	Brown
14.	P + Picric acid	Black	Black	Greenish brown	Yellow brown
15.	P + Methanol	Black	Black	Greenish brown	Creamy brown
16.	P + Ethyl acetate	Black	Black	Greenish black	Creamy brown
17.	P + Benzene	Black	Black	Greenish black	Reddish brown
18.	P + Acetone	Black	Black	Dark brown	Creamy brown
19.	P + Chloroform	Black	Black	Greenish brown	Reddish Brown
20.	P + 1N Silver nitrate	Black	Black	Greenish brown	Creamy brown
21.	P + Ammonia	Black	Black	Greenish brown	Creamy brown
22.	P + Acetic acid	Black	Black	Brown	Creamy brown

Table 3: Phytohemical screening of *DA*

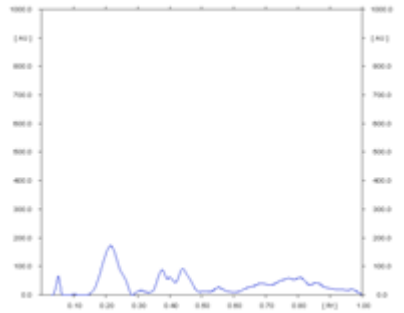
TEST	CHF	AC	EA	AE
Alkaloids				
Draggendorf's test	-	-	-	-
Hager's test	-	-	-	+
Wagner's test	+	+	+ + +	+ + +
Mayer's test	-	+	+ + +	+ +
Froehde's test	-	+ + +	+	+ +
Marquis test	-	+ +	+	+ +
Carbohydrates				
Molish test	+	+	+ + +	+ + +
Fehling's test	-	+	-	-
Flavanoids				
Shinoda test	-	+	-	-
Lead acetate test	-	+ + +	+ + +	-
Alkaline reagent test	-	+	-	-
Proteins & amino acids				
Xanthoproteinic test	-	-	+ +	-
Ninhydrin test	-	-	-	-
Biuretic test	-	+	+ +	+ +
Phenols				
Ferric chloride test	-	+ + +	+ + +	+ + +
Glycosides				
Modified Borntrager's test	-	-	-	-
Legal's test	-	+ + +	-	+ + +
Keller Killiani test	-	-	-	-
Saponins				
Froth's test	-	+ + +	-	-
Foam test	-	+	-	-
Phytosteroids				
Salkowski's test	-	+ +	+ + +	-
Leibermann Burchard's test	-	+	-	-
Fixed oils and fats				
Stain test	+	+ +	-	+ + +
Resins				
Acetone water test	+ + +		-	-
Tannins				
Gelatin test	-	+ + +	+ +	-

Table 4: Rf values and number of spots of DAME at 254nm and 366 nm

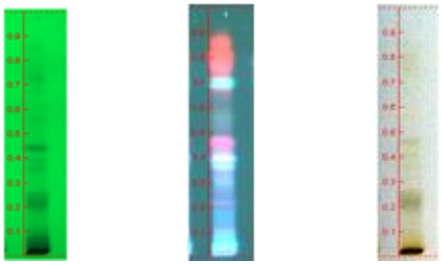
254 nm	366 nm
0.22	0.05
0.36	0.22
0.39	0.31
0.44	0.38
0.56	0.40
0.68	0.44
0.73	0.55
0.84	0.68
	0.77
	0.81
	0.85
	0.97



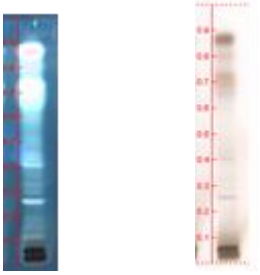
At 254 nm



At 366 nm



At 254nm At 366nm At white light



Derivatization at 366nm Derivatization at white light

Figure 1,2,3,4,5,6,7: HPTLC Profile of *DA* sample

Table 5: Mean reduction in CFU's in DA and HK groups

Group	*BT*	AT
DA	26.44±15.3	9.6±9.7[a]
HK	28.04±18.9	23.8±18.1[b]

Data is expressed as mean± S.D (n=50), a- significant at P<0.001, b-insignificant (P>0.05)

Effect of *Dhatakyadi agada(DA)* and Hiora-K herbal mouthwash(HK) in reducing oral microbial load by the unpaired comparison in volunteers.

Table 6: Mean difference in CFU's in DA and HK groups

Group	Mean ± S.D
DA	16.84±16.7
HK	4.24±16.9

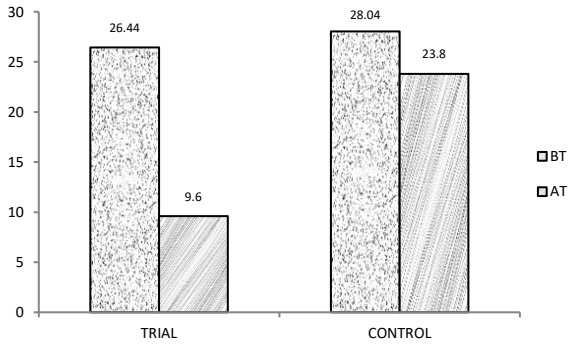


Figure 8: Reducing in CFU in DA and HK groups

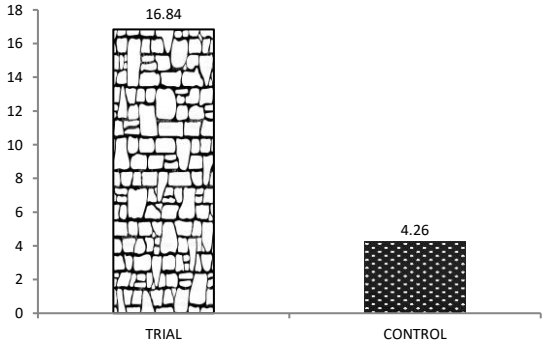


Figure 9: Mean reduction in CFU's in DA and HK groups

Meanwhile some patients reported with adverse drug reactions (ADR) in the form of burning of oral mucosa and erythema while using HK. The complaints were noted and the incidence was reported to the Pharmaco-Vigilance Unit of the institution. The subjects were withdrawn from the study and were given expert medical attention in the same hospital where the study was undertaken. It was recorded as no ADR (20%), mild(24%),moderate(24%) and severe(32%).

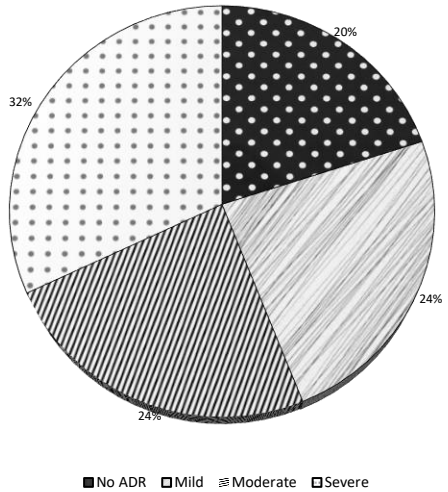


Figure 10: Percentage of ADR in control group

Figure 11,12: Adverse drug reaction in the control group

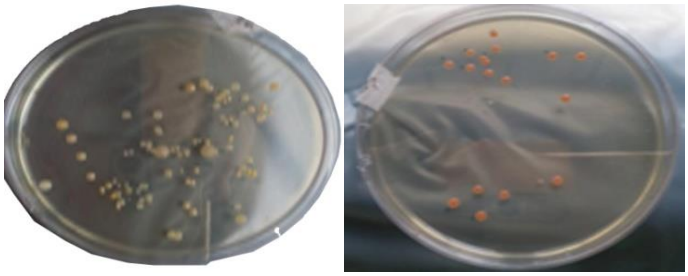


Figure 13,14: Sample before and after treatment

DISCUSSION

Poly-herbal formulations are a whole mark of traditional health systems like ayurveda. Specific formulations utilized in cases of poisonous etiology are termed 'agada'. The escalating death toll from infectious diseases is on the rise. Though antibiotics form the mainstay of treatment in the control of infections, the emergence of multi antibiotic resistant strains of microbes poses a new threat to human survival. Multi drug resistant forms as well as 'superbugs' are now paving way to the re-emergence of once contained and eradicated diseases. Scientific world is now turning their attention to other natural sources for deriving lead compounds with which they can fight this menace. Among them plant sources form the major chunk of contributors of several compounds with potent bactericidal and bacteriostatic activity [14]. In humans, the entire body forms the habitat for many microorganisms many of them are deadly pathogenic. They are being contained from proliferating and cause deadly diseases by a complex mechanism. It involves the role of many signaling pathways and complex immunological mechanisms. Oral cavity being the primary portal of entry of microbes into our body, it becomes our primary target of prevention [15]. Oral microbes gain entry into the gut through various sources like food, water, air etc. Oral hygiene plays a pivotal role in the maintenance of a positive health. Several commercial preparations are available in the market for this purpose. Hiora K is one such preparation marketed by Himalaya Herbal Healthcare Ltd. It is proved as a efficacious drug in reducing oral microbial load [16]. In this study Hiora K was compared against *Dhatakyadi Agada*, a formulation detailed in Susruta Samhita. The formulation made out of three drugs, *Woodfordia fruiticosa, Terminalia chebula* and *Syzygium cumini* was used for gargling along with honey as a hot infusion. Bacterial infections determine the onset as well as severity of disease depending on the potency of the

toxins elaborated. These toxins which are micro-molecular and target specific can enter into the cells or macrophages and remain unidentified by the immune system of the body. Common mechanisms of anti cytotoxicity, bactereostatic, bactereocidal activities and antioxidant defence mechanisms of individual plant sources provides added protection to normal mucosa [17].

Dhātaki, paṭya, jambu have antibacterial effect. Also these drugs contains many active principles like tannin, alkaloids etc which may contribute to this action. Tannins are a group polyphenolic substances and the plant extract of *T. chebula*, with tannin as its active compound, is well recognized for its microbial activity and its astringent property [18, 19]. *Harītaki* is one among thriphala, and it is using in the inhibition of pathologically elevated collagenases and hence may be used as an alternative adjunct in the management of oral diseases [20]. Studies have shown that Essential oil present in *dhātaki* promotes its antibacterial activity [21]. *Sizygium cumini* is also rich in flavonoids, alkaloids, glycosides, steroids, phenols, tannins and saponins. These phytochemicals confer their antimicrobial activity. The various phytochemical compounds detected are known to have beneficial importance in medical sciences. Flavanoids may pocessess their inherent capacity to modify the body's reaction to allergies, viruses and many other microbes, hence it acts as a good antimicrobial, anti allergic and anti-inflammatory compound. Saponin is used as mild detergents and in intracellular histo-chemical staining. It is also known to have antifungal properties. However, some kinds of tannins can reduce the mutagenicity of a number of mutagens and display antimicrobial and antioxidant activities. The medicinal plants containing phenolic compounds, including tannins, as major constituents are used topically for care and repair of skin wounds. The advantage of the use of topical antimicrobials is their ability to deliver high local concentrations of antibiotic irrespective of vascular supply [22].

From the study it is evident that the drug possesses 'clearing' quality of and is also having wound healing property. And this formulation also possess probable molecular target mechanisms in the level of cell wall, cell replication, receptor binding, DNA binding and antibiotic resistance impeding activity related to plasmoids [19, 23]. All these factors are contributing to its antibacterial activity. Thus we can say that *Dhātakyādi Agada* pocessess significant antimicrobial activity.

CONCLUSION

Oral health affects people physically and psychologically and influences their social life. Many bactereae residing in the oral cavity are beneficial, but may change into pathological. The oral toileteries available in the market are chemicals, which are neither capable of resisting these type of organisms nor devoid of side effects. Ayurveda has its own uniqueness in every field of medicine; it also provided evidence based quality medicines to the field of oral microbiology.

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REFERENCES

1. Joseph B, Sujatha S. Role of the oral micro flora in human population: A brief review. Int J Pharm Life Sci. 2012; 3(11):2138-48.
2. Marsh PD, Martin MV. Oral Microbiology-fifth edition. Churchill Livingstone Elsevier-UK. 2009; 25-44 p.
3. Subramaniam P, Gupta T. Efficacy of a herbal mouthrinse on oral microbial load in Down syndrome children. Int J Herb … [Internet]. 2013; 1(4):50-4. Available from: http://florajournal.com/vol1issue4/nov2013/11.1.pdf
4. Saily SP, Sobhana C, VPSV ayurveda college K. A Randomized Controlled Trial on the Efficacy of Vyōṣatrivargacūrṇam on Oral Hygiene. Doctor of medicine (ayurveda) in Swasthavritta, 2013.
5. Jangid K, Doraiswamy JN, Saji VS, Malaiappan S. International Journal of Efficacy of Herbal Mouthwashes Compared to Chlorhexidine in Gingivitis- A Systematic Review. 2014; 1-12.
6. Joy Jeethu Elsa, S.Gopikrishna, N.J Jeena, VPSV ayurveda college K. A comparative *in-vitro* antimicrobial study of *Dhatakyadi agada* in selected oral microorganisms against chlorhexidine and a RCT to assess its efficacy against a commercially available herbal mouthwash in reducing oral microbial load in volunteers. Doctor of medicine (ayurveda) in Agadtantra;2018.
7. Prashant Tiwari B, Kumar MK, Gurpreet Kaur HK. Phytochemical screening and extraction - A review. Int Pharm Sci [Internet]. 2011; 1(1):98-106. Available from: http://www.ipharmsciencia.com.
8. Pharmacopoeia I. Government of India. Ministry of health and family welfare. 2007; 2:726.
9. Gupta AK, Tandon N, Sharma M. Quality standards of Indian medicinal plants. Vol. I. New Delhi: Indian Council of Medical Research. 2010; 172-79.
10. World Health Organization. WHO Expert Committee on Specifications for Pharmaceutical Preparations. World Health Organization technical report series. 2012; (970):1.
11. Harborne JB. Methods of plant analysis. In: Phytochemical methods. Dordrecht. Springer, 1984: 1-36.
12. Benil PB, *et al*. *Elephantopus scaber* linn. enhances membrane stability in human erythrocytesin in vitro models. Int. J. Res. Ayurveda Pharm. 2018; 9(6).
13. Dubey RC MDK. Practical microbiology;Methods for study of microorganisms. 2(ed). Newdelhi: S. Chand &company ltd. 2006; 25-30 p.
14. Abdallah M Emad. Plants: An alternative source for antimicrobials. Journal of Applied Pharmaceutical Science. 2011; 1(06):16-20.
15. Zarco MF, Vess TJ, Ginsburg GS. The oral microbiome in health and disease and the potential impact on personalized dental medicine. Oral Dis. 2012; 18(2):109-20.
16. Subramaniam P, Gupta T. Efficacy of a herbal mouthrinse on oral microbial load in Down syndrome children. Int J Herb … [Internet]. 2013; 1(4):50-4. Available from: http://florajournal.com/vol1issue4/nov2013/11.1.pdf.
17. Takahashi K etal. Pilot study on antiplaque effects of mastic chewing gum in the oral cavity. J Periodontol. 2003; 74:501-5.
18. Carounanidy U. Use of an aqueous extract of Terminalia chebula as an anticaries agent: A clinical study. Indian J Dent … [Internet]. 2007; 152-6. Available from: http://www.ijdr.in/article.asp?issn=0970-9290;year=2007;volume=18;issue=4;spage=152;epage=156;aulast=Carounanidy.
19. Journal W, Pharmacypharmaceutical of. a review on antimicrobial activities of triphala and its constituents. 2016.
20. Desai A, Anil M, Debnath S. A clinical trial to evaluate the effects of triphala as a mouthwash in comparison with chlorhexidine in chronic generalised periodontitis patient. Indian J Dent Adv. 2010; 2(3):243-8.
21. Rajandeep Kaur HK. The Antimicrobial activity of essential oil and plant extracts of Woodfordia fruticosa. Arch Appl Sci Res. 2010; 1(2):302-9.
22. Shyamala S, Gowri KV. Phytochemical Screening and Antibacterial Activity of *Syzygium cumini* (L.) (Myrtaceae) Leaves Extracts., Int J PharmTech Res coden ijprif. 2(2):1569-73.
23. Das PK, *et al*. Woodfordia fruticosa : Traditional uses and recent findingse. J Ethnopharmacol. 2007; 110:189-199.

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