RESEARCH ARTICLE

Received 27 Dec 2022 | Accepted 30 Dec 2022 | Published Online 31 Dec 2022 https://doi.org/10.32948/ajpt.2022.12.30

Evaluation and formulation of antiseptic herbal hand wipes

S.M. Stefi[1], Jerrine Joseph[2], Mary Shamya A[2], V. Ramesh Kumar[1], Arumugam Suresh[3], Rajasekar Thirunavukarasu[4]

Cite this article: Stefi SM, Joseph J, Shamya AM, Kumar VR et al. Evaluation and formulation of antiseptic herbal hand wipes. Asia-Pac J Pharmacother Toxicol 2022; 2: 11-17. https://doi.org/10.32948/ajpt.2022.12.30

Abstract

Background Wet wipes have been commercially used for various purposes, to clean hard surfaces such as floors or kitchen surfaces including personal cleansing. Wet wipes were impregnated with a synthetic lotion for cleaning the skin, these wipes are hygienic because they are disposable and are normally discarded after their first use. Commercially available antiseptic Wet wipes have strong antimicrobial agents Triclosan and other chemicals. The present objective is to alternate the strong antimicrobial agents by using Herbal formulation in Wet wipes. With Aloe barbadensis miller gel and essential oils based antimicrobial formulation was used to prepare antiseptic wet wipes.

Methods Antimicrobial (Agar well diffusion method), antioxidant (DPPH assay), toxicity studies (using Zebra fish embryo) were carried out in this study.

Results The 0.5-2µg/ml of essential oil showed promising antimicrobial activity against standard routine bacterial pathogens, and also for Mycobacterium smegmatis wild strains. 100µg/ml of Tea tree oil and cinnamon oil were toxic to zebrafish embryo. However, sub-lethal doses of oils is (100 µg/mL) against Zebrafish embryo.

Conclusion Thus, an eco-friendly, safe, low-cost herbal hand wipes are formulated for human use for prevention and protection against microbial infection and for maintaining personal hygiene.

Key words aloe vera, essential oils, herbal wet wipes, antioxidant, embryo toxicity

1. Department of Biotechnology, Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu, India.
2. Centre for Drug Discovery and Development, Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu, India.
3. Central Research Laboratory Meenakshi Medical College Hospital & Research Institute, Chennai, Tamil Nadu, India.
4. Meenakshi Academy of Higher Education and Research Institute, Raasi Nagar, Karrapettai Post, Enathur, Tamil Nadu, India.
Correspondence: Jerrine Joseph (Centre for Drug Discovery and Development, Sathyabama Institute of Science and Technology, Chennai 600119, Tamil Nadu, India; E-mail: jerrine.jj@gmail.com).

Introduction

Wipes are subjected to light rubbing on skin or friction, to remove the dirt or liquid from the surface of the purchaser. The main aim of the Consumers is they want wipes to absorb, retain, or release dust or liquid on demand [1]. Records show that wipes are constantly used by consumers in various aspects and its demand is increasing vigorously. In recent days, their portability and convenience, coupled with the hygiene aspect of their single usage have made them very popular with all types of consumers. Wet wipes are made up of porous and adsorbent sheets such as non-woven materials, impregnated with a soothing or antiseptic agent [2, 3]. Commercial routinely used wipes are used to clean the hard surface including skin, for cosmetic purposes, Most of the commercially available wet wipes were susceptible to microbial contamination and require microbial inhibitors or preservatives and Antiseptic wet wipes usually comprise of FDA approved antimicrobial agents such as Triclosan or benzalkonium chloride or chlorhexidine gluconate used to cleansing the microbial contamination in hard surfaces and skin [4]. For easy use herbal wipes can be used which will be a greatest choice and can inhibit the growth. Efterpi et al [5] explained that Aloe vera is used for mankind in various fields. Aloe vera latex and gel have physiologically active substances with biological effects. The identification of these substances is very important for the effective use of the plant. The chemical composition of Aloe vera varies and depends on climate, region, growing conditions and the age of the plant or the processing method. Many researchers reported that Aloe vera is a potent therapeutic application in skin disorders. Maan et al [6] indicated the therapeutic applications of Aloe vera in pharmaceutical, food and cosmetic side. He also explained aloe vera has good anticancer activity, anti diabetic activity, anti inflammatory, anti ulcer effects etc. Because of its wide variety of applications the plant can be used in antiseptic wipes preparation which will give a better and safe hygiene for the consumers who use it. Where Hamid et al [7] indicated the medicinal and pharmacological use of essential oils. These oils are produced from different parts of the plant and each one has different activity. The microbial activity of essential oils was tested with the reference of Swamy et al [8] against P.aeruginosa, C.albicans, and other different pathogens. Present study aimed about the Aloe vera based herbal formulation along with essential oil to study the efficacy against various pathogenic bacteria for the application of wet wipe preparation and also evaluate the toxicity study on zebra fish embryos.

Materials and methods

Chemicals required

Chemicals and solvents used in this experiment are of analytical grade.

Collection and processing of Aloe vera

The plant Aloe Veras were collected from Chintadripet, Nimilicherry and Mandampakkam of Chennai, Tamil Nadu in November 2019 and the voucher specimen was identified and validated by a botanist. The freshly harvested leaves of aloe vera were cut manually for experimentation. To avoid biodegradation the leaves are pulled from the mother plant carefully not to break the rind and packed in an icebox at 4-5°C and transported to the laboratory. The leaves were washed completely with fresh water. The outer layer of aloe vera was cut using a knife and the gel inside was taken separately and placed in a beaker. After taking the full gel in the beaker it was homogenized by blender to form the viscous solution.

Formulation of herbal solution

To the Aloe vera solution with preservatives, essential oils were added. This was done in four combinations. In 65% prepared solution 0.5% of tea tree oil and 0.5% of cinnamon oil were mixed separately and added. Lavender oil, basil oil and lemon oil were added based on fragrance. Also, a guar gum of 0.05% was dissolved with 30ml water and added to the solution. 0.5% of Quercetin was mixed with 2ml of acetone or ethanol and this was also separately added in the solution. 0.5ml of DMSO dissolved in 1.5ml of distilled water and added to the solution to the makeup of 100ml.To the other three combinations, different concentrations of EO's, gum, and quercetin with the stabilizing agent was added.

Addition of preservatives

Figure 1. Extraction and preparation of viscous solution.

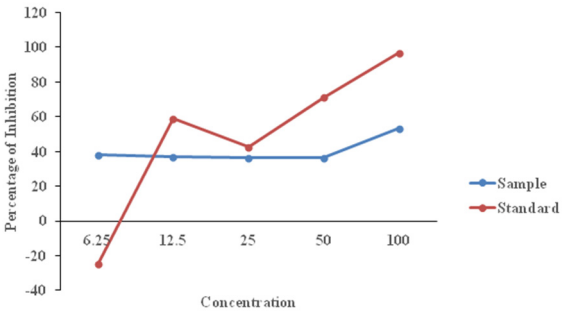


Figure 2. The antioxidant activity of the crude Aloe vera extract by DPPH method.

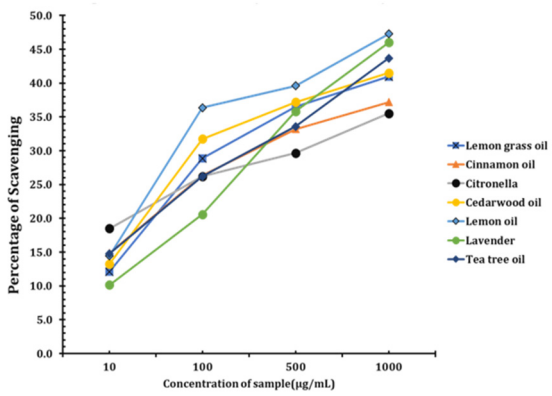


Figure 3. The antioxidant activity of the essential oils by DPPH method.

Preservatives are added to promote the shelf life of the gel and its stability. Preservatives such as potassium sorbate of 0.1%, sodium benzoate of 0.1%, glycerine 0.1%, vitamin E 3mg/100ml, vitamin D 3mg/100ml, citric acid of 0.1% was added to the processed viscous solution of Aloe vera [9].

Antioxidant assay

DPPH radical scavenging activity. The free radical scavenging capacity of the plant extract was measured based on the method delineate by Brand-Williams et al [10]. with slight modification. It is based on electron-transfer that produces a violet solution in methanol. This free radical, which is stable at room temperature, is reduced to colorless methanol solution in the presence of an antioxidant molecule [11]. 0.1 mM DPPH solution was mixed with 1ml of plant extract solution of varying concentration (10, 100, 500, 1000µg/ml) tubes was incubated at room temperature for 30 minutes in dark. Then the absorbance was measured using a UV-Vis spectrophotometer at 517 nm. Gallic acid was used as the standard [12]. The capability of scavenging the DPPH radical was calculated by using the formula:

DPPH scavenging effect/activity
(%inhibition or %scavenging) = {(A0-A1)/A0)x100}

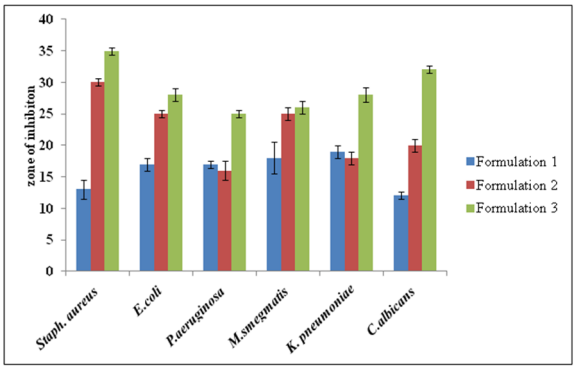


Figure 4. Antimicrobial assay of different formulations against various pathogens.

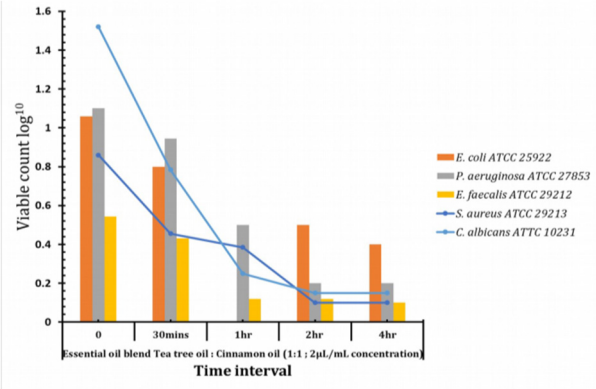


Figure 5. Time kill assay for essential oil blend.

Table 1. Classification of Aloe vera.

Kingdom	Plantae
Clade	Tracheophytes
Clade	Angiosperms
Clade	Monocots
Order	Asparagales
Family	Asphodelaceae
Subfamily	Asphodeloideae
Genus	Aloe
Species	A. vera

Preparation and evaluation of antimicrobial herbal formulation

(1) Agar Well diffusion method. The antimicrobial activity of formulated solutions was screened by well diffusion method. The test strains used are S. aureus ATCC 29213, E.coli ATCC 25922, P.aeroginosa ATCC 27853, E.faecalis ATCC 29212, C.crusei ATCC 6258, C.albicans ATCC, and Mycobacterium smegmatis culture were collected from Lingam Microbiological Laboratory. The plates prepared with 15ml of Muller Hinton agar, lawn culture were made in each plate with standard testing strains, by using well puncture, well was made 15µl of formulated solution were added to the well. Gentamicin was used as a positive control and water as a negative control. The plates were incubated at 37°C for 24 h. After 24 hours of incubation, the test determines the efficacy of the formulation in terms of the zone of inhibition of the organism. The higher the zone of inhibition, the test sample will be more effective [13].
(2) Minimum inhibitory concentration. The MIC is defined as the lowest concentration which completely inhibits the growth of microorganisms for 24 hrs incubation. Determination of minimum inhibitory concentration of formulation was determined by preparing different concentrations 200µg, 400µg, and 800µg were added respectively to the Muller Hinton broth. A 50µl volume of each dilution was added aseptically into the wells of Mueller Hinton agar plates that were already inoculated with test bacteria. The agar plates were incubated at 37°C for 24 hours. The lowest concentration of oils showing a clear zone of inhibition was considered as the MIC.

Zebra fish embryo toxicity studies

Zebra fish embryos were purchased from the zebrafish aquarium in the Kanchipuram district. For toxicity studies, 10 healthy posts hatched zebrafish were transferred to the wells of a 24-well plate along with 1ml of embryo water (60 mg of sea salt/liter of ultrapure water). Different concentrations of wet wipe formulation (5, 10, 25, 50 and100 µg ml-1) were added to the wells and incubated for 72hrs at 28.5°C [14]. Mortality of the zebrafish was noted after 24, 48 and 72 hrs. At the end of the incubation period, the embryos were photographed using the stereomicroscope.

Statistical analysis

All invitro assay data signify the mean ± standard deviation of triplicates and IC50 was calculated by using one way analysis of variances (ANOVA). The inhibition concentration for each microorganism was analyzed using one-way analysis of variance (ANOVA). P value < 0.05 was considered as significant.

Results

Collection and extraction of Aloe vera gel

The plant was collected from different places of Chennai and was processed at Centre for Drug and Discovery Development, Sathyabama Institute of Science and Technology and the classification is represented in **Table 1**. The plant was sliced and the gel was separated and placed in a beaker. The gel was ground and made into a viscous solution in **Figure 1**.

Antioxidant activity by DPPH assay

(1) Antioxidant activity of Aloe vera extract and essential oils. The free radical scavenging capacity of the plant extract was measured with DPPH (2,2-diphenyl-1-picrylhydrazyl) method. It is a rapid and simple method to measure the ability of essential oils and the crude extract of Aloe vera act as free radical scavengers. In this method the maximum level of crude extract of Aloe vera inhibition was found to be 50% at 1000µg/ml. This can be seen in **Figure 2**. And for the essential oil Lemon grass oil showed maximum level of inhibition of 48% at 1000 µg/ml) and Citronella showed minimum level of inhibition and the IC50 value was also determined in **Figure 3**.
(2) Formulation of herbal solution. The formulation was prepared in four combinations with different concentrations of EO's, gum, quercetin were shown in **Table 2**.

Microbial assay

(1) Anti microbial assay of different formulation. The microbial activity of different formulation was performed against different pathogens (Staph aurues, E.coli, P.aeruginosa, K.pneumoniae and C.albicans after 24 hours of incubation the zone of inhibition

Table 2. Optimization of Essential oil concentration in antiseptic formulations.

Essential oil	Formulation 1	Formulation 2	Formulation 3
Tea Tree oil	1±0.1	0.3±0.3	0.01±0.2
Cinnamon oil	1±0.2	0.3±0.1	0.01±0.1
Constant Base ratio	Aloe vera with stabilizing agent-50v/v; Guar gum-0.1%; Vitamin E & D-0.01%; Quercetin-1%		

Toxicity study using zebra fish embryo

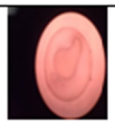
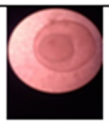
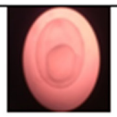
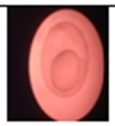
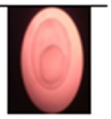
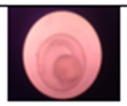
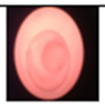
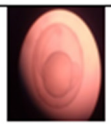
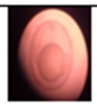
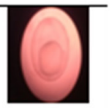
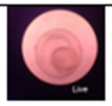
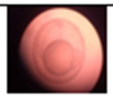
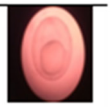
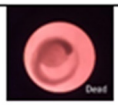
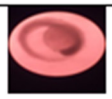
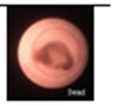
Days	Different concentration of herbal formulation						
Day-1							
	Control	20µl	40µl	60µl	80µl	100 µl	500 µl
Day-2							
	Control	20µl	40µl	60µl	80µl	100 µl	500 µl
Day-3							
	Control	20µl	40µl	60µl	80µl	100 µl	500 µl
Day-4							
	Control	20µl	40µl	60µl	80µl	100 µl	500 µl

Figure 6. Fabrication and packing of herbal wipes.

was noted. Whereas M.smegmatis took 24-48 hours to show the zone of inhibition. Formulation one showed the highest zone of inhibition against Klebsiella pneumonia, formulation 2 and 3 showed the highest zone against Staphylococcus aureus represented in **Figure 4**.

(2) Minimum inhibitory concentration. This method is used to measure the lowest concentration of the sample which inhibits the growth of the specific pathogen. However formulation 1 showed the lowest inhibitory concentration (100 µg/ml) against C.albicans, where formulation 2 showed minimal inhibitory activity against C.albicans at (250µg/ml), S.aureus at (62.5µg/ml) and E.coli (500µg/ml) and formulation 3 showed lowest concentrations against S.aureus at (100µg/ml). According to statistical analysis results of one way ANOVA of Staphylococcus aureus the P-value was 0.306, Candida albicans; P-value was 0.808, Escherichia coli P-value was 0.530. However the P-values were greater than 0.05 were described in **Table 3**. As a result, we fail to reject the null hypothesis and conclude that there is no significant variation in the MIC at 95% CI for each of the test organisms.

Time kill assay

This assay is used to determine the bacetriacidal or bacteriostatic activity of the essential oils over time. Based on the above results formulation 2 and 3 were chosen to check the bactericidal activity against Escherichia coli, Staphylococcus aureus, P.aeruginosa ATCC 27853, E.faecalis ATCC 29212 and C.albicans ATCC. The results from this study show the potential of EO's (Essential oil) as a blend in the ratio of 1:1:2µg/ml concentration that is formulation 3 acts as a therapeutic option to reduce bacterial colonization and infections from clinically resistant pathogens in **Figure 5**.

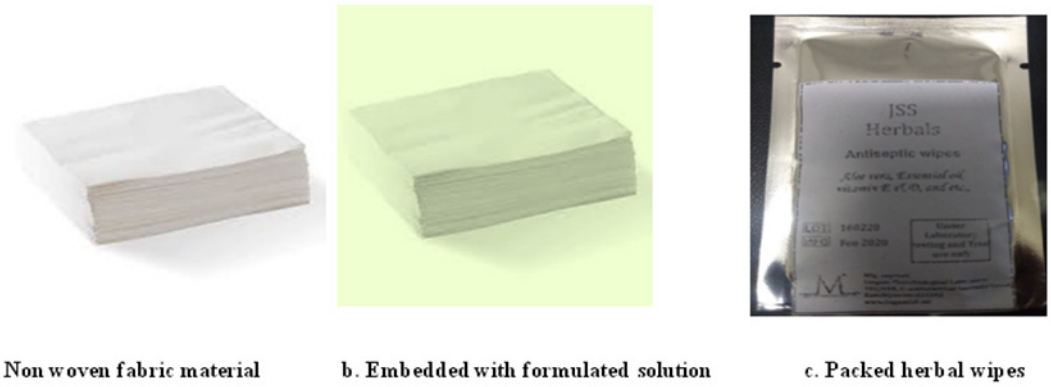
Toxicity on embryo of zebra fish

Toxicity was seen in the embryos of zebra fish from 24 to 72 hours and every day the picture was taken in a microscope. At this period different stages of zebrafish and also the movement was recorded. The zygote, cleavage, blastula, gastrula and segmentation were seen. This was seen for different concentration for the formulation 3 such as 20 µl, 40 µl, 60 µl, 80 µl, 100 µl and 500 µl were no toxicity was observed. Even in higher concentration also after 72 hours the embryos able to survive in the herbal formulation solution in all concentration **Figure 6** and live rate of zebra fish embryo.

Fabrication and packing of wipes

The non woven fabric material was cut into 10cm and autoclaved to make the wipes sterile. The sterile wipes are placed on a tray and the formulated solution is poured on the material and allowed to settle for 5-10min so that the wipes absorbs the solution. It is then folded and placed inside silver pack and it is sealed in **Figure 7**.

Trademark registration



a. Non woven fabric material b. Embedded with formulated solution c. Packed herbal wipes

Figure 7. Zebrafish embryo toxicity of herbal formulation.

The future scope of the study is to register for the trademark for the product. Protocol for trademark are Receiving International applications, Verification of International applications, Certification and transmission of International application, Receiving irregularities if any, from the wipe and responding to them, Communication as to ceasing of effect, Receiving international registrations designating India, Examination of international registrations designating India, Publication of the international registration and Opposition proceedings.

Discussion

The present study showed that the herbal wipes formulated from Aloe vera plant has good ability to kill the microbes. These types of wipes help doctors specially while consulting patients in order to prevent them from getting rid of diseases or contamination. The plant Aloe vera is used by man for thousands of years in medicine mainly for therapeutic uses especially on skin [5]. It has found to be effective against various diseases like wound healing, asthma, HIV infections, teeth and gum protection and genital herbs. Also shows good results against anticancer, antioxidant, antidiabetic and anti inflammatory activities [15]. Bashir et al [16] denoted that aloe vera extract showed 100% active against all gram negative isolates and 75.3% active against gram positive pathogens. The microbial and antioxidant activity of Aloe vera extract 50% at 1000µg/ml and essential oils 48% at 1000 µg/ml showed good percentage of inhibition. Similar report denoted by Sultana et al [17] the antioxidant activity of Aloe vera extract is 53% at 1000µg/ml. Hamid et al [7] indicated the medicinal and pharmacological use of essential oils. Essential oils like lemon oil, tea tree oil, basil oil, cinnamon oil were extracted by distillation process and tested against different pathogens. The microbial activity of essential oils was tested with the reference of Swamy [8] against P.aerogenosa, C.albicans, and other different pathogens. Essential oils were extracted by distillation process. Formulation 1 showed maximum zone of inhibition around 35mm against Staph. aureus. It was used as blend for time kill assay. Time kill assay determined the death of bacteria for certain period. In DPPH assay Aloe gel exhibited radical in a concentration dependent approach and its IC50 value was found to be 572.14 µg/mL, lower value of IC50 predicts the higher antioxidant activity of a test substance [18]. In our study the crude extract of Aloe vera act as free radical scavengers inhibition was found to be 50% at 1000µg/ml. The sterility of the wipes was checked for two months which showed there was no bacterial or fungal growth at this period. The results from this study show the

Table 3. Minimum inhibitory concentration of different herbal formulations against three different pathogens.

Formulation 1			Formulation 2			Formulation 3			P value
S.aureus MIC µg/ml	E. coli MIC µg/ml	C.albicans MIC µg/ml	S.aureus MIC µg/ml	E. coli MIC µg/ml	C.albicans MIC µg/ml	S.aureus MIC µg/ml	E. coli MIC µg/ml	C.albicans MIC µg/ml	
-	-	-	-	500	-	-	-	-	0.05
-	-	-	-	-	250	100	-	-	
-	-	100	-	-	-	-	-	-	
-	-	-	62.5	-	-	-	-	-	

potential of EO's (Essential oil) as a blend in the ratio of 1:1:2µg/ml concentration that is formulation 3 acts as a therapeutic option to reduce bacterial colonization and infections from clinically resistant pathogens. And the toxicity study is conducted with the help of zebra fish embryos toxicity study was observed after 24 to 72hrs for different concentrations 20, 40, 60, 80,100 and 500 µg/ml along with the control. During this time the embryo was developed fish model with head and tail. By this study formulated solution was proved as nontoxic which can be used for hands trial. At last, the wipes was sterilized to kill the presence of any microbes present over it and the formulated solution was poured on to wipes in a tray. It was soaked for 10-15mins then folded and packed into 8 x 3cm silver pack.

Conclusion

Antiseptic herbal hand wipes were developed from Aloe vera. The antimicrobial and antioxidant activity showed promising activity. The shelf life and stability of the product were checked under different temperatures where there was no change of color and phase separation was found. The toxicity studies were done in invitro under embryos of zebrafish. During this period the embryo was developed into the segmentation period after 72hrs and no toxicity was seen. Thus a herbal based wipes were formulated with full microbial testing. The future scope is based on the Institutional Review Boards (IRB) which will be applied for the product and tested under 20-50 volunteers in their right and left thumb. Thus the developed wipes can be taken to the market for the welfare of the society.

Acknowledgments

The authors are grateful to the management of Sathyabama Institute of Science and Technology (deemed University) Chennai for the providing the infrastructure facility.

Ethics approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors.

Funding

Not applicable.

Author contributions

SMS carried out the entire lab work assays for the manuscript; JJ prepared draft document and invigilation for the manuscript; MSA carried out the extract preparation and antioxidant assay for the manuscript and VRK carried out correction and invigilation for the manuscript; AS carried out the trade marketing and antimicrobial study and RT carried out the grammer and correction for the manuscript.

Competing interests

All authors declare no competing interests.

References

1. Siegert W. Microbiological quality management for the production of wet-wipes. Household and Personal Care Today 2008; 2.
2. Acharya SB, Ghosh S, Yadav G, Sharma K, Ghosh S, Joshi S. Formulation, evaluation and antibacterial efficiency of water-based herbal hand sanitizer gel. bioRxiv 2018: 373928.
3. Al-Zahrani SH, Baghdadi AM. Evaluation of the efficiency of Non alcoholic-Hand Gel Sanitizers products as an antibacterial. Nature and Science 2012; 10(6): 15-20.
4. Ahmad A, Husain A, Khan SA, Mujeeb M, Bhandari A. Synthesis, antimicrobial and antitubercular activities of some novel pyrazoline derivatives. Journal of Saudi Chemical Society 2016; 20(5): 577-554.
5. Christaki EV, Florou-Paneri PC. Aloe vera: a plant for many uses. J Food Agric Environ. 2010; 8(2): 245-249.
6. Maan A, Nazir A, Khan M, Ahmad T, Zia R, Murid M, Abrar M. The therapeutic properties and applications of Aloe vera: a review J Herb Med 12: 1-10.
7. Hamid AA, Aiyelaagbe OO, Usman LA. Essential oils: its medicinal and pharmacological uses. International journal of Current research 2011; 33(2): 86-98.
8. Swamy MK, Akhtar MS, Sinniah UR. Antimicrobial properties of plant essential oils against human pathogens and their mode of action: an updated review. Evidence-Based Complementary and alternative medicine. 2016: 2016
9. Nazemi N, Monfared A. Processing and stabilization of Aloe Vera leaf gel by adding chemical and natural preservatives. Research Journal of Pharmacognosy 2017; (4): 24-24.
10. Brand-Williams W, Cuvelier ME, Berset CL. Use of a free radical method to evaluate antioxidant activity. LWT-Food science and Technology 1995; 28(1): 25-30.
11. Miladi S, Damak M. In vitro antioxidant activities of Aloe vera leaf skin extracts. J Soc Chim Tunisie 2008; 10(10): 101-109.
12. Garcia EJ, Oldoni TL, Alencar SM, Reis A, Loguercio AD, Grande RH. Antioxidant activity by DPPH assay of potential solutions to be applied on bleached teeth. Brazilian dental journal 2012; 23: 22-27.
13. Aref HL, Salah KB, Chaumont JP, Fekih A, Aouni M, Said K. In vitro antimicrobial activity of four Ficus carica latex fractions against resistant human pathogens (antimicrobial activity of Ficus carica latex). Pak J Pharm Sci 2010; 23(1): 53-58.
14. Stephens WZ, Burns AR, Stagaman K, Wong S, Rawls JF, Guillemin K, Bohannan BJ. The composition of the zebrafish intestinal microbial community varies across development. The ISME journal 2016; 10(3): 644-654.
15. Pellizzoni M, Molinari GP, Lucini L. Stability of the main Aloe fractions and Aloe-based commercial products under different storage conditions. Agrochimica 2011; 55(5): 288-296.
16. Bashir A, Saeed B, Mujahid TY, Jehan N. Comparative study of antimicrobial activities of Aloe vera extracts and antibiotics against isolates from skin infections. African Journal of Biotechnology 2011; 10(19): 3835-3840.
17. Sultana R, Begum R, Rahman MN, Hasan MR, Haque MA. Development of Aloe Vera Jelly for Diabetic Patients and Analysis of Its Physicochemical Properties. International Journal of Food Science and Biotechnology 2020; 5(1): 1.
18. Ray A, Gupta SD, Ghosh S. Evaluation of anti-oxidative activity and UV absorption potential of the extracts of Aloe vera L. gel from different growth periods of plants. Industrial Crops and Products 2013; 49: 712-719.