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DEVELOPMENT AND ANTIBACTERIAL CHARACTERIZATION OF HAND SANITIZER GEL FROM MINT LEAF EXTRACT (*Mentha arvensis* L)

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ABSTRACT

Formulation of hand sanitizers with herbs extract to enhance safety and quality while maintaining effective antibacterial properties. This study aimed to formulate hand sanitizers with different concentrations of ethyl acetate extracts of Mentha arvensis leaves and assess their physiochemical properties and antibacterial activities. The plant material was collected in Jaffna and allowed to shade dry. The dried plant material was macerated with ethyl acetate for 48 hrs at room temperature. Two different formulations (A and B) were prepared with 5% and 10% plant extract. Organoleptic characteristics, pH, homogeneity, turbidity, and antibacterial activity were evaluated. The antimicrobial activity of formulations was estimated using the agar well diffusion method against Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli by employing a formulation excluding plant extract as control and WHO standard hand sanitizer used as a positive control. Results indicated that Formulation B, containing 10% extract, demonstrated satisfactory physiochemical properties and antibacterial activity against the tested bacteria. Formulation A showed a maximum inhibition of 10.60 ± 0.58 mm against E. coli at 800 µg/ml, with no activity against S. aureus and P. aeruginosa at lower concentrations. In contrast, Formulation B demonstrated greater efficacy, achieving inhibition zones of 14.17 ± 1.04 mm for E. coli, 10.17 ± 1.04 mm for S. aureus, and 3.83 ± 0.76 mm for P. aeruginosa at 800 µg/ml. These results indicate that Formulation B shows significant potential as an effective hand sanitizer. Further stability evaluation of formulation B will ensure the evaluation of the clinical usage.

KEYWORDS: *Mentha arvensis L, hand sanitizer gel formulation, antibacterial activity*

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1. INTRODUCTION

Microorganisms, such as fungi, bacteria, viruses, and protozoa, are abundant in nature but invisible to the unaided visible. They cause diseases in healthy individuals, especially bacteria causing a wide variety of diseases such as throat infection, cholera, meningitis, pneumonia, and urinary and GIT infections (Shaloo *et al*., 2017). Among these, Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli are important players in nosocomial infections. *Staphylococcus aureus* grows and survives in nasal secretions, and on the skin and causes minor to fatal diseases (Yaun and Vasquez, 2017). *Escherichia coli* is an opportunistic microorganism, causes urinary tract infections, certain strains produce enterotoxins that can cause traveler's diarrhea and occasionally cause serious foodborne disease (Baruah and Leclercq, 1993). *Pseudomonas aeruginosa* is commonly found as a nosocomial infectious agent and it causes disease in hospitalized patients and immunosuppressed people (Wani *et.al*., 2013).

Hand hygiene plays crucial preventive measure against the chain transmission of microorganisms. (Baruah and Leclercq, 1993). The historical evolution of hand cleansing practices emerged back in the 19th century, with alternative options like soap, water, and hand sanitizers containing at least 70% alcohol (Baruah and Leclercq, 1993). Several studies demonstrated that the presence of 70% of alcohol in sanitizer kills 99.9% of the bacteria on hands (Acharya *et,al*., 2018). As per WHO and CDC guidelines, hand sanitizer formulations may include one or more alcohols such as ethanol, isopropanol, or propanol, along with other active ingredients, excipients, and humectants. To ensure effective sanitization, it is recommended that hand sanitizers should contain between 60% to 95% alcohol, which serves as both an antimicrobial and antiseptic agent (Sommatis *et.al*., 2023) Sanitizers, available commercially in various formulations, offer a convenient and effective means of reducing bacterial counts, particularly associated with the reduction of traveler's diarrhea and vomiting. Studies exploring herbal hand sanitizers highlight the potential synergistic antimicrobial effects of secondary metabolites present in plant extracts, such as alkaloids, terpenoids, tannins, and flavonoids (Patankar and Chandak, 2018). In comparison to liquid (spray) or foam hand sanitizers, gel-based hand sanitizers offer several benefits. One significant advantage is that gels can form a protective layer on the skin, providing longer-lasting protection. Additionally, hand sanitizing gels have a higher retention time on the skin, resulting in better adherence and a more moisturizing feel compared to other forms of hand sanitizers (Booq *et.al*., 2021)

Mentha arvensis L. is an herbal plant belonging to the family of Lamiaceae, features a dark green, quadrangular stem that grows 55-91 cm tall with opposite leaves. Known for its strong peppermint scent and pleasant taste, it is cultivated widely, originating in Japan and spreading to India, Australia, and beyond (Sharma *et.al*., 2013) (Chetia and Saikia, 2020). Various types of phytochemicals such as menthol, menthofuran, and cineol are present in different parts of the plant, and they can be used for different purposes including pharmaceutical formulations. These phytochemicals can be extracted by using organic solvents like chloroform, methanol, and ethyl acetate. According to a previous study, ethyl acetate leaf extract showed more anti-bacterial activity than other solvents (Sujana *et.al*., 2013). Further, *M. arvensis*, the plant contains various flavor compounds and is recognized for its cold-relieving properties, making it valuable in cosmetics and pharmaceuticals (Kapp et.al., 2020). Its leaves are particularly rich in flavonoids, alkaloids, and other secondary metabolites (Patel *et.al*., 2021). Additionally, *M. arvensis* exhibits antifungal, antiseptic, antioxidant, anti-inflammatory, sedative, and antitumor activities (Thawkar *et.al*., 2016) (Sevindik *et.al*., 2018). Considering the extensive tradition of using plants for medical purposes, *M. arvensis* shows promise as a natural and efficient option for hand sanitizers, perhaps protecting against microbial hazards.

There are several studies related to the formulation of hand sanitizer using the combination of herbal plant

extract and evaluation of their anti-microbial and physical parameter. Since there were no studies performed using a single plant extract of *M. arvensis L.* for the formulation of hand sanitizer, this study focused on the formulation and evaluation of *M. arvensis L*. leaf extract containing hand sanitizer.

2. METHODOLOGY

Collection of Plant Material

The *M. arvensis* L. (mint) leaves were collected from Inuvil, Jaffna, Sri Lanka and the plant was authenticated by Prof. Priyangani Senanayake at the Department of Plant and Molecular Biology at the University of Kelaniya using morphological characters.

Preparation of Plant Material

Fresh leaves were washed thoroughly with running tap water to get rid of mud sand, and other dirty particles completely and kept under the shade to dry and ground finely using a clean electric grinder to obtain a homogenous powder sample. It was macerated with ethyl acetate for 48 hours at room temperature. The supernatant was filtered using Whatman No 1 filter paper with a pore size of 11 µm using a vacuum suction pump. The filtrate was removed under reduced pressure below 45 °C using a rotary evaporator for dryness. The resulting crude extract was stored in a refrigerator at 4 °C until further use (John De Britto, Sebastian and Mary Sujin, R., 2012).

Preparation of Formulation

Formulations were prepared according to previous studies and standard industrial guideline in two forms (Surini, Amirtha and Lestari, 2018) (17. Lubrizol, 2011).

- Formulation A- containing 5% (W/V) mint extract and 60% ethanol.
- Formulation B- containing 10% (W/V) mint extract and 60% ethanol.
- Formulation C- Control hand sanitizer
- Formulation D- Standard hand sanitizer (WHO)

The required amount of carbopol 940 was weighed and transferred into the beaker glass containing deionized water. This solution was stirred using a magnetic stirrer after 24 hours, and tri-ethanolamine was added slowly with stirring until get proper gel consistency. Meanwhile, Plant extract was added to the ethanol along with glycerin, and polysorbate-20 into a separate beaker. This mixture was poured into a gel base and stirred vigorously using a magnetic stirrer. Finally, methyl paraben was added and finally, the volume was adjusted up to 30 mL using deionized water (Rahmasari *et.al*., 2020). A similar procedure was repeated for the preparation of negative control without having the plant extracts. The 70% alcohol-based hand rub was prepared based on the WHO guideline which was used as positive control. The composition of ethyl acetate in cooperated hand sanitizer is given in Table 1.

EVALUATION OF PHYSICAL PARAMETERS

Physical evaluation tests were done for all the prepared hand sanitizer formulations.

Organoleptic characteristics

Oduor and color of the formulation were observed manually (visual method).

Homogeneity and turbidity

It was ensured manually.

pH

pH of the hand sanitizer was determined by using a digital pH meter. The formulation was dissolved in 100 mL of distilled water and stored for two hours. The measurement was taken using a previously calibrated pH meter (Afsar and Khanam, 2016).

Table 1: Composition of ethyl acetate extract incorporated hand sanitizer.

Ingredients	Quantity		
	Formulation A (30 mL)	Formulation B (30 mL)	Formulation C (30 mL)
Ethanol (95%) (ml)	18.947	18.947	18.947
Plant extraction (g)	1.5	3	-
Glycerin (ml)	0.69	0.69	0.69
Carbapol 940 (g)	0.15	0.15	0.15
Triethanolamine	0.21	0.21	0.21
Polysorbate 20 (ml)	0.15	0.15	0.15
Methyl paraben (mg)	0.15	0.15	0.15
Deionized water (ml)	Up to 30 ml	Up to 30ml	Up to 30ml

ANTIMICROBIAL ACTIVITY

Test organisms.

In-vitro anti-microbial activity of prepared hand sanitizer formulations was evaluated by using the good diffusion method in Mueller-Hinton agar medium against the selected microorganism *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 9027).

Dilution procedure for test sample

A stock solution of formulation A was prepared by using 16 mg of prepared formulation and it was transferred into a 20 mL volumetric flask. Then 10 mL of sterile distilled water was added into each flask, and it was shaken well until all the gel dissolved completely. Finally, the volume was made up to 20 mL. From the stock solution various concentrations of sample (800 µg/ml, 400 µg/ml, 200 µg/ml) were prepared using serial dilution method. The same procedure was repeated for formulation B.

Determination of antimicrobial activity by agar well diffusion method

All above mentioned species of bacterial inoculum were prepared by suspending bacterial colonies in sterile normal saline directly to achieve the same density of the 0.5 McFarland standard (1-2 x 10^8 CFU/ml).

The agar plate was inoculated with the standard bacterial suspension described above. Using a sterile cotton swab, the bacterial suspension was carefully applied by rotating the swab several times and streaking it across the entire surface of the agar plate in a methodical manner. This process was repeated two additional times, with the agar plate rotated approximately 60 degrees each time to ensure a thorough and even distribution of the inoculum. Subsequently, the agar plate was left undisturbed to air dry for 3-5 minutes to allow the bacterial cells to adhere to the agar surface. Following inoculation, each plate was meticulously perforated with a 6 mm diameter sterile cork borer, creating precisely five bores on each plate (Patankar and Chandak., 2018).

Each test sample, consisting of 100 µL, was carefully introduced into the wells present in the inoculated Mueller-Hinton agar medium. Subsequently, the plate was placed in an incubator overnight at 37°C. As part of the experimental controls, a 0% mint extract (formulation C) and a 70% alcohol-based hand rub (formulation D) were employed as positive controls in this study. The experiment was conducted in triplicate to ensure the accuracy and reliability of the results. The antibacterial activity was quantified by measuring the mean zone of inhibition (in mm) produced by each formulation.

Statistical Analysis

The diameter of the zone of inhibition (in mm) was used as the indicator for the antimicrobial activity of samples. The results were expressed as Mean ± Standard Deviation of the mean. The antimicrobial activity of the formulations was analyzed with one-way ANOVAs followed by Tukey's test using

software, SPSS. Differences between means were considered significant if P-values lower than 0.05 ($p<0.05$)

3., RESULTS AND DISCUSSION

Yield percentages of extract of *M. arvensis L.*

The yield percentage of *M. arvensis L.* is shown in Table 2.

Table 2 Yield percentages of different extracts

Parts of plant	Type of solvent	Weight of crude extract (g)	Yield percentage (% w/w)
Leaf	Ethyl acetate	6.89	1.72

M. arvensis L. leaves were extracted successively with ethyl acetate using the maceration method exhibiting a yield percentage of 1.72%. Ethyl acetate was selected as the solvent for extraction as it exhibited strong antibacterial activity (Sujana *et.al*., 2013). The low yield percentage might be due to solvent selection, extraction condition, method of extraction, variation in plant parts and variation of secondary metabolites in accordance with geographical area (Khan *et.al*., 2022).

The results of the organoleptic characteristics and pH of the two formulations are shown in Table 3. The prepared sanitizer gel showed good gel characteristics and the color of the formulation was observed as dark green. The odor of the hand sanitizer was the odor of the mint leaves. The two formulations had a homogeneous consistency, characterized by an absence of turbidity or cloudiness. Its visual aspect was smooth and translucent, enhancing its overall aesthetic appeal. The pH values of the formulation A and formulation B gels were 4.43 and 5.54 respectively. pH value of B formulations is high compared to formulation A might by the presence of acidic compounds. Generally, hand sanitizer should have a pH similar to that of skin which falls a range of 4.5 to 6.5 (Fallica, *et.al.*, 2021). While formulation B falls within this acceptable range, formulation A is slightly below the lower limit but still close enough to be considered suitable for skin compatibility though prolonged use could cause minor skin irritation (Malarvarnan, Sivasinthujah and Gnanakarunyan, 2023). This slightly higher pH in Formulation B may result from the presence of acidic compounds in the extract, but it aligns well with the skin's natural pH, offering better skin compatibility and potentially more stable antimicrobial efficacy. Given that both formulations demonstrate effective antibacterial activity, with Formulation B outperforming Formulation A, it is plausible that the pH of Formulation B contributes positively to its stability and enhanced antibacterial performance, particularly against bacteria like *E. coli* and *S. aureus*. Maintaining a pH closer to the skin's natural range likely ensures that Formulation B is more suitable for prolonged use while retaining its antimicrobial properties. The antimicrobial efficacy of various compounds can be influenced by pH levels. Studies on the antibacterial activity of plant extracts like *M. arvensis* often show that pH can affect the stability and bioavailability of active compounds. For ethanol-based hand sanitizers, a pH closer to the skin's natural pH promotes stability and enhances user comfort during long-term use (Gama *et.al*., 2023).

The organoleptic characteristics and pH results of the two formulations are presented in Table 3. The prepared sanitizer gel exhibited desirable gel characteristics, with the formulation displaying a distinct dark green colouration. The scent of the hand sanitizer was reminiscent of mint leaves, providing a refreshing olfactory experience. Both formulations demonstrated a uniform consistency, devoid of any turbidity or cloudiness, and presented a visually appealing smooth and translucent appearance. The pH values of formulation A and formulation B gels were measured at 4.43 and 5.54, respectively. The higher pH value of formulation B compared to formulation A may be attributed to the presence of acidic compounds. It is noteworthy that hand sanitizers ideally maintain a pH level similar to that of the skin, typically falling within the range of 4.5 to 6.5 (Fallica, *et.al*., 2021). Thus, both formulation A and B are within the acceptable standard pH range for hand sanitizers.

Table 3: Results of Physiochemical parameters

Parameter	Formulation	Observation
Colour	A	Dark green
	B	
Odour	A	Odour of the mint leaves
	B	
Homogeneity	A	Homogenous
	B	
Turbidity	A	No turbidity
	B	
Appearance	A	Smooth and translucent
	B	
pH	A	4.43
	B	5.54

Evaluation of antimicrobial activity of formulations

The test was conducted against three bacterial species *E. coli, S. aureus and P. aeruginosa*. The mean and standard deviation of the zone of inhibition are shown in Table 4.

Based on the results, both formulations A and B showed antimicrobial activity at 800 µg/ml. However, E. coli, S. aureus and P. aeruginosa showed resistance to formulation A at both 400 µg/ml and 200 µg/ml, except for E. coli, which was sensitive at 200 µg/ml. In contrast, E. coli, S. aureus, and P. aeruginosa were sensitive to formulation B at 400 µg/ml and 200 µg/ml, with the exception of P. aeruginosa at 200 µg/ml. The zone of inhibin of formulations A, B, Positive control and negative control is shown in figure 1, 2 and 3.

Table 4: The inhibitory effect of M. arvensis L. at different formulations on E. coli (ATCC 25922), S. aureus (ATCC 25923) and P. aeruginosa (ATCC 9027).

The type of hand sanitizer	Concentration (µg/ml)	Mean ± Std deviation of the zone of inhibition (mm) for *E.coli*	Mean ± Std deviation of the zone of inhibition (mm) for *S. aureus*	Mean ± Std deviation of the zone of inhibition (mm) for *P. aeruginosa*
Formulation A	800 µg/ml	10.60 ±0.58^{c}	9.00 ± 1.00^{c}	2.50 ±0.5^{c}
	400 µg/ml	7.67 ± 1.5^{d}	00±00^{e}	00±00^{d}
	200 µg/ml	00±00^{f}	00±00^{e}	00±00^{d}
Formulation B	800 µg/ml	14.17 ±1.04^{b}	10.17 ±1.04^{b}	3.83 ±0.76^{b}
	400 µg/ml	7.33 ±0.57^{d}	5.83 ±0.28^{d}	3.67 ±0.28^{b}
	200 µg/ml	4.16 ±0.28^{e}	2.33 ±0.57^{d}	00 ±00^{d}
Formulation C (Negative control)	800 µg/ml	00 ±00^{f}	00 ±00^{e}	00 ±00^{d}
	400 µg/ml	00 ±00^{f}	00 ±00^{e}	00 ±00^{d}
	200 µg/ml	00 ±00^{f}	00 ±00^{e}	00 ±00^{d}
Formulation D (Positive control)	70% alcohol-based hand sanitizer	27.00 ±6.42^{a}	26.00 ±3.60^{a}	12.00 ±0.57^{a}

In the table 4 Values are represented as mean±SD; Values with different superscripts in the same column differ significantly ($P<0.05$).

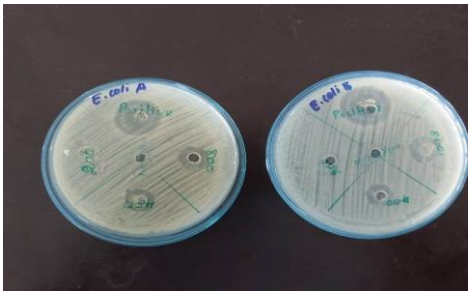

Figure 1: Zone of Inhibition in *E.coli.*

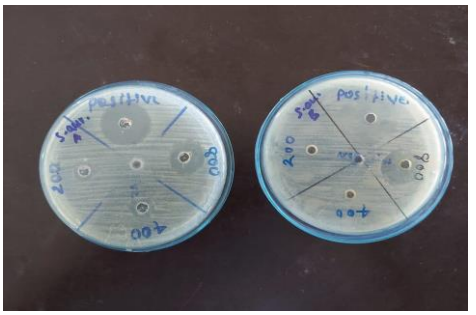

Figure 2: Zone of Inhibition in *S. aureus*

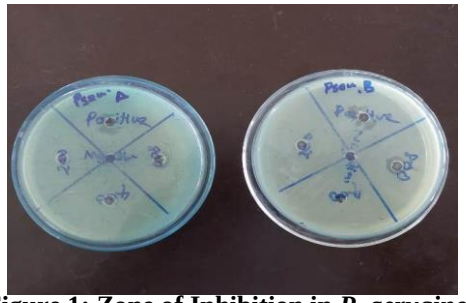

Figure 1: Zone of Inhibition in *P. aeruginosa*

The zone of inhibition for formulation A was found to be 10.60±0.577 mm, 9.00±1.00 mm and 2.50±0.5 mm for *E. coli*, *S. aureus* and *P. aeruginosa* respectively at 800 µg/ml. Likewise, formulation B showed 14.17±1.04 mm, 10.17±1.04 mm and 3.83±0.76 mm respectively at 800 µg/ml. Among both, formulation B showed a better antibacterial efficacy compared to formulation A.

When comparing Formulation A and Formulation B with the WHO-recommended hand sanitizer (formulation D), both formulations showed less antibacterial activity, particularly at higher concentrations. For *E. coli*, Formulation B achieved a 14.17±1.04 mm zone of inhibition, which, while lower than the positive control's 27.00±6.42 mm, still indicates considerable antibacterial potential. Formulation A also performed moderately well, with an inhibition zone of 10.60±0.58 mm. Formulation B again showed 10.17±1.04 mm zone while 26.00±3.60 mm for the positive control against *S. aureus*. Formulation A followed closely formulation B with 9.00±1.00 mm, suggesting both could serve as viable antibacterial agents. Even for the more resistant *P. aeruginosa*, Formulation B recorded 3.83±0.76 mm, while Formulation A showed 2.50±0.5 mm, though still trailing behind the WHO recommended sanitizer's 12.00±0.57 mm. These results highlight that while Formulation D remains the most effective, Formulation A and B demonstrate sufficient antibacterial activity, making them promising candidates for further development and optimization. In contrast, the negative control (Formulation C) did not show any antimicrobial activity, confirming that the observed inhibition zones for Formulations A and B are due to their active ingredients.

In comparing formulated hand sanitizer formulations using *M. arvensis* with the referenced herbal sanitizer study, higher concentration formulation (B) showed a stronger zone of inhibition against *E. coli* (14.17±1.04 mm) compared to their polyherbal sanitizer (7±0.7 mm), suggesting superior efficacy of mint extract at higher concentrations. For *S. aureus*, both studies demonstrated similar antibacterial activity, with the polyherbal formulation showing 11±0.01 mm inhibition and formulation B showing 10.17±1.04 mm. However, the polyherbal sanitizer outperformed *P. aeruginosa*, showing a 9 mm inhibition zone compared to results of 2.50±0.5 mm for Formulation A and 3.83±0.76 mm for Formulation B. This suggests that while *M.arvensis* is effective against certain strains, a polyherbal approach incorporating other plant extracts, as used in the referenced study, may provide broader antibacterial efficacy, particularly against more resistant bacteria like *P. aeruginosa* (Acharya *et,al.*, 2018).

4. CONCLUSION

It concludes that formulation B with 10% of ethyl acetate extract demonstrated favorable physicochemical properties and proved effective reduction in bacterial counts. Further stability tests

should be done for the formulation B to evaluate the potential activities.

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