Research Article

www.ijrap.net

(ISSN Online:2229–3566, ISSN Print:2277–4343)

# AN *IN VITRO* ANTIBACTERIAL STUDY OF NIMBADI ARKA: A NON-ALCOHOL BASED HERBAL HAND SANITIZER

Bhoomika R [1], Prasanna Kulkarni [2]*, Priyanka BV [3], Kiran M. Goud [4]

[1] Research Scholar, Sri Kalabhyraveshwaraswamy Ayurvedic Medical College Hospital and Research Centre, Bangalore, Karnataka, India

[2] Professor & HOD, Department of Swasthavritta, Sri Kalabhyraveshwaraswamy Ayurvedic Medical College Hospital and Research Centre, Bangalore, Karnataka, India

[3] Assistant Professor, Department of Samhita and Siddhanta, Sri Kalabhyraveshwaraswamy Ayurvedic Medical College Hospital and Research Centre, Bangalore, Karnataka, India

[4] Professor, Department of Panchakarma, Sri Kalabhyraveshwaraswamy Ayurvedic Medical College Hospital and Research Centre, Bangalore, Karnataka, India

Received on: 12/04/22 Accepted on: 16/05/22

***Corresponding author**
E-mail: swasthaprasanna@gmail.com

**DOI: 10.7897/2277-4343.130351**

**ABSTRACT**

Hand hygiene is crucial as it gets contaminated easily from direct contact with airborne microorganism droplets and droplet nuclei from coughs and sneezes. In situations like a pandemic outbreak of COVID-19, it is imperative to interrupt the transmission chain of the pathogens by the practice of proper hand sanitization. It can be achieved with contact isolation and strict infection control tools like maintaining good hand hygiene in the house, in hospital settings, and in public. The success of hand sanitization solely depends on practical hand disinfecting agents formulated in various types and forms, such as antimicrobial soaps and water-based or alcohol-based hand sanitiser, with the latter being widely used in hospital settings and by common people. Most effective hand sanitiser products are alcohol-based formulations containing 62%–95% of alcohol as they can denature the proteins of microbes and the ability to inactivate pathogens. Considering the need, we prepared five herbal hand sanitizers in Arka form using drugs of krimighna gana dravyas that have an antimicrobial property and are volatile. Among all the five preparations, it was noticed from the statistical analysis, that there was a significant reduction in the bacterial count in the 'immediate application' of Batch I (Tulsi Arka), and Batch II (Tulsi, Nimba Arka) showed a significant decrease in the bacterial count in 'after 30 minutes of application'. However, Batch III (Tulsi, Nimba, Haridra arka) gave an intermediate result in 'immediate application' and 'after 30 minutes of application'. None of the preparations showed any sort of irritation, dryness or discomfort to the subjects even after 30 minutes while conducting the study.

**Keywords**: Hand Sanitization, Ayurveda, Arka, Distillation, Colony-forming unit, Anti-microbial action.

## INTRODUCTION

Hand sanitation plays an important role in the prevention of diseases. Many gastrointestinal diseases are transmitted from the environment to the body through unhygienic hands. Hence adequate hand sanitation will minimize the incidences of such diseases. Hand sanitizer is generally a liquid substance used to decrease infectious agents on the hands. In the current practices, Alcohol-based hand sanitizers are widely used that contain Triclosan as one of the essential ingredients. The most common adverse health effects of alcohol- and non-alcohol-based hand sanitizers are ocular irritation, vomiting, conjunctivitis, oral irritation, cough, and abdominal pain. Rare effects included coma, seizure, hypoglycaemia, metabolic acidosis, and respiratory depression[1]. Over to this, Triclosan is a known endocrine disruptor and a suspected carcinogen. There is good reason to believe that the overuse of products with triclosan has contributed to bacterial resistance in the same way we are cautioned against the use of antibiotics[2]. The emergence of the novel virus, SARS-CoV-2, has posed challenges to public health worldwide. Presently, strategies planned to deal with COVID-19 are purely supportive and preventative to reduce transmission. A practical and simple method for reducing transmission of infections in public or healthcare settings is hand hygiene[3].

Even certain Herbal hand sanitizers available on the market are alcohol-based. Hence an ideal, non-toxic, non-alcohol herbal hand sanitizer is the need of the hour. Here is an attempt to prepare herbal hand sanitizer by applying Ayurvedic techniques with significant antimicrobial properties. Ayurveda has described certain drugs that process antimicrobial actions like Nimba (*Azadirachta indica*), Haridra (*Curcuma longa*), Tulasi (*Ocimum sanctum*), Vasa (*Adhathoda vasica*), Nirgundi (*Vitex negundo*), Shirisha (*Albizia lebbeck*), Almadi Sambrani (*Plectranthus amboinicus*). These herbs also contain volatile oils and can be best used in the Arka (distillation) process.

Different combinations from the above drugs were made, and Arkas was prepared and applied to subjects to see its efficacy. Statistically, the preparations showed highly significant results.

Hand sanitizer is a substance that reduces the number of bacterial contaminants to safe levels as judged by public health requirements. Hand sanitizers are two large categories Non-alcohol-based hand sanitizers (NABHS) and Alcohol-based hand sanitizers (ABHS)[3].

The most common primary active ingredient of NABHS, benzalkonium chloride, quaternary ammonium, is a commonly used disinfectant. Disinfectants with benzalkonium chloride are

generally less irritating than those with alcohol, though more recent evidence suggests it may cause contact dermatitis. Although ABHS are less user-friendly on the skin than NABHS, ABHS predominates in healthcare given their low cost and efficacy in reducing infectious transmission. NABHS, however, are less problematic regarding their flammability and abuse potential.

Hand sanitizer preparations containing alcohol, on the other hand, can include ethanol, isopropyl alcohol, *n*-propanol, or a combination of these, water, as well as excipients and humectants. Solutions containing alcohols between 60% and 95% in the volume are most prevalent and effective. Humectants are included to prevent skin dehydration, and excipients help stabilize the product and prolong the time needed to evaporate alcohol, increasing its biocidal activity.

The limitations of alcohol-based hand sanitizer are minimizing the severity of flu, but any type of hand sanitizer does not kill some important organisms; doesn't kill anthrax, is not effective against *Clostridium difficile,* which has become another antibiotic-resistant organism; may be hazardous for small children; frequent use results in stomach ache, vomit, itching of eyes significantly below five years of age[1]; Causes eye infections; kill even good and bad bacteria, explosive in nature, so always kept away from flammable objects; Weakens immunity; causes alcohol poisoning; Antibiotic-resistant by triclosan[2]; Hormonal disruption by triclosan; Adverse impact on skin and promotes ageing; Alcohol used is responsible for drying out the skin, they strip away natural oils of the skin and causes irritation and acids used causes dehydration of skin cells and can leads to contact dermatitis[4].

Though many alcohol-based sanitisers are effective against many micro-organisms due to their side effects, they need to prepare a non-toxic, non-alcohol-based hand sanitizer.

## MATERIALS AND METHODS

This study was planned to determine the anti-bacterial effect of Nimbadi Arka, prepared in 5 variants to design a good non-alcohol based Ayurvedic hand sanitizer.

The Arka was prepared, and its efficacy was checked on the most contaminated hands, i.e. on group D workers working in the department of Rasa shastra and Bahishajya Kalpana SKAMCH & RC, Bangalore, Karnataka, India.

The identified raw drugs, i.e. Nimba (*Azadirachta indica*), Haridra (*Curcuma longa*), Tulasi (*Ocimum sanctum*), Vasa (*Adhatoda vasica*), Nirgundi (*Vitex negundo*), Shirisha (*Albizia lebbeck),* Almadi Sambrani (*Plectranthus amboinicus*) required for the study were purchased from the approved vendors of Sri Kalabhyraveshwaraswamy Ayurvedic Medical College Hospital and Research centre. Raw drugs were authenticated by the experts in the department of Dravya Guna, SKAMCH & RC, Bangalore.

After getting approval from Institutional Ethics Committee (SKAMCH & RC/ IEC/003/2020), the Healthy volunteers with no skin abrasion, wounds and infections on palms and hands and aged between 18-60 years were included in the study. Volunteers who were not willing to participate in the study and had sensitive reactions in the past when certain chemicals were applied to their body surface were excluded from the trial.

For the study, sanitizers were prepared (Table 1) using dry drugs (Tulsi, Nimba, Haridra, Shireesha, Nirgundi, Vasa & Almadi Sambrani) were taken cleaned for physical impurities. All the drugs were weighed and made into a coarse powder. The coarse powder was taken in a bowl and soaked in water (sufficient quantity, i.e. until the drugs are completely immersed in water) overnight. The following day-soaked drugs were well macerated, and the same was transferred to the round bottom flask with ten parts of water[5,6]. The round bottom flask, thus filled with soaked and macerated drugs, was kept on the burner and heated at the temperature of 100°C. On heating, the volatile principles in the drugs get vaporised, and on passing over the condenser, it gets liquefied and collected in the conical flask. The collected Arka was then stored in an airtight container and further used for the studies.

The study was undertaken in three Phases.

**Phase I**: Before the commencement of the trial, four healthy volunteers (to select the most contaminated hands for applying the prepared Arka) without any signs of abrasion, wound or infection of skin hands were included in this study. The four volunteers selected were, Intern who was posted in the hospital OPD, a Group D worker working in the pharmacy, Office staff and teaching faculty. 4 swabs were taken with sterile cotton swab stick from the healthy volunteer that was inoculated and cultured.

Samples were collected by just wiping the swab stick over palms and hands. Then the swab stick was placed back into the swab sample container containing 1% NaCl, and gently it was tapped against the palms just for the bacteria to get into the NaCl solution.

Analysis of the collected samples was done at Azyme Bioscience – Bangalore, Karnataka, India.

200µL of the sample were used to Check Bacterial Colonies.

Luria Bertani (LB) agar media (Tryptone 10g, sodium chloride 10g, yeast extract 6g, agar 15g, distil water 1000mL) 200mL was prepared and autoclaved at 121°c for 15mins. 200µL of the sample (1A, 1B, 2A, 2B, 3A, 3B, 4A and 4B) were poured into the sterilized Petri plate and spread thoroughly. Approximately 25mL of the LB agar was poured into the plate allowing for solidification (pour plate method), and incubated at 37°C for 24 hours. After 24 hours, the plates were observed, and the colony-forming units (CFU) were recorded (Table 2) (Graph 1).

The result showed that the 2$^{nd}$ volunteer, i.e., the group D worker, showed the highest contamination. Therefore, a Group D worker was selected for the human trial purpose.

**Phase II:** 4 humans were selected from different professions to conduct the study. Out of them, one subject got the highest infectivity, a group D worker. All the five prepared Arka was applied swabs collected immediately after application and after 30 minutes.

The prepared Arka (5 samples) was applied to the highest contaminated hands, and swab samples were taken immediately and at the interval of half an hour and swabs were taken with sterile cotton swab sticks from the healthy volunteer that was inoculated and cultured.

**Phase III:** In this phase, the swab samples were collected from the hands and to the collected sample, 1ml of the prepared Arka was poured and sent for analysis.

**Table 1: Arka of five different drug combinations**

| Name | Ingredients | Quantity (in gm) | Water (in ml) | Yield (in ml) |
|---|---|---|---|---|
| Tulsi Arka (Batch I) | Tulsi | 30 | 300 | 150 |
| Tulsi Nimba Arka (Batch II) | Tulsi<br>Nimba | 20<br>20 | 400 | 200 |
| Tulsi Nimba Haridra Arka (Batch III) | Tulsi<br>Nimba<br>Haridra | 11<br>11<br>12 | 350 | 200 |
| Tulsi Nimba Haridra Shireesha Arka (Batch IV) | Tulsi<br>Nimba<br>Haridra<br>Shireesha | 10<br>10<br>10<br>10 | 400 | 200 |
| Tulsi Nimba Haridra Shireesha Nirgundi Vasa Almadi Sambrani Arka (Batch V) | Tulsi<br>Nimba<br>Haridra<br>Shireesha<br>Vasa<br>Almadi sambrani | 7<br>7<br>7<br>7<br>7<br>7 | 500 | 200 |

**Table 2: Colony-forming Units**

| Sample Plates | Colony-forming units per 1mL | |
|---|---|---|
| | Plate 1 | Plate 2 |
| 1-Int | $0.63 \times 10^3$ | $0.305 \times 10^3$ |
| 2- Mal | $10.6 \times 10^3$ | $11.4 \times 10^3$ |
| 3-Pura | $0.165 \times 10^3$ | $0.985 \times 10^3$ |
| 4-Sha | $1.96 \times 10^3$ | $2.94 \times 10^3$ |

1-Int: intern who was posted in the hospital OPD; 2-Mal: Group D worker working in the pharmacy; 3-Pura: Office staff; 4-Sha: Teacher faculty

**Table 3: Physical Examination of Arka**

| Arka | Specific gravity | pH |
|---|---|---|
| Tulsi Arka (Batch I) | 1.030 | 6 |
| Tulsi nimba Arka (Batch II) | 1.010 | 6 |
| Tulsi nimba haridra Arka (Batch III) | 1.010 | 6 |
| Tulsi nimba haridra shireesha Arka (Batch IV) | 1.010 | 6 |
| Tulsi nimba haridra shireesha nirgundi Vasa almadi sambrani Arka (Batch V) | 1.010 | 6 |

**Table 4**

| Batches | BT to 1 minute | | | BT to after 30 minutes | | |
|---|---|---|---|---|---|---|
| | Mean | t. | p. | Mean | t. | p. |
| **I** | 6.78 | 2.38 | >0.05 | 0.38 | 21.77 | <0.05 |
| **II** | 5.22 | 33 | <0.05 | 4.14 | 19.87 | <0.05 |
| **III** | 0.88 | 12.35 | <0.05 | 0.37 | 15.2 | <0.05 |
| **IV** | 4.38 | 6.23 | <0.05 | 3.29 | 5.11 | <0.05 |
| **V** | 6.38 | 4.47 | <0.05 | 4.36 | 3.301 | <0.05 |

t – t-test ; p – p score; BT – before treatment

**Table 5: Reduction in CFU after treated with BATCH I and BATCH II Arka**

| Plates | Mean Batch I | Mean Batch II | T | P |
|---|---|---|---|---|
| Immediate | 6.78 | 5.22 | 1.41 | >0.05 |
| After 30 min | 0.38 | 4.14 | 9.51 | <0.05 |
| *In vitro* study | 1.36 | 6.24 | 55.07 | <0.05 |

**Table 6: Reduction in CFU after treated with BATCH I and BATCH III Arka**

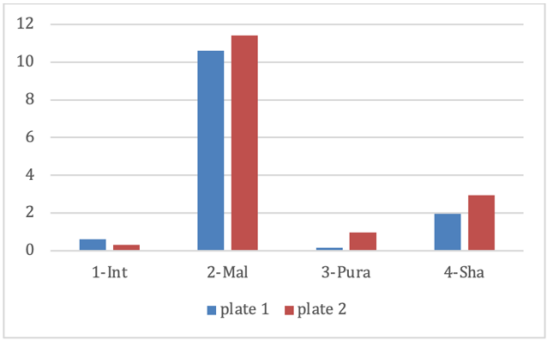
| Plates | Mean Batch I | Mean Batch III | T | P |
|---|---|---|---|---|
| Immediate | 6.78 | 0.88 | 5.4 | <0.05 |
| After 30 min | 0.38 | 0.31 | 0.18 | >0.05 |
| *In vitro* study | 1.36 | 1.37 | 0.03 | >0.05 |

**Table 7: Reduction in CFU after treatment with BATCH I and BATCH IV Arka**

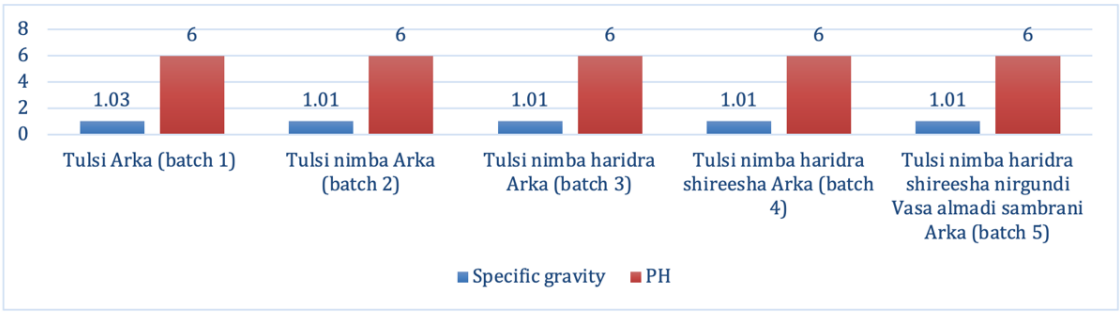
| Plates | Mean Batch I | Mean Batch IV | T | P |
|---|---|---|---|---|
| Immediate | 6.78 | 4.38 | 2.01 | >0.05 |
| After 30 min | 0.38 | 3.29 | 2.99 | >0.05 |
| *In vitro* study | 1.36 | 2.62 | 5.12 | <0.05 |

**Table 8: Reduction in CFU after treated with BATCH I and BATCH V Arka**

| Plates | Mean Batch I | Mean Batch V | T | P |
|---|---|---|---|---|
| Immediate | 6.78 | 6.38 | 0.35 | >0.05 |
| After 30 min | 0.38 | 4.36 | 2.83 | >0.05 |
| *In vitro* study | 1.36 | 2.85 | 5.32 | <0.05 |




**Graph 1: Observation of results before the application of the Arka**




**Graph 2: Specific gravity and pH of the prepared Arka.**

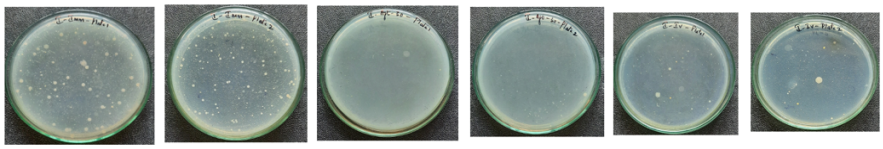


**Figure 1: Batch I, CFU: Plate 1 and 2 immediate, after 30 minutes and *In vitro* studies**

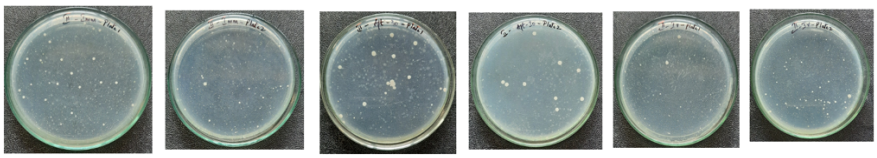


**Figure 2: Batch II, CFU: Plate 1 and 2 immediate, after 30 minutes and *In vitro* studies**

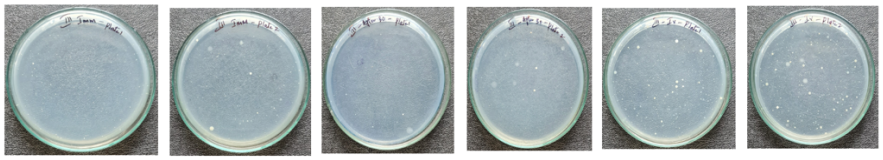


**Figure 3: Batch III, CFU: Plate 1 and 2 immediate, after 30 minutes and *In vitro* studies**

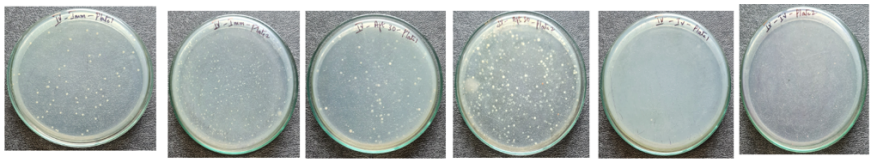


**Figure 4: Batch IV, CFU: Plate 1 and 2 immediate, after 30 minutes and *In vitro* studies**

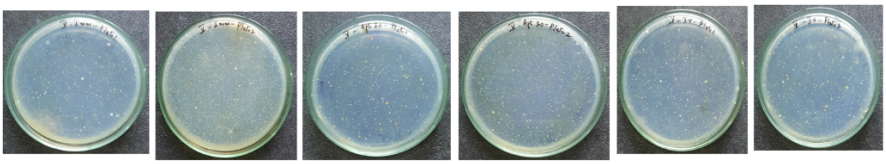


**Figure 5: Batch V, CFU: Plate 1 and 2 immediate, after 30 minutes and *In vitro* studies**

## OBSERVATION AND RESULTS

The Arka prepared was crystal clear and had the odour of the used drugs and was stored in the airtight container, and the container was placed so that it was away from direct sunlight. (Table 3) (Graph 2)

The pH of all the samples was the same, and specific gravity was also almost the same. (Table 3)

IN BATCH I, reduction in CFU was insignificant, whereas in Batch II to Batch V, a significant decrease in CFU (p=0.05) immediately and after 30 minutes was observed. (Table 4)

BATCH I (Tulasi Arka) showed an insignificant reduction in CFU (p=0.05) immediately, whereas after 30 minutes and in *In vitro* analysis, more reduction was found in Batch II (Tulasi Nimba Arka), which was statistically significant. (Table 5, Figure 1 and 2)

In BATCH I (Tulasi Arka) showed an insignificant reduction in CFU (p=0.05) immediately, whereas, after 30 minutes, reduction CFU was more in Batch I, which was statistically insignificant and in *In vitro* analysis, more reduction was found in Batch III (Tulsi, Nimba, Haridra Arka) which was statistically insignificant. (Table 6, Figure 1 and 3)

In BATCH I (Tulasi Arka) showed an insignificant reduction in CFU (p=0.05) immediately, whereas, after 30 minutes reduction, CFU was more in Batch IV (Tulsi, Nimba, Haridra, Shireesha Arka), which was statistically insignificant and in *In vitro* analysis, more reduction was found in statistically significant Batch IV. (Table 7, Figure 1 and 4)

In BATCH I (Tulasi Arka) showed an insignificant reduction in CFU (p=0.05) immediately, whereas, after 30 minutes of reduction, CFU was more in Batch V (Tulsi, Nimba, Haridra, Shireesha, Vasa, Nirgundi, Almadi sambrani Arka) which was statistically insignificant and in *In vitro* analysis, more reduction was found in Batch V which was statistically significant. (Table 8, Figure 1 and 5)

Out of the results:
By the statistical analysis, it was evident that there was a significant reduction in the bacterial count in the immediate application of Batch I (Tulsi Arka), and it showed a substantial decrease in the bacterial count in Batch II (Tulsi, Nimba, Haridra after 30 minutes of application. The 3rd sample prepared out of Tulsi Nimba Haridra (Batch III) gave a moderate result immediately after 30 minutes of application.

## DISCUSSION

As presently available herbal hand sanitizer contains less alcohol percentage, there is a need to prepare the hand sanitizer, which is non-alcohol based. Therefore, some antimicrobial property drugs were selected, and Arka was prepared.

The drugs selected are predominant Katu, Tikta and Kashaya Rasa. Actions of the Rasas as explained in Samhitas are: Katu Rasa has Krimighna property[7]; Tikta Rasa has Vishagna and Krumigna properties[8,] and Kashaya rasa has Ropana effect[9]. The prepared Arka also has volatile principles. It fulfils some of the criteria of hand sanitizer, i.e., the liquid form contains anti-microbial, volatile principles, easy to use, non-toxic, environmentally friendly, etc.

The phytochemical constituents of Tulsi, Nimba and Haridra are proven to be effective against a wide variety of gram-positive and gram-negative bacterias, fungi and viruses.

Some of the phytochemical constituents of Tulsi are Ursolic acid flavonoids such as apigenin, polyphenols, anthocyanins and luteolin, eugenol, thymol, or sesquiterpene alcohols proved to be effective against a wide variety of gram-positive and gram-negative bacterias, fungi and viruses. Aqueous and ethanolic extracts have immunomodulatory properties, and leaves of the plant, when taken, can induce cytokine secretions.[10]

Nimba contains Phenols, unsaturated sterols, triterpenes and Phenolic saponin diterpenoids, limonoids), c-secomeliacins, c-secolimonoids, polysaccharides etc. A minimum Bactericidal Concentration (MBC) value of 5 mg/l was obtained with *Azadirachta indica* against *S. typhi, K. pneumoniae.*[11]

Haridra contains curcuminoids, desmethoxycurcumin, bisdemethoxycurcumin, dihydro curcumin, phytosterols, fatty acids and polysaccharides. Curcumin showed strong against four genera of bacteria, including Gram-positive (*Staphylococcus aureus* and *Enterococcus faecalis*) and Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) antibacterial property[12,13].

The drugs were selected to contain volatile principles and have Krumigna gunas (anti-microbial property). The drugs should be fresh and healthy and not affected by any insects. The selected drugs predominantly have Katu, Tikta, Kashaya Rasa, Laghu, Ruksha, Tikshna gunas and Ushna Virya.

The study was done considering four healthy volunteers, and the person with the most contaminated hands was selected to test the efficacy of all the five prepared Arkas. All the prepared five arkas did not show any discomfort or untoward effects on the volunteer.

## CONCLUSION

Hand and place hygiene is not new to the traditional medicine-Ayurveda. People used to sanitize the operation theatre, houses, Kumara gara (a place where a newborn child and post-partum mother stays) by the Dhupana Karma (fumigation) and some used to do the Prakshalana (sprinkling) with medicated water. By considering various references in the classical texts, a set of drugs has been selected to prepare Arka and see their safety and efficacy as Hand sanitizer.

Katu dominated selected drugs, Tikta and Kashaya Rasas, and these Rasas are known for their Krumigna and Vishagna properties. The prepared Arkas possessed essential criteria for Hand sanitizer.

By exploring the knowledge of ancient science and modern technology, in this study, we tried to demonstrate that the drugs that are explained in Ayurvedic classics, which possess anti-microbial (Krimighna) and volatile properties, can be best used as hand sanitizers. The current study also showed its effective action on hand microbes. Among all the five preparations, it was noticed that the statistical analysis, it was evident that there was a significant reduction in the bacterial count in the immediate application of Batch I (Tulsi Arka) and showed a significant decrease in the bacterial count in Batch II (Tulsi, Nimba, Haridra Arka) after 30 minutes of application. The 3rd sample prepared out of Tulsi Nimba Haridra (Batch III) gave an intermediate result immediately and after 30 minutes of application.

**ACKNOWLEDGEMENT**

We would like to thank Rajeev Gandhi University of Health Sciences, Bangalore, for the financial support and encouragement. We would like to thank all the concerned staff and Management of Sri Kalabhyraveshwaraswamy Ayurvedic medical college Hospital and Research Centre for all the encouragement and support.

**REFERENCES**

1. Santos C, Kieszak S, Wang A, Law R, Schier J, Wolkin A. Reported Adverse Health Effects in Children from Ingestion of Alcohol-Based Hand Sanitizers - the United States, 2011-2014. MMWR Morb Mortal Wkly Rep. 2017;66(8):223-226. Published 2017 Mar 3. doi:10.15585/mmwr.mm6608a5.
2. Mighty nest, Dangers of triclosan, May 23, 2012
3. Golin AP, Choi D, Ghahary A. Hand sanitizers: A review of ingredients, mechanisms of action, modes of delivery, and efficacy against coronaviruses. Am J Infect Control. 2020 Sep;48(9):1062-1067. DOI: 10.1016/j.ajic.2020.06.182.
4. Santos C, Kieszak S, Wang A, Law R, Schier J, Wolkin A. Reported Adverse Health Effects in Children from Ingestion of Alcohol-Based Hand Sanitizers - the United States, 2011-2014. MMWR Morb Mortal Wkly Rep. 2017;66(8):223-226. Published 2017 Mar 3. doi:10.15585/mmwr.mm6608a5
5. Lankapathi Ravana, Arka Prakasha, Hindi teeka by Indradeva Tripati, revised ed., (1:74) Choukamba press, Varanasi, p 14.
6. The Ayurvedic Formulary of India, part 3, first ed., The controller of publications civil lines, Delhi, p 27
7. Charaka Samhita of Agnivesa, elaborated by Charaka, sutra sthana 26/42(4), Charaka Chandrika Hindi Commentary by Dr Brahmananda Tripathi, Chaukhamba Surbharati Prakashana, Varanasi reprint 2011: p 859.
8. Charaka Samhita of Agnivesa, elaborated by Charaka, sutra sthana 26/42(5), Charaka Chandrika Hindi Commentary by Dr Brahmananda Tripathi, Chaukhamba Surbharati Prakashana, Varanasi reprint 2011: p 859.
9. Charaka Samhita of Agnivesa, elaborated by Charaka, sutra sthana 26/43, Charaka Chandrika Hindi Commentary by Dr Brahmananda Tripathi, Chaukhamba Surbharati Prakashana, Varanasi reprint 2011: p 859.
10. Kumar A, Rahal A, Chakraborty S, Tiwari R, Latheef SK, & Dhama K. *Ocimum sanctum* (Tulsi): A miracle herb and boon to medical science - A review. International Journal of Agronomy and Plant Production, 2013;4*:1580-1589.*
11. Joshi, Bishnu & Sah, Govind & Basnet, Buddha & Bhatt, Megh & Sharma, Dinita & Subedi, Krishna & Janardhan, Pandey & Malla, Rajani. Phytochemical extraction and antimicrobial properties of different medicinal plants: *Ocimum Sanctum* (Tulsi), *Eugenia caryophyllata* (clove), *Achytanthes Bidentata* (Datiwan) and *Azadirachta Indica* (Neem). Journal of Microbiology and Antimicrobials. 2011;3:1-7.
12. Tyagi P, Singh M, Kumari H, Kumari A, Mukhopadhyay K. Bactericidal activity of curcumin I is associated with damaging of bacterial membrane. PLoS One. 2015 Mar 26;10(3):e0121313. DOI: 10.1371/journal.pone.0121313.
13. Jing JLJ, Pei Yi T, Bose RJC, McCarthy JR, Tharmalingam N, Madheswaran T. Hand Sanitizers: A Review on Formulation Aspects, Adverse Effects, and Regulations. Int J Environ Res Public Health. 2020;17(9):3326. DOI:10.3390/ijerph17093326

**Cite this article as:**

Bhoomika R *et al*. An *In vitro* antibacterial study of nimbadi arka: A non-alcohol based herbal hand sanitizer. Int. J. Res. Ayurveda Pharm. 2022;13(3):21-26 http://dx.doi.org/10.7897/2277-4343.130351

Source of support: Rajeev Gandhi University of Health Sciences, Bangalore, India (Project code: UG20AYU106), Conflict of interest: None Declared