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NOVEL AYURVEDIC PULLING OIL WITH POTENT ANTIVIRAL ACTIVITY

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ABSTRACT

Herpes Simplex Virus (Type-1) (HSV-1) causes infection of the oral cavity. Herpes causes cold sores which are small painful blisters that can appear around the mouth, face, or nose. This disease could be life threatening in people who are immunocompromised. Standard treatment for HSV-1 includes taking antiviral oral medications, such as acyclovir, famciclovir and valacyclovir and over-the-counter creams such as docosanol. However, repeated use of these medications could result in development of resistant virus. Thus, there exists a need for a natural alternative based on herbs which can be helpful in avoiding the risk of developing drug resistance that exists with single-chemical agents. Oil Pulling is a method that uses oil-based mouthwashes and it generates antioxidants which can cause some abnormalities in the structure and function of proteins in the membranes of Vero cells or HSV-1 envelope, which consequently inhibit the binding and penetration of the virus into the cells. In accordance with the principles of Ayurveda, the test substance, Dabur pulling oil Mouthwash was formulated using oils from selected medicinal herbs. The anti-viral efficacy of the test substance was evaluated by Virucidal activity assay against 10TCID50 virus challenge dose of HSV-1 virus. It was found in the Virus neutralization study that Dabur Pulling Oil Mouthwash at a concentration of 0.1% (v/v) and after a contact time of 5 mins exhibited 3.00 Log reduction against HSV-1 virus challenge dose of 10TCID50 (Virus 3.71 TCID50). The study revealed that Dabur pulling oil Mouthwash reduced 99.9% of HSV-1 Enveloped Virus against untreated Virus control.

KEYWORDS: *Herpes simplex virus-1, HSV-1, anti-viral, oil pulling, Dabur Pulling Oil Mouthwash, ayurveda, natural alternative, herbal remedies, herbal mouthwash.*

1. INTRODUCTION

Maintaining a good oral hygiene is an important aspect of one's health. Regular oral care helps toprotect against various oral diseases. However, certain oral diseases create problems to human beings and one such disease is infection of Herpes Simplex Virus (Type-1) (HSV-1). Oral herpes is a common infection of the mouth area. It has been observed that this virus becomes latent after the primary infection in the nerve tissues in the face and may reactivate at a later stage causing cold sores. In 2016, an estimated 3.7 billion people under the age of 50, or 67% of the population,had HSV-1 infection and estimated prevalence of the infection was highest in Africa (88%) and lowest in the America (45%).[1] HSV-1 is transmitted by viral shedding into saliva and can occur by direct contact with saliva. Viral shedding into saliva may occur during asymptomatic infection but it is thought that the risk of infection is much lesser than during symptomatic infection. Viralshedding can occur up to 60 hours after the onset of symptoms.[2] Oral Herpes may be life threatening in people who are immunocompromised. Standard treatment protocols for oro-facial HSV infections include over-the-counter creams such as docosanol and pharmaceutical anti-viral agents such as acyclovir.[3] However, there is a mixed level of success of these treatments and repeated use may lead to development of resistant virus. Mouthwashes are one such product which are often used for prevention and treatment of severe oral conditions. The common active ingredients in mouthwashes are chlorhexidine, cetylpyridinium chloride, sodium fluoride, zinc oxide, zinc chloride etc. Edible oils from herbs are also used in mouthwashes. Chlorhexidine gluconate, PCP-1, C31G (an equimolar mixture of alkyl dimethyl glycine and alkyl dimethyl amine oxide) are certain active ingredients of mouthwashes that have a wide range of antimicrobialeffects, however, frequent use of these mouthwashes may lead to development of resistantmicrobes. Therefore, there exists a need for a natural alternative. The use of herbal alternatives is gaining popularity these days due to their low side effects and low cost of production.

Ayurveda is an ancient Indian system of medicine which utilizes the inherent power of herbs for formulating herbal remedies. In Ayurveda, herpes can be correlated with *Pittaj visarpa*. In traditional Ayurvedic medicine, the use of oil mouthwashes is called "*Kavala* " ("oil swishing") or"*Gandusha*".[4] *Kavala* refers to gargling, swishing the liquid in the mouth while *Gandusha* refers to holding the liquid in the mouth without any movement. The procedure of oil pulling involves swishing a measured volume of oil around the mouth for a period of time, forcing the oil in between all the teeth and around the mouth. Oil pulling

generates antioxidants which damage the cell wallof microorganisms and kill them. Examples of organic oils that are used include sunflower oil, sesame oil, and coconut oil.[5] Moreover, most of the oils used in Oil pulling have no side effectsor lingering after taste. Sesame oil is an edible oil obtained from sesame seeds. Sesame oil containsthree lignans (sesamin, sesamolin and sesaminol). These lignans contain Vitamin E and polyunsaturated fats. The components of sesame oil are known to be powerful antioxidants and possess antibacterial and antiviral properties. Coconut oil is obtained from the nut (fruits) of the coconut palm *(Cocos nucifera)*. Coconut oil is composed mostly of medium chain fatty acids and approximately 50% of these medium-chain fatty acids are lauric acid. Monolaurin, a monoester formed from lauric acid, has been reported to have the ability to fight various types of viruses, especially enveloped viruses, including various influenza viruses.[6] *Mentha piperita L.*, a medicinally important plant belongs to the Family Lamiaceae and commonly known as peppermint. Peppermint oil has been found to exhibit high levels of virucidal activity against HSV-1 and HSV-2 in viral suspension tests.[7] Similarly, Clove oil which is an essential oil that's derived from clove trees, known as *Syzygium aromaticum*, is traditionally known as a dental pain reliever. Clove oil is known to possess antioxidant, antifungal and antiviral activity. Cinnamon is another herb that has been traditionally used as tooth powder and to treat toothaches, dental problems, oral microbiota, and bad breath. Cinnamon oil is derived from the bark or leaves of *Cinnamomum cassia* tree. Cinnamon oil has antifungal, antibacterial, antiviral, antiparasitic, larvicidal, nematocidal, insecticidal, anti-inflammatory, and antioxidant properties.[8] The main component of Thyme Oil is Thymol. Thymol (2-isopropyl-5-methylphenol) belongs to the phenolic monoterpenes and mostly occurs in thyme species. Thymol has been shown to possess antiseptic, antibacterial, antifungal, anthelmintic, antiviral, antioxidant, expectorant, antispasmodic, carminative, diaphoretic, sedative, anti-rheumatic, and even anti-cancer, anti-hyperlipidemic and anti-hyperglycemic action.[9] *Alkanna tinctoria* is a medicinal herb belonging to the Boraginaceae family, and is widely known for its medicinal and pharmaceutical properties, since the ancient times. The roots of this plant are the most effective part. Its roots yield a water-insoluble red dye used to colour fat, oil, perfume, wood, marble, and pharmaceutical products. Its roots contain up to 5% alkannins and these alkannins have antimicrobial and wound-healing properties and are non-toxic in mice.[10] Among the traditional medicinal herbs, Tulsi, *Ocimum sanctum*, is the most revered medicinal herb in Ayurveda. Tulsi is well known for its myriad medicinal properties viz. antibacterial, antifungal, antipyretic, antiviral, antioxidant, antiseptic and anticancer.

Accordingly, a unique blend of oils of medicinal plants/herbs was selected to prepare Dabur Pulling Oil Mouthwash. The present study was carried out to assess the antiviral efficacy of the test substance (Dabur Pulling Oil Mouthwash) against Herpes Simplex Virus (Type-1) (HSV-1). This study accentuates the utilization of Ayurveda based alternative treatments in treating the viral infections of oral cavity. The present study demonstrates that Dabur Pulling Oil Mouthwash is a potent oral care Ayurvedic formulation.

2. MATERIALS AND METHODS

2.1. Materials: Vero cells (ATCC CCL-81) (NCCS), Pune, India), Culture Media- MEM (HiMedia, India), MTT (HiMedia, India), PBS (HiMedia India), Trypsin (HiMedia India), Fetal bovine serum (Gibco, USA) were used in the study. Dabur Pulling Oil Mouthwash was obtained from Dabur India Limited, Ghaziabad, Uttar Pradesh, India. The active ingredients of the Dabur Pulling Oil Mouthwash are provided in Table 1.

Table 1: Ingredients of dabur pulling oil mouthwash.

S. No.	Active Ingredients
1.	Sesame Oil
2.	Coconut Oil
3.	Clove Oil
4.	Cinnamon Oil
5.	Peppermint Oil
6.	Thyme Oil
7.	Tulsi Oil

2.2. Preparation of test Solution and Standard

For the studies, Dabur Pulling Oil Mouthwash was taken as provided and serial two-fold dilutions were prepared from this for carrying out cytotoxic studies. The Standard (Acyclovir) was weighed separately, dissolved in DMSO (Dimethyl sulfoxide) and volume was made up with MEM (minimum essential medium)-supplemented with 2% inactivated FBS to obtain a stock solution of 10mg/ml concentration and sterilized by filtration. Non-toxic dilution was prepared from this for carrying out Anti-viral activity.

2.3. Cell Line and Culture medium

Vero (ATCC-CCL-81) cell line was procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in DMEM high Glucose supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/mL), and amphotericin B (5 µg/mL) in a humidified atmosphere of 5% CO_2 at 37°C until

confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 well microtitre plates.

2.4. *In vitro* cytotoxicity assay

Dabur Pulling Oil Mouthwash was studied for its *in vitro* cytotoxicity activity by MTT assay. The monolayer cell culture was trypsinized and the cell count was adjusted to 100,000 cells/ml using DMEM High Glucose containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension was added. After 24h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100µL of different test concentrations of test drugs (Test substance, Dabur Pulling Oil Mouthwash and the standard Acyclovir) were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37°C for 3 days in 5% CO_2 atmosphere. Microscopic examination was carried out and observations were noted at every 24 h interval. After 72 h, the drug solutions in the wells were discarded and 50 µL of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) in PBS (phosphate buffered saline) was added to each well. The plates were gently shaken and incubated for 3 h at 37°C in 5% CO_2 atmosphere. The supernatant was removed and 100 µL of DMSO (Dimethyl sulfoxide) was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated and concentration of test drug needed to inhibit cell growth by 50% (CTC_{50}) values was generated from the dose-response curves for each cell line.

2.5. Anti HSV-1 studies

Anti HSV-1 activity of test substance (Dabur Pulling Oil Mouthwash) was evaluated by Virucidal activity assay against $10TCID_{50}$ virus challenge dose of HSV-1. Prior to this, virus stock was standardized by titration. In the present experiment, virus challenge dose ($10TCID_{50}$) was prepared by suitable dilution technique (End Point method) and used as virus challenge dose against different doses of test substance.

2.5.1. Virucidal assay

Vero cells were cultivated as $1x10^5$ cell/well in 96-well flat bottom culture plates in MEM culture medium at 37°C in a humidified 5% CO_2 atmosphere for 24 h. One non-toxic

concentration of test substance, Dabur Pulling Oil Mouthwash (0.1%v/v), i.e., lower than CTC_{50}, was tested for antiviral property by virucidal assay against virus challenge dose of $TCID_{50}$. The virus suspensions ($10TCID_{50}$) with desired concentrations of test substance were incubated at 37°C for 5 min (Test Substance + Virus Suspension). In addition, the virus without test substance was kept as virus control (Pathogen Control). After incubation, 2.5% cell culture containing 10% inactivated fetal bovine serum was added into each tube to neutralize the test substance at room temperature. The neutralized solution was diluted from 10 to 10^8 times with cell culture solution, and 100 µL of each mixture (Test substance + Virus suspension) were added to the monolayer cultures grown in 96 well microtitre plates. The CPE (cytopathogenic effect) was observed every 24 hours for 72 hours and compared with controls, which was expressed as the protection offered by the test samples to the cells and the virus titer was estimated by endpoint titration method as $TCID_{50}$/ml.

3. RESULTS AND DISCUSSION

The Test Substance i.e., Dabur Pulling Oil Mouthwash, when evaluated for its cytotoxicity activity by MTT assay in Vero cells at different concentrations from 0.5 to 0.038 % (v/v) showed a dose dependent toxicity against Vero cell as is shown in Table 2.

Table 2: Cytotoxic properties of the test substance (Dabur Pulling Oil Mouthwash) on Verocells.

S. No.	Name of Test Substance	Test Conc. (% v/v)	% Cytotoxicity
1.	Dabur Pulling Oil Mouthwash	0.5	62.44
		0.15	32.19
		0.125	30.28
		0.1	25.33
		0.075	15.59
		0.038	7.70

The test substance (Dabur Pulling Oil Mouthwash) exhibited moderate cytotoxicity; hence the non-toxic concentration (0.1% v/v) was taken for further virus neutralization studies.

The first step in screening compounds for their anti-viral activity involves conducting Virus Neutralization assay. The Virus Neutralization Assay is based on the observation that virus infection and multiplication results in cytopathic effects due to either release of virus or induction of apoptosis as a result of host immune responses. Inhibition of Virus CPE in presence of test compound could be due to inhibition of virus replication.

Table 3 shows the virucidal activity of the test substance wherein acyclovir is taken as the standard for comparison of activity. It was found that Dabur Pulling Oil Mouthwash after a contact time of 5 mins and 0.100 % (v/v) concentration exhibited a Log reduction value of 3.00 against challenge virus dose TCID50. Further, log reduction against HSV-1 virus challenge dose of 10TCID50 was comparable between the test substance at 0.1% (v/v) concentration and the Standard Acyclovir at10 µg/mL concentration.

Table 3: Virucidal activity of Test Substance (Dabur Pulling Oil Mouthwash) against HSV-1.

Virus	Name of Test Substance	Viral Load (TCID)	Test Conc.	Log TCID50 reduction
HSV-1	Dabur Pulling Oil Mouthwash	10	0.100 (% v/v)	3.00
	Acyclovir (std)	10	10 µg/mL	3.25

CONCLUSION

Through this short communication, the anti-viral efficacy of the ayurvedic test substance towards HSV-1 virus is being reported. Oral herpes is a common infection of the mouth area caused by HSV-1 virus. There exists a need for herbal alternatives in management of this disease. Pulling Oil is an Ayurvedic therapy which is known to be anti-microbial. The objective of the study was to evaluate the anti-viral efficacy of Dabur Pulling Oil Mouthwash, prepared from a unique blend of oils of medicinal herbs, against HSV type 1 Virus. In Virus Neutralization assay, the test substance, Dabur Pulling Oil Mouthwash, exhibited a 3.0 log reduction against HSV-1 virus challenge dose of 10TCID50 (Virus-3.71 TCID50) at 0.1% (v/v) concentration and contact time of 5 Mins. Based on the results, it can be concluded that Dabur Pulling Oil Mouthwash reduced 99.9% of HSV-1 Enveloped Virus against untreated Virus control.

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Conflicts of interest

The authors declare no conflict of interest.

REFERENCES

1. World Health Organization. Herpes Simplex Virus. https://www.who.int/news-room/fact-sheets/detail/herpes-simplex-virus
2. https://patient.info/doctor/oral-herpes-simplex#ref-1
3. Amir J, Harel L, Smetana Z, Varsano I. Treatment of herpes simplex gingivostomatitis with aciclovir in children: a randomised double blind placebo controlled study. BMJ, 1997;21, 314(7097): 1800-3.
4. Singh A and Purohit B. Tooth brushing, oil pulling and tissue regeneration: A review of holistic approaches to oral health. Journal of Ayurveda and Integrative Medicine, 2011; 2(2): 64–8.
5. Shanbhag VK. Oil pulling for maintaining oral hygiene - A review. J Tradit Complement Med, 2016; 6, 7(1): 106-109.
6. Subroto, E. and Indiarto, R. Bioactive monolaurin as an antimicrobial and its potential to improve the immune system and against COVID-19: a review. Food Research., 2020; 4(6): 2355 – 2365.
7. Schuhmacher A, Reichling J, Schnitzler P. Virucidal effect of peppermint oil on the enveloped viruses herpes simplex virus type 1 and type 2 in vitro. Phytomedicine, 2003; 10(6-7): 504-10.
8. Vangalapati M, Satya NS, Prakash DS, Avanigadda S. A Review on Pharmacological Activities and Clinical Effects of Cinnamon Species. Res J Pharm Biol Chem Sci, 2012; 3(1): 653-63.
9. Francis D and Rita L. "rapidcolorometric assay for cell growth and survival modifications to thetetrazolium dye procedure giving improved sensitivity and reliability". Journal of Immunological Methods, 1986; 89: 271-277.
10. Hu JM, Hsiung GD. "Evaluation of new antiviral agents: I. In vitro perspectives". Journal of Antiviral Research, 1989; 11(5-6): 217-32.
11. Vijayan, Prabha; Hariharapura, Raghu; Godavarthi, Ashok; So, Dhanaraj & Suresh, B Antiviral activity of medicinal plants of Nilgiris. Indian Journal of Medical Research, 2004; 120: 24-29.
12. Behbahani M. Anti-viral activity of the methanolic leaf extract of an Iranianmedicinal plant "Hyssopus officinalis" against herpes simplex virus. Journal of Medicinal Plants Research, 2009; 3: 1118–25.
13. Kowalczyk A, Przychodna M, Sopata S, Bodalska A, Fecka I. Thymol and Thyme Essential Oil—New Insights into Selected Therapeutic Applications. Molecules, 2020;

25(18): 4125.

14. Khare CP. Indian Medicinal plants: An Illustrated Dictionary (ed.), New York; Springer Science+Business Media, LLC, 2007; 32.