

## “ Research On Formulation And evaluation of Polyherbal Hand Sanitizer ”

Name of Resarchers :- Pranali Vilas Bodake ,  
Nilima Dnyaneshawar Gurule ,  
Sakshi Arun Narode ,  
Ankita Sudam Shinde

Name of Guide :- Sangita.N.Bhandare .

Submitted: 15-05-2023

Accepted: 30-05-2023

### ABSTRACT :-

Hands are the first mode of transmission of microbes and infections. Hand hygiene is a key principle and exercise in the prevention, control and reduction of infections. Due to COVID pandemic the need of hand sanitizer has increased which causes less dryness to hand. Considering the need, we prepared a polyherbal sanitizer using seven plant extracts (Lantana Camara -Gultura, Tridax Procumbens-tantani, Ocimum sanctum(linn) -Tulsi, Azadirachta Indica-Neem) with other ingredients including isopropyl alcohol, camphor, hydrogen peroxide, glycerol and water. The ingredients were selected on the basis of their antimicrobial property. The ingredients and sanitizer were evaluated for antimicrobial property and showed potent activity against gram positive bacteria *S. aureus*, whereas mixture of extracts showed potent activity against all the bacterial strains used except *B. cereus*. The sanitizer also showed potent activity against all the strains used in the ascending order of *Klebsiella* spp. > *B. cereus* > *S. aureus* > *S. pyogenes* > *P. aeruginosa* > Dermatophyte > *E. coli* and gave zone of inhibition of  $18 \pm 0.05$ ,  $12 \pm 0.03$ ,  $11 \pm 0.01$ ,  $10 \pm 0.01$ ,  $9 \pm 0.5$ ,  $9 \pm 0.01$  and  $7 \pm 0.001$  mm respectively. The antimicrobial activity was compared with other commercial hand sanitizer and maximum activity was showed against *Klebsiella* spp. and minimum against *E. coli* and *P. aeruginosa* by all the sanitizer used. The efficacy of hand sanitizer was checked on hands and 12 volunteers of laboratory workers, patients and their relatives with written and oral consent. The sanitizer reduced or eliminated the growth of pathogens isolated from hands. Time interval effect was also checked at a time gap of two hours also showed the effect of hand sanitizer for longer time with reduction in bacterial growth. The sanitizer pH was alkaline

with good shelf, texture and odor. No turbidity was seen when kept at higher temperature for 3 months and showed no skin dryness with soothing effect after using sanitizer on different volunteers.

**KEYWORDS:** Lantana Camara -Gultura, Tridax Procumbens- tantani, Ocimum sanctum(linn) - Tulsi, Azadirachata Indica-Neem, s. Aureus,, sanitizer ,polyherbal,

### I. INTRODUCTION

Hand hygiene is disturbed by repeated use of Alcohol Based sanitizer is increased recently due to this the study of sanitizer effect on skin is important. Hands are the first mode of transmission of microbes and infections. Hand hygiene is a key principle and exercise in the prevention, control and reduction of infections.[35 ] Due to COVID pandemic the need of hand sanitizer has increased which causes less dryness to hand. The stratum corneum is in the outer layer of the epidermis, and it contains several proteins and lipids that are important for skin health. This skin layer can be disrupted by agents that cause skin dryness and irritation, resulting in a dysfunctional skin barrier when there is loss of filaggrin breakdown products, also referred to as natural moisturizing factor (NMF), and lipids. This then leads to the unwanted dermatologic effects of skin dermatitis, and in some patients, contact dermatitis. While it is known that frequent hand washing and hand sanitizer use causes hand dermatitis, the effects on skin barrier proteins and lipids has not been studied. We have prepared Polyherbal hand sanitizer which having relatively less side effects and having best results for the kill the bacterial species by using herbs Lantana Camera -Gultura,Tridax Procumbens-tantani ,Ocimum sanctum(linn) -Tulsi,Azadirachta Indica-Neem

### Need of Herbal Sanitizer

Currently, people across the globe are using alcohol based hand sanitizers on massive scale to stop or reduce the spread of coronavirus. Most of the available hand rubs used as sanitizers composed up of isopropyl alcohols, H<sub>2</sub>O<sub>2</sub> and ethanol in different combinations. Misuse of these provisions may leads to the toxicity in human well beings and to environment.

Formulations and preparations of hand sanitizers by herbal plants have been proved effective against pathogens and results have also been compared and found effective with alcoholic based formulations of hand sanitizers. These herbal formulations have been considered safe for human health as far as to environment.

### PLAN OF WORK :

Following is the plan for research project for preparation of polyherbal hand sanitizer :-

- Collection of Data which researched previously
- Preparation of plant extract for hand sanitizer
- Collection of Excipients for polyherbal hand sanitizer
- Testing Anti-microbial Activity
- Evaluation test for polyherbal hand sanitizer
- Checking compatibility of preparation
- Stability testing

### METHODOLOGY AND MATERIAL

#### Collection of selected leaves of the plant :-

Collection of leaves of the plant The plants leaves were collected for the preparation of sanitizer from in and around the campus The hospital premises. The plant selected on the basis of its potent antimicrobial activity reported In research articles. The plants used for the study were Lantana Camara -Gultura, Tridax Procumbens-tantani, Ocimum sanctum(linn) -Tulsi, Azadirachta Indica-Neem. The Plants leaves collected were weight, washed, cleaned and shade dried in laboratory. After drying Plant extract was prepared in ethanol and used for the preparation of hand sanitizer

#### Preparation of Extract For Hand Sanitizer :

- 1] collection of leaves of herbs of lantana camara, tridax Procumbens, ocimum sanctum, Azadirachta indica from the farms.
- 2] Dry the leaves for 24 hrs. In this process plants leaves become dry as it is capable of convert into fine powder.

3] Powdered the plant leaves which are dried previously.

4] Keep the powdered plants leaves weigh all plants powdered leaves and take 50gm of each plant leaves powder for extraction.

5] Take 50 gm of powdered leaves and poured into 100ml volumetric flask add 100ml 99.9% pure ethanol to it and kept soaked for 24 hrs.

6] Filter the extract form the volumetric flask.

#### Preparation of Polyherbal Hand Sanitizer

The herbal sanitizer was prepared by the following ingredients given below-

1. Plant extracts prepared from 50 gram dried plant were added in equal amount
  2. Hydrogen peroxide -0.2 ml
  3. Glycerol -2. 5 ml
  4. Ethanol 10 ml
  5. Kapoor - 2 Nos
  6. Isopropyl alcohol - 10 ml
  7. Distilled water was used to make up to 100 ml
- The pH of the hand sanitizer was checked using pH strips.

#### Antimicrobial activity of hand sanitizer:-

The antimicrobial activity of hand sanitizer was evaluated by disc diffusion method using **Mueller hinton media**. The commercial sanitizers were used for comparative study with the Sanitizer prepared in laboratory. The commercial sanitizers used were Sterilium, Savlon, Purest, Hand safe, Genius, Lab alcohol (70% isopropyl alcohol as hand disinfectant).

#### Disc Diffusion Method :-

This method is based on the principle that antibiotic-impregnated disk, placed On agar previously inoculated with the test bacterium, pick-up moisture and the Antibiotic diffuse radially outward through the agar medium producing an antibiotic Concentration gradient. The concentration of the antibiotic at the edge of the disk Is high and gradually diminishes as the distance from the disk increases to a point Where it is no longer inhibitory for the organism, which then grows freely. A clear Zone or ring is formed around an antibiotic disk after incubation if the agent inhibits Bacterial growth.

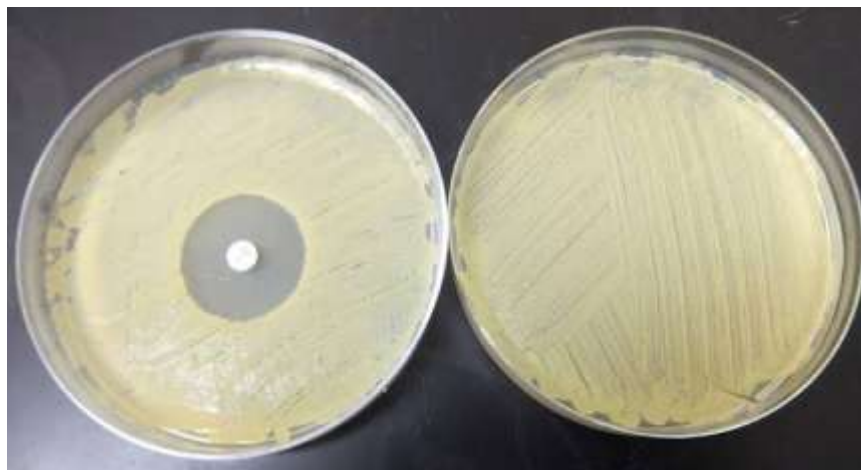
The disk diffusion method is performed using Mueller-Hinton Agar (MHA), which Is the best medium for routine susceptibility tests because it has good reproducibility, Low in sulfonamide, trimethoprim, and tetracycline inhibitors, and gives satisfactory Growth of most bacterial

pathogens. The inoculum for the disk diffusion method is prepared using a suitable broth such as tryptic soy broth. This medium is prepared according to manufacturer's instructions, dispensed

in tubes at 4-5 ml and sterilized. Sterile 0.9% salt solution may also be used. Media are supplemented with 1-2% sodium chloride (NaCl) if intended for marine organisms

**Observations of anti microbial activity -**

Sr.No	Test Organisms	Zone of inhibition			
		Lantana camara	Tridax Procumbens	Azadirachta indica	Ocimum sanctum
1.	E.coli	19 ± 1.41	8.1 ± 0.6	17 ± 0.5	32.02 ± 1.98
2.	Staphylococcus Aureus	13.5 ± 0.71	7.2 ± 0.6	17 ± 3	18.68 ± 0.95
3.	Pseudomonas Aeurogenosa	6 ± 00	8 ± 0.4	16 ± 0.6	25.1 ± 0.75
4.	Proteus Vulgaris	13.2 ± 0.71	7.4 ± 0.6	20 ± 0.1	32.02 ± 1.76
5.	Bacillus Cereus	15 ± 1.41	6.8 ± 1.2	19.5 ± 0.5	18.02 ± 0.88



**Fig. Antimicrobial testing**

**Evaluation test for Polyherbal Hand Sanitizer  
 Physical stability of hand sanitizer**

The physical changes were determined by observing color, odor and pH of sanitizer weekly. After sanitizer preparation the pH of the sanitizer was checked. The pH of sanitizer was 8, alkaline. Alkaline pH is good for skin and maintains moisture and does not cause dryness of skin. The sanitizer was prepared in June and its color, pH and odor were checked every week. The sanitizer was

found stable for 3 months without change in color, odor and pH.

Odor - Alcoholic  
 Color - Light Orange  
 PH - 8

**Shelf life of hand sanitizer**

The sanitizer was kept at different room temperature and observed for 3 months with no physical change. The sanitizer was kept at high temperature at 40 ± 2°C, room temperature at 27 ±

2°C and low temperature in refrigerator at  $4 \pm 2^\circ\text{C}$  for three months. The sanitizer was found stable at all the three different temperatures. The physical stability including pH, color and odor was stable. As well as consistency and viscosity was also found stable. The turbidity was checked in sanitizer and no turbidity was also seen. High temperature promotes the growth of bacteria and fungus, but sanitizer made by us did not showed any turbidity or growth in sanitizer. [5]

### Skin exposure to sanitizer

Skin sensitivity of the sanitizer was checked on different individuals and feedback was collected in consent form. The individuals gave positive response with mesmerizing odor and soothing effect after using sanitizer. The individuals were asked to observe redness, irritation, burning sensation and dryness. But no side effects were seen in any individuals after using sanitizer. Like other commercial sanitizer, our sanitizer gave soothing effect and no dryness was observed.

### Stability Testing

#### Antimicrobial Activity; Table:

Sr. No	Test Organisms	Zone of Inhibition			
		Lantana camara	Tridax procumbens	Ocimum sanctum	Azadirachta Indica
1.	E.Coli	$19 \pm 1.41$	$8.1 \pm 0.6$	$17 \pm 0.5$	$32.02 \pm 1.98$
2.	Staphylococcus Aureus	$13.5 \pm 0.71$	$7.02 \pm 0.6$	$17 \pm 3$	$18.68 \pm 0.95$
3.	Pseudomonas Aeurogenosa	$6 \pm 0$	$8 \pm 0.4$	$16 \pm 0.6$	$25.1 \pm 75$
4.	Protease Vulgaris	$13.2 \pm 71$	$7.4 \pm 0.6$	$20 \pm 0.1$	$32.2 \pm 0.76$
5.	Bacillus Cereus	$15 \pm 1.41$	$6.8 \pm 1.2$	$19.5 \pm 0.5$	$18.2 \pm 0.88$

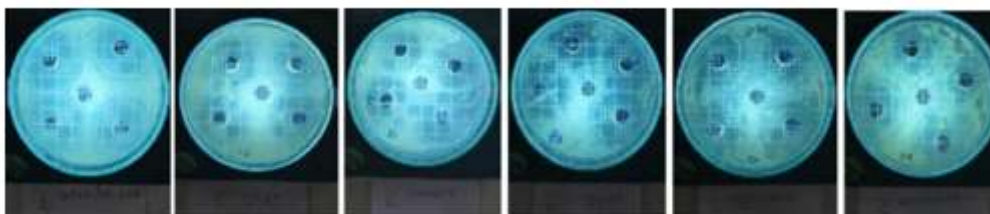


Fig. Results of antimicrobial testing against various microorganisms

### Physical stability of hand sanitizer

The physical changes were determined by observing color, odor and pH of sanitizer weekly. After sanitizer preparation the pH of the sanitizer was checked. The pH of sanitizer was 8, alkaline.

Liquid sanitizer was allowed to stand at 37°C for two months. The stability of liquid sanitizer was observed during this period. The sample which was stable liquid after standing was indicated as stable and the sample in which precipitation were caused; then liquid was said to be as unstable [34]

## II. RESULTS:

The polyherbal sanitizer was prepared in the laboratory and its efficacy was checked, its shelf life, pH and other physical parameters were evaluated. The antimicrobial activity of the sanitizer and its ingredients were evaluated on different bacterial strains and its efficacy on different individuals was also evaluated.

The ingredients used for the preparation of sanitizer were isopropyl alcohol, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), ethanol, glycerol, camphor, mixture of all plant extracts and distilled water. The plant extract used for the preparation of sanitizer were Ocimum sanctum (Shyama tulsi), Lantana camara(Gultura)and Azadirachta indica (Neem), Tridax Procumbens(tantani,jakhamkhudi)The plant details with family name are given in introduction.

Alkaline pH is good for skin and maintains moisture and does cause dryness of skin. The sanitizer was prepared in June and its color, pH and odor were checked every week. The sanitizer was

found stable for 3 months without change in color, odor and pH.

Odor - Alcoholic

Color – Light Orange

PH – 8

#### Shelf life of hand sanitizer

The sanitizer was kept at different room temperature and observed for 3 months with no physical change. The sanitizer was kept at high temperature at  $40 \pm 2^\circ\text{C}$ , room temperature at  $27 \pm 2^\circ\text{C}$  and low temperature in refrigerator at  $4 \pm 2^\circ\text{C}$  for three months. The sanitizer was found stable at all the three different temperatures. The physical stability including pH, color and odor was stable. As well as consistency and viscosity was also found stable. The turbidity was checked in sanitizer and no turbidity was also seen. High temperature promotes the growth of bacteria and fungus, but sanitizer made by us did not showed any turbidity or growth in sanitizer [ as per ICH guideline]

#### Skin exposure to sanitizer

Skin sensitivity of the sanitizer was checked on different individuals and feedback was collected in consent form. The individuals gave positive response with mesmerizing odor and soothing effect after using sanitizer. The individuals were asked to observe redness, irritation, burning sensation and dryness. But no side effects were seen in any individuals after using sanitizer. Like other commercial sanitizer, our sanitizer gave soothing effect and no dryness was observed.[8]

#### Stability Testing

Liquid sanitizer was allowed to stand at  $37^\circ\text{C}$  for two months. The stability of liquid sanitizer was observed during this period. The sample which was stable liquid after standing was indicated as stable and the sample in which precipitation were caused; then liquid was said to be as unstable.[34]

### III. DISCUSSION

The skin of human provides nutrients and suitable growth conditions for opportunistic microbes and other pathogens and these resist most of cleaning regimen and contribute to their existence in ecosystem. Hand hygiene is a simple and least expensive means of preventing hospital acquired infections specially derived from environmental surfaces. There are different types of sanitizers available in the market commercially

which includes alcohol and non alcoholic based sanitizer. Different sanitizer has different effect of bacterial growth and leads biocidal activity. Nowadays, due to COVID 19 pandemic, there is an urgent need and demand of hand sanitizer not only in medical professionals, but also in common man. Alcohol based sanitizer if used regularly leads dryness of skin and irritation also in sensitive people .So we focused on polyherbal sanitizer. In the present study we selected seven plants and prepared a sanitizer using alcohol, glycerol, hydrogen peroxide and camphor. Every ingredient has a specific role in inhibiting the growth of bacteria and fungus also. Our study focused on antibacterial activity of hand sanitizer and was found potent as compared to other well known commercial sanitizer. The plants selected for the study were Lantana Camera -Gultura, Tridax Procumbens-tantani ,Ocimum sanctum(linn) - Tulsi, Azadirachta Indica-Neem.

The selection of ingredients was on the basis of its medicinal and antibacterial properties reported. The ingredients were also checked for its antibacterial property against bacterial strains *Pseudomonasaeruginosa*, *Bacillus cereus*, *Streptococcus pyogenes*, *Klebsiella spp.*, *Escherichia coli*, *Staphylococcus aureus* and *Dermatophyte* and gave potent activity against all pathogens used for the study. The use of methanol in hand scrub is not recommended due to its toxic effects and cause severe systemic toxicity, even deaths can occur after oral, pulmonary or skin exposures leads to chronic toxicity (e.g., visual disturbances) if used regularly. Therefore, in place of methanol ethanol, isopropyl alcohol, n-propyl alcohol, or their combinations can be used in alcohol based hand rub .Therefore, isopropyl and ethanol was selected in the present study to prepare sanitizer.

*Ocimum sanctum* common Indian aromatic plant, belongs to the family of *Lamiaceae*. The plant is reported for antimicrobial property against *Actinobacillusactinomycetemcomitans*, a periodontal pathogen responsible for human dental plaque with 22 mm zone of inhibition. The plant used in preparation of hand sanitizer with different ingredients .and showed antimicrobial property against *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* and *Fungi-Saccharomyces cerevisiae* and *Candida albicans* *Ocimumgratissimum* L. (*Labiatae*) is widely distributed in tropical and warm temperature regions and commonly used in folk medicine to treat different diseases, e.g. upper respiratory tract

infections, diarrhea, headache, ophthalmic, skin diseases, pneumonia, and also as a treatment for cough, fever and conjunctivitis.

#### IV. CONCLUSION:

Hands are the most common mode of transmission Of pathogens to patients and proper hand hygiene Can prevent health care-associated infections and the Spread of antimicrobial resistance. Scientific Evidence and ease of use support of alcohol-based Hand sanitizers during patient care. It may be Concluded that Herbal Hand Sanitizer has a Significant anti-microbial effect on the specified Microorganisms except Ps. Aeruginosa and S.Cerevisiae. Thus, there is immense potential in Establishing the use of antimicrobial herbal products As a measure to control the multidrug resistant through Microbes Microbes as well as check their spread through Hands from one geographical region to another.

#### REFERENCES

- [1]. R. Kapil, H. K. Bhavsar and M. Madan, "Hand hygiene in reducing transient flora on the hands of healthcare workers: an educational intervention," *Indian J Med Microbiol.*, vol. 33, no. 1, (2015), pp. 125-128.
- [2]. C. N. Stanley, V. B. Alobari and K. M. Ezealisiji, "Formulation and evaluation of the effectiveness of a novel hand sanitizer using *Pleurotusostreatus* Oyster mushroom extracts," *Int J Pharma Res Review.*, vol. 6, no. 1, (2017), pp. 7-15.
- [3]. J. M. Boyce and D. Pittet, "Guideline for hand hygiene in health care settings," *Morbidity Mortality Weekly Report.*, vol. 51, no. 16, (2002), pp. 1-44.
- [4]. R. Hirose, T. Nakaya, Y. Naito, T. Daidoji, R. Bandou, K. Inoue, O. Dohi, N. Yoshida, H. Konishi and Y. Itoh, "Situations leading to reduced effectiveness of current hand hygiene against infectious mucus from Influenza virus infecte patients," *MSphere.*, vol. 4, no. 5, (2019).
- [5]. Y. A. Ali, "To study the effect of hand sanitizers used in Kingdom of Saudi Arabia against the common bacterial pathogens," *Int Res J Nat Appl Sciences.*, vol. 2, no. 2, (2015), pp. 2349-4077.
- [6]. F. Alderees, R. Mereddy R, D. Webber, N. Nirmal and Y. Sultanbawa, "Mechanism of action against food spoilage yeasts and bioactivity of *Tasmania Lanceolata*, *Backhousia citriodora* and *Tasmania Lanceolata*, *Backhousia citriodora*," *Plant Solvent Extracts Foods.*, vol. 11, no. 179, (2018).
- [7]. P. G. Andrew, C. Dexter and G. Aziz, "Hand Sanitizers: A review of ingredients, mechanisms of action, modes of delivery and efficacy against coronaviruses," *Am J Infect Control.*, (2020).
- [8]. G. Kampf and A. Kramer, "Epideriologic background of hand hygiene and evaluation of the most important agents for scrubs and rubs," *Clinical microbiology review.*, vol. 17, (2004), pp. 863-893.
- [9]. K. Pedersen, H. Elisabeth, D. J. Jeanne and A. Tove, "Short-term effects of alcohol-based disinfectant and detergent on skin irritation," *Contact Dermatitis.*, vol. 52, no. 2, (2005).
- [10]. O. M. David, "Assessment of soap skin-substantively and other hygiene regimens for skin disinfection," *Int J Biosci.*, vol 4, no. 2, (2009), pp. 89-94.
- [11]. P. A. Jumaa, "Hand hygiene: simple and complex," *International Journal of Infectious Diseases.*, vol. 9, (2005), pp. 3-14.
- [12]. K. Deepak, K. K. Satyendra, K. Gopal, P. Vidyut, P. Pradyot and N. Gopal, "Evaluation of the antibacterial activity of commonly used alcohol based hand sanitizers on common pathogenic bacteria," *Indian J Appl Res.*, vol. 5, no. 3, (2015).
- [13]. S. Silvia, I. A. Nurul and C. L. Delly, "Formulation and effectiveness of a hand sanitizer gel produced using salam bark extract," *Int J App Pharm.*, vol. 10, no. 1, (2018).
- [14]. S. Maryam, K. Sanja, K. S. Judith and R. Thomas, "The effectiveness of a skin care program for the prevention of contact dermatitis in health care workers (the healthy hands project): study protocol for a cluster randomized controlled trial," *Trials.*, vol. 18, no. 92, (2017).
- [15]. O. M. David, D. Ayeni, I. B. Fakayode and O. Famurewa, "Evaluation of antibacterial properties of various hand sanitizers wipes used for cosmetic and hand hygiene purposes in Nigeria," *Int J*

- Microbiol Res., vol. 1, no. 2, (2013), pp. 22-26.
- [16]. P. L. C. Alan and Y. K. C. Thomas, "Methanol as an unlisted ingredient in supposedly alcohol-based hand rub can pose serious health risk," *Int J Environ Res Public Health.*, vol. 15, no. 1440,(2018).
- [17]. M. A. Hossain, M. D. Shah and M. Sakari, "Gas chromatography–mass spectrometry analysis of various organic extracts of *Merremiaborneensis* from Sabah," *Asian Pacific J Trop Med.*, vol. 4, no. 8, (2011), pp. 637-641.
- [18]. T. R. Govindachari, G. Suresh, G. Gopalakrishnan, B. Banumathy and S. Masilamani, "Identification of antifungal compounds from the seed oil of *Azadirachta indica*," *Phytoparasitica.*, vol. 26, no. 2, (1998), pp. 109-116.
- [19]. U. Bandyopadhyay, K. Biswas, A. Sengupta, P. Moitra, P. Dutta, D. Sarkar, P. Debnath, C. K. Ganguly and R. K. Banerjee, "Clinical studies on the effect of Neem (*Azadirachta indica*) bark extract on gastric secretion and gastroduodenal ulcer," *Life Sciences.*, vol. 75, no. 24, (2004), pp. 2867–2878.
- [20]. P. E. Ebong, I. J. Atangwho, E. U. Eyong and G. E. Egbung, "The antidiabetic efficacy of combined extracts from two continental plants: *Azadirachta indica* (A. Juss) (Neem) and *Vernonia amygdalina* (Del.) (African Bitter Leaf)," *Am J BiochemBiotechnol.*, vol. 4, no. 3, (2008), pp. 239–244.
- [21]. R. Paul, M. Prasad and N. K. Sah, "Anticancer biology of *Azadirachta indica* L (neem): a minireview," *Cancer BiolTher.*, vol. 12, no. 6, (2011), pp. 467–476.
- [22]. F. Uwimbabazi, J. Uwimana and J. P. Rutanga, "Assessment of antibacterial activity of Neem plant (*Azadirachta indica*) on *Staphylococcus aureus* and *Escherichia coli*," *J Med Plants Stud.*, vol. 3, no. 4, (2015), pp. 85-91.
- [23]. S. Dipti and S. Kamna, "Formulation of an herbal substitute for chemical sanitizer and its evaluation for antimicrobial efficiency," *Int J ChemTech Res.*, vol. 12, no. 3, (2019), pp. 114-120.
- [24]. S. P. Rutuja and C. Nayan, "Formulation of herbal sanitizer and determining their antimicrobial activities against skin Pathogens," *Int J Innov Sci Res Technol.*, vol. 3, no. 8, (2018).
- [25]. R. Kumar, A. K. Singh, A. Gupta, A. Bishayee and A. K. Pandey, "Therapeutic potential of Aloe vera – A miracle gift of nature," *Phytomedicine.*, vol. 60, (2019).
- [26]. P. P. Athiban, B. J. Borthakur, S. Ganesan and B. Swathika, "Evaluation of antimicrobial efficacy of Aloe vera and its effectiveness in decontaminating gutta percha cones.," *J Conserv Dent.*, vol. 15, no. 3, (2012), pp. 246-248.
- [27]. B. F. Nejatizadeh, "Antibacterial activities and antioxidant capacity of Aloe vera," *Org Med Chem Lett.*, vol. 3, no. 1, (2013).
- [28]. S. Patel, V. Sharma, N. S. Chauhan and V. K. Dixit, "An updated review on the parasitic herb of *Cuscutareflexa* Roxb.," *Chin J Integr Med.*, vol. 10, no. 3, (2012), pp. 249-255.
- [29]. F. B. Inamdaee, R. Oswal, T. V. Chorage and K. Garje, "In vitro antimicrobial activity of *Cuscutareflexa* Roxb.," *Int Res J Pharm.*, vol. 2, no. 4, (2011), pp. 214-216.
- [30]. A. B. Behrooz, F. T. Yazdi, M. Ali, Z. Fatemeh, M. G. Mohammad and V. Alireza, "Effect of aqueous and ethanolic extract of *Eucalyptus camaldulensis* L. on food infection and intoxication microorganisms in vitro," *JPS Summer.*, vol. 4, no. 3, (2013).
- [31]. O. Ayepola and B. deniyi, "The antibacterial activity of leaf extracts of *Eucalyptus camaldulensis* (Myrtaceae)," *J Appl Sciences Res.*, vol. 4, no. 11, (2008), pp. 1410-1413.
- [32]. P. Horvath and J. Koscova, "In vitro antibacterial activity of *Mentha* essential oils against *Staphylococcus aureus*," *Folia veterinaria.*, vol. 61, no. 3, (2017), pp. 71-77.
- [33]. D. Deepak, K. Gaurav, K. P. Rakesh and S. Vinod, "Antimicrobial activity of *Mentha arvensis* against clinical isolates of human cariogenic pathogens – an in vitro study," *IJPSR.*, vol. 3, no. 5, (2012), pp. 1355-1360.
- [34]. Vijaya .B .Surwase , Mansi .M .Savale , Ranjit .S . Jadhav ,Akshaykumar .B. Kadam , Pradnya .P. Shinde. JUSCT 1007 -6735