



Formulation of Antibacterial Hand Gel using *Mentha spicata*

Krishnamoorthy Sreenikitha¹, Ravichandran Menaga¹, Dhilip Gowri¹, Shankaran Sruthi¹, Shilpa J^{1*}

¹ Department of Biotechnology, Sri Shakthi Institute of Technology, Coimbatore, Tamil Nadu, India

Abstract:

Hygiene is one of the main factors involved in leading a better and healthier lifestyle. Hygiene is directly or indirectly influenced by hands' sanitization. Hands are the foremost route of transmission of infections, so maintaining hand hygiene is very important. In this study, an economically viable antibacterial gel was made using Aloe Vera in combination with aqueous extract of *Mentha spicata* (spear mint), which is an herb that has wide range of applications in therapeutics like anti-ulcer, wound healing and anti-bacterial properties. The extract of the plant was obtained by drying followed by crushing and extraction using methanol. The water content of the gel was analyzed for maintaining the gel consistency. The minimum inhibition concentration of the extract on *E. coli* was studied. The antibacterial activity of the gel was studied by introducing *E. coli* in nutrient agar incorporated with gel. The efficiency of the gel to suppress the growth of *E. coli* was studied by performing spread plate on media with gel and disc diffusion method. Corresponding positive (Dettol sanitizer) and negative controls were introduced to compare the gel efficiency. A light green colored gel was obtained with bactericidal property. In the study, number of colonies formed in the presence of antibacterial gel was less when compared to the gel without the herbal extract. The zone of inhibition of bacterial growth was compared with commercially available antibiotics and sanitizers. In conclusion, the combination of the aqueous extract of *Mentha spicata* and Aloe Vera can actively work against microbial infections and can have wide range of applications in personal hygiene.

Keywords: Antibacterial gel, Aloe Vera, *Mentha spicata*, hand sanitization.

Introduction:

Hygiene plays a major role in leading a better and healthier lifestyle. Sanitization of hand can have a direct or indirect impact on hygiene. Skin is the largest sensory organ in our body and a part of integumentary system. The most crucial, straightforward, and affordable method of reducing nosocomial infections is practicing good hand hygiene. Microorganisms may be transmitted through the contaminated hands [1]. Hand washing is a crucial safety measure to protect the skin from dangerous microorganisms and to stop the spread of many deadly diseases [2]. Hand washing eliminates visible dirt from hands and lowers the quantity of dangerous bacteria like *Salmonella* and *E. coli* that are associated with people, animals or equipment's. Humans obtain life-saving medications from medicinal plants, especially in underdeveloped nations. In developing nations, more than 80% of the world's population relies mostly on herbal medicine for basic health care, according to estimates from the World Health Organization [3]. *Mentha spicata* is commonly known as spearmint or mint which is one of the perennial aromatic herb species that belongs to the family of *Lamiaceae* (mint family) and the subfamily of

Napithoidae. This plant has a wide of application in culinary like the fresh and dried leaves are used in preparing beverages like tea and candies, jellies, syrups, ice creams and mint sauce and also helps in the production of essential oils for flavoring purposes, toothpaste, cosmetics, and chewing gum[4]. The strong smell of spearmint makes it simple to recognize. Apart from the applications in culinary it also plays a major role in the medicinal fields. The herb is thought to have diuretic, stomachic, stimulant, carminative, and antispasmodic effects. This is extensively used in the Persian medicines for their unique properties. It helps in reducing nerve strains, stress, fatigue, respiratory problems like asthma and headaches by in taking the decoction of the leaves [5]. The cream and lotions containing mint extract is used to reduce the itching, reduces the growth of hair from the body due to its anti-androgenic property and the antibacterial activity [5]. A well-known medical plant called *Aloe barbadensis* Miller is utilized for a variety of therapeutic applications [6] [7] [8]. It naturally contains a variety of beneficial chemicals with the potential to be used in the treatment of numerous ailments. Aloe Vera has an antibacterial, antiseptic, anti-inflammatory and wound healing property [6] [8]. Aloe Vera extracts are known to cure skin damage. Aloe Vera's antibacterial effects ease itching and skin swellings and helps to cure burns, skin irritations, cuts, and insect bites. It is well recognized to actively repair the damaged skin cells that create the outward signs of ageing and can help slow down the development of wrinkles. Aloe is an effective detoxifier, antibacterial, and nervous system tonic. Additionally, it possesses immune-stimulating and antiviral qualities [7] [9]. The Aloe Vera gel act as germ-retarding agent and moisturizing agent. It inhibits the growth of some bacterial strains [2] [10]. When dissolved in a liquid phase, gelling agents are the substances that produce a weakly cohesive internal structure in a colloidal combination. They are either hydrophilic inorganic compounds or organic hydrocolloids. Gelling agents are utilized in semisolid dosage forms at a concentration of 0.5%–10%. This study helps us to minimize the effect of bacteria and improve hand sanitation by applying the herbal gel which is rich in antibacterial property, anti-inflammatory property to the site of action. In addition, gel based hand sanitizers has longer protection time and moisture content on the surface of the skin [2] [11]. The application of the herbal hand gel is easy and can be opted by everyone. The present work highlights the combination of mint extract and aloe Vera extract to develop an herbal hand sanitation gel, which can combat with the widely demanding commercially available hand sanitizer.

Materials and methods:

Extraction of bioactive compounds from *Mentha spicata*:

The leaves of the spearmint was collected from the garden, washed properly to remove dirt's and soil particles and weighed. It was allowed to shade dry for 48 hours. After complete drying, the leaves were ground into fine coarse particles with the help of mortar and pestle. Five gram of the ground powder was mixed with 50 mL of methanol. This mixture was placed in the stirrer with constant stirring for 24 hours. The bioactive compounds present in the spearmint gets dissolved in the methanol [4]. Then it was filtered using Whatmann filter paper and the extract is stored at 4°C.

Preparation of Aloe Vera gel:

Healthy leaves from the Aloe Vera plant was collected from the garden and washed thoroughly using water. The thick epidermis of the leaves were transversely cut and the center gel portion is obtained and made into small pieces. These pieces were thoroughly ground using mixer and the paste was obtained [8]. This mixture was filtered using cheese cloth. Filtrate and the liquid was collected separately. Gelling agent (gelatin) was added to the extracted liquid to make a consistent gel.

Antibacterial studies of the extract:

Disc diffusion method:

E. coli was sub cultured from the mother culture which was inoculated in nutrient broth. Nutrient agar was prepared and sterilized using autoclave at 15 lbs pressure at 121°C. After sterilization, the media was poured into sterile petri plates which were neatly marked and allowed to solidify. Bacterial culture was streaked in the solidified media and left undisturbed for 15 minutes. After drying, the discs were placed in the media. Disc 1- commercial antibiotic (amoxicillin), disc 2- Commercial hand sanitizer (Dettol), disc 3- Aloe Vera gel, disc 4- methanolic mint extract, disc 5- combination of aloe Vera gel and methanolic mint extract in the ratio 1:2, disc 6- combination of Aloe Vera gel and methanolic mint extract in the ratio 1:3. These were made in triplicates. The plates were incubated overnight in the incubator for 37°C, and the zone of inhibition was observed [1].

Spread plate method:

Nutrient agar was prepared and sterilized. After sterilization, the herbal extract of different concentration was mixed with the nutrient agar in a conical flask. This mixture was poured in the sterile petri dishes and allowed to solidify. Another petri plate with media alone was taken as a control. After solidification, the bacterial sample was spread on both of agar medium with the help of sterile L-rod. The plates were incubated overnight and the results were observed after 24 hours of incubation.

Water content analysis:

This analysis was carried out to find the consistency of the gel. The prepared hand gel was weighed and it was allowed to dry for 15 days. After complete drying, hand gel was completely free from moisture content. The weight of the dehydrated hand gel was noted and the results were interpreted. The moisture content of the gel was calculated using the formula given below,

$$\text{Moisture content, MC} = (w-d/w) \times 100$$

Where w = wet weight of the gel

d = weight of the gel after drying.

Results:

Light green colored spearmint methanolic extract and Aloe Vera gel were obtained. The zone of inhibition was studied for different concentrations of gel with the herbal extracts and also it was compared with commercially available hand sanitizer and antibiotic present in the market (Fig 1; Table 1). The diameter of the zone of inhibition was noted for different concentrations of the extracts.

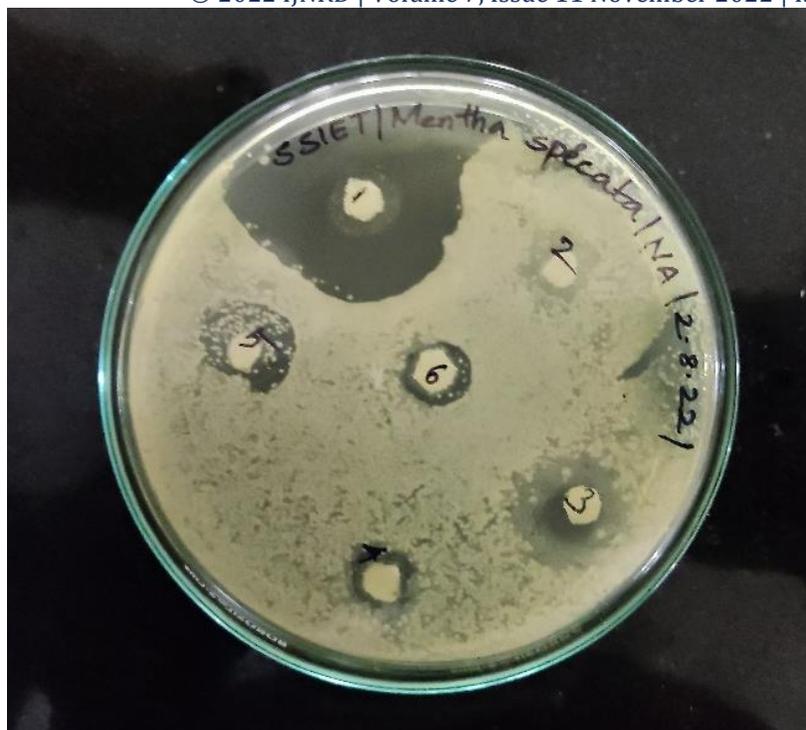


Fig.1 The antibacterial activity of the hand gel by Kirby Baur disc diffusion method

(Disc 1- commercial antibiotic (amoxicillin), disc 2- Commercial hand sanitizer (Dettol), disc 3- Aloe Vera gel, disc 4- methanolic mint extract, disc 5- combination of aloe Vera gel and methanolic mint extract in the ratio 1:2, disc 6- combination of aloe Vera gel and methanolic mint extract in the ratio 1:3)

Table 1 - The mean diameter of zone of inhibition of the samples in disc diffusion method

Sample	Zone of inhibition (cm)
Disc 1- commercial antibiotic (amoxicillin)	2.0
Disc 2- Commercial hand sanitizer (Dettol)	0.7
Disc 3- Aloe Vera gel	1.8
Disc 4- methanolic herbal extract	0.8
Disc 5- combination of aloe Vera gel and methanolic extract in the ratio 1:2	0.9
Disc 6- combination of aloe Vera gel and methanolic extract in the ratio 1:3	1.0

The spread plate technique was carried out to understand the minimum concentration required by the extracts to inhibit the growth of *E. coli*. Here the extracts and the control were compared and differentiated to conclude the results. The 5mL of methanolic extract of mint was showing maximum inhibition of bacteria when compared to 1mL and 2mL of extracts (Fig 2).

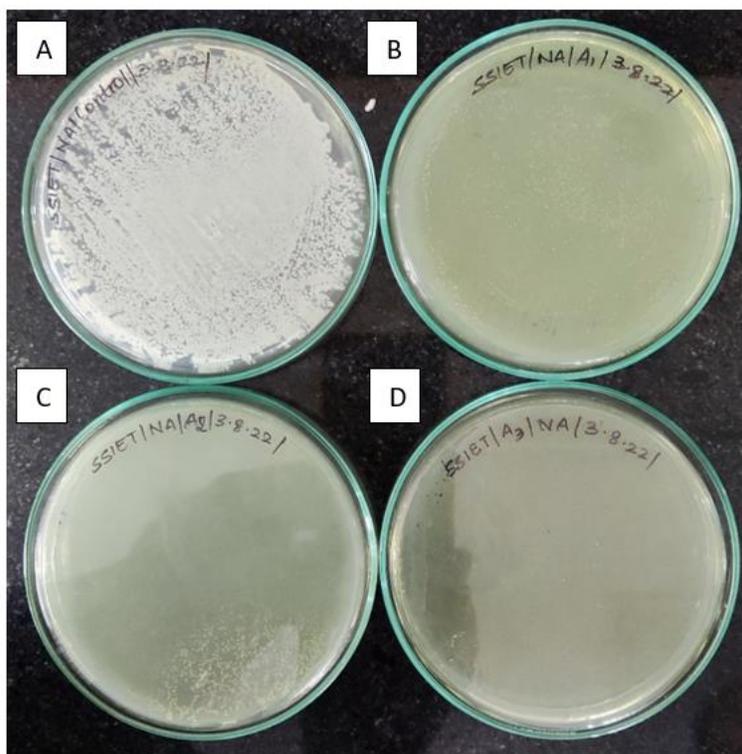


Fig. 2 The minimum inhibition concentration of the herbal extract to inhibit the growth of *E. coli* using spread plate method.

A - Control (nutrient agar + *E. coli*), B - (nutrient agar + 1 ml of methanolic extract + *E. coli*), C - (nutrient agar + 2 ml of methanolic extract + *E. coli*), D - (nutrient agar + 5 ml of methanolic extract + *E. coli*)

The water content analysis was also determined to understand the water content in the antibacterial gel. Sufficient water is required for maintaining the consistency of the gel. Considering the initial and dry weights of the gel, the percent water content was observed as 87%.

Discussion:

The aloe Vera exhibits antibacterial activity against many gram positive and gram negative bacteria like *Klebsiella pneumonia*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pyogenus* by breaking their cell wall and causing death to the organism [13] [14] [15]. *Mentha spicata* essential oil shows the antibacterial activity which has been documented using the diffusion method and the minimal inhibitory concentration against *Escherichia coli* and *Staphylococcus aureus* [5]. In the present study, the Aloe Vera gel and the extract of *Mentha spicata* was experimented against the infectious microorganism like *E. coli* and compared with commercially available hand sanitizer. The Aloe Vera and *Mentha spicata* extract is found to have an antibacterial activity by rupturing the cell wall of *E. coli* which is studied in terms of zone of inhibition by disc diffusion method. The hand gel with natural agents minimizes the toxicity, allergies and side effects, which has global importance in the current scenario.

Conclusion:

Hands are the primary mode of spreading various infections. Poor hand hygiene among people might be a concern. No matter the location, prevention and control of infectious activities are intended to stop the spread of infection and create a secure atmosphere for everyone. Effective infection control techniques, such hand sanitizing is crucial to prevent the advent of antibiotic-resistant pathogens. Comparing with chemically prepared hand sanitizer, herbal

aloe Vera and mint extract hand sanitizer is effective against the prominent bacteria on the hand, *E. coli* and also is environment friendly. In order to create an effective antibacterial herbal hand gel with little to no synthetic chemicals, the biochemical extracts from aloe Vera and spearmint may be incorporated. This may be commercialized further. Thus, with the use of antibacterial gel, germ-free hands are maintained to promote safe and healthier living. The skin sensitivity of aloe vera and mint extract combination need to be addressed before commercializing the gel, which will be considered in future.

References:

1. Acharya, Shri Balakrishna, et al. "Formulation, evaluation and antibacterial efficiency of water-based herbal hand sanitizer gel." *bioRxiv* (2018): 373928.
2. Booq, Rayan Y., et al. "Formulation and evaluation of alcohol-free hand sanitizer gels to prevent the spread of infections during pandemics." *International Journal of Environmental Research and Public Health* 18.12 (2021): 6252.
3. Ekor, Martins. "The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety." *Frontiers in pharmacology* vol. 4 177. 10 Jan. 2014, doi:10.3389/fphar.2013.00177
4. Jain, P. K., et al. "Phytochemical analysis of *Mentha spicata* plant extract using UV-VIS, FTIR and GC/MS technique." *J Chem Pharm Res* 8.2 (2016): 1-6.
5. Mahendran, Ganesan, Sanjeet Kumar Verma, and Laiq-Ur Rahman. "The traditional uses, phytochemistry and pharmacology of spearmint (*Mentha spicata* L.): A review." *Journal of Ethnopharmacology* 278 (2021): 114266.
6. Surjushe, Amar, Resham Vasani, and D. G. Saple. "Aloe vera: a short review." *Indian journal of dermatology* 53.4 (2008): 163.
7. Danish, P., et al. "Antifungal and antibacterial activity of aloe vera plant extract." *Biological and Clinical Sciences Research Journal* 2020.1 (2020).
8. Khan, Abdul Wadood, et al. "Formulation development, optimization and evaluation of aloe vera gel for wound healing." *Pharmacognosy magazine* 9.Suppl 1 (2013): S6
9. Rajeswari, R., et al. "Aloe vera: the miracle plant its medicinal and traditional uses in India." *Journal of Pharmacognosy and Phytochemistry* 1.4 (2012): 118-124.
10. Saoo, K.; Miki, H.; Ohmori, M.; Winters, W.D. Antiviral Activity of Aloe Extracts against Cytomegalovirus. *Phytother. Res.* 1996, 10, 348–350.
11. Greenaway, R.E.; Ormandy, K.; Fellows, C.; Hollowood, T. Impact of hand sanitizer format (gel/foam/liquid) and dose amount on its sensory properties and acceptability for improving hand hygiene compliance. *J. Hosp. Infect.* 2018, 100, 195–201
12. Bimakr, Mandana, et al. "Comparison of different extraction methods for the extraction of major bioactive flavonoid compounds from spearmint (*Mentha spicata* L.) leaves." *Food and bioproducts processing* 89.1 (2011): 67-72.

13. Reynolds T, Dweck AC. Aloe Vera leaf gel: a review update. *J Ethnopharmacol.* 1999; 68 (1): 3-37.
14. Urch D. Aloe Vera the plant. In: *Aloe Vera nature's gift.* 1st ed. Blackdown Publication, Bristol, United Kingdom; 1999; 8-17.
15. Pugh N, et al. Characterization of Aloeride, a new high molecular weight polysaccharide from Aloe Vera with potent immunostimulatory activity. *J Agri Food Chem.* 2001; 49 (2): 1030-4