RESEARCH ARTICLE ISSN 2456-0170

EVALUATION OF ANTIMICROBIAL ACTIVITY OF *HARIDRA* (*Curcuma longa)* VIS-À-VIS *MADHU* SAMSKARA

[1]Dr Harshitha.S [2]Dr Satish Pai [3]Dr S. Satish

[1]PG scholar, [2]Corresponding author, Reader & Head, Department of PG studies in Dravyaguna Vignana, JSS Ayurveda Medical College, Mysuru, Karnataka [3]Professor and Chairperson, Department of Studies in Microbiology, Manasagangotri University of Mysuru, Mysuru, Karnataka

ABSTRACT

A microorganism or microbe is a unicellular creature which has the potential to spread infection very rapidly and cause widespread epidemics. A wound is a pathological discontinuity of the skin or other tissues like muscles, bone, among others, which is capable of spreading to neighboring organs or tissues. Microbial strains such as Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Salmonellatyphi and Pseudomonas aeruginosa are responsible infecting healthy wounds in patients, complicating the healing process. Due to the distinctive nature of the microbes and extremely intricate wound healing mechanism, effective and targeted cures are still needed to prevent and cure acute and chronic microbial wound infections. Haridra(Curcuma longa), Known for its Krimighna activity is often used as a potent antimicrobial agent. Honey is said to possess, Vranashodana property as Samanya Guna. Madhu is Yogavahi Dravya and has the ability to Potentiate the activity of Dravya with which it is used. Kaiyadeva mentioned Madhu Samskarita Haridra as Vranashodhaka. With this background, the present study is carried out with the objective for evaluation of the antimicrobial capability of madhu when used in Samskara with Haridra.

KEYWORDS: *Haridra, Madhu Samskara,* Antimicrobial, *Krimighna*

INTRODUCTION

Spices are a group of esoteric nutraceutical supplements that have been used for thousands of years. Among the physiological influences, spices are documented to show their hypolipidemic, antimicrobial, and anti-oxidant properties have a wide range of health implications.(1) Despite these positive effects, an important criticism of Curcumin, one of the principal constituents of Haridra, is its low bioavailability. Indeed, some researches have supported the claim; other clinical trials do not provide justification. Curcumin has been confirmed to exhibit very low bioavailability, sometimes even undetectable in blood plasma and extra-intestinal tissue. The main reasons being poor gut absorption, rapid metabolism, chemical instability and rapid systemic elimination.(2)

Traditionally, raw extracts from various parts of medicinal plants, including roots, stems,

flowers, fruits and twigs, were used extensively for treatments of some human diseases.[3] Medicinal plants contain several phytochemical compounds such as flavonoids, alkaloids, tannins and terpenoids, which possess antimicrobial and antioxidant properties.[4]

When Haridra is combined with honey, the effects are multiplied because honey/Madhu has the quality of Krimighna. Madhu combined with Haridra and subjected to paka may result in differences in the chemistry and utility of the formulation.

MATERIALS AND METHODS:

Preparation of trial drug:

Sample 1: *Kevala M*adhu

Sample 2: *Ardra Haridra Swarasa: Haridra Swarasa*- juice extracted from a fresh *Haridra*, pounded and squeezed through a cloth and then lyophilized for obtaining dried mass. [5]

Sample 3: *Shushka Haridra Rasa- Sushka Haridra* soaked overnight, mixed with eight parts of water (v/v), boiled, and reduced to 1/4th of the original quantity[6].

Sample 4: *Madhu Samskarita Ardra Haridra Swarasa***:** *Madhu* and *Ardra Haridra Swarasa* were mixed in equal proportion and then subjected to *Madhupaka*. (Boiling over *Mandagni* till *Paka Lakshanas* are observed).[7]

Sample 5: *Madhu Samskaritha Shushka Haridra Rasa: Madhu* and *Suska Haridra Rasa* were mixed in equal proportion and then subjected to *Madhupaka*. (Boiling over *Mandagni* till *Paka Lakshanas* are observed).

Anti-microbial activity was assessed with below mentioned samples

1. Kevala Madhu

2. Ardra Haridra Swarasa

3. Shushka Haridra Rasa

4. Madhusamskaritha Ardra Haridra Swarasa

5. Madhusamskaritha Shushka Haridra Rasa

Test bacteria:

The antibacterial activity was assessed against four microbial species:

→ *Staphylococcus aureus* ATCC 25923

→ *Escherichia coli* ATCC 25922

→ *Bacillus septilis* ATCC 25925

→ *Salmonella typhi* ATCC 25924 maintained in Nutrient broth (Mueller-Hilton broth) at 20ºC.

Dilution methods have been used for the determination of MIC values since they provided the possibility to estimate the concentration of the tested antimicrobial agent in the agar (agar dilution) or broth medium macro dilution.

Microbial culture:

→20µL of each stock culture was added to 5 mL of Nutrient Broth.

→Cultures were kept for 24 h at 36ºC ± 1ºC.

→After 24 h of incubation, bacterial suspension (inoculum) was diluted with a sterile physiological solution to 10^8 CFU/mL (McFarland standard)

→Bacterial suspension will be diluted with a sterile physiological solution to 10^8 CFU/mL (McFarland standard) to adjust it to the required or desired amount of bacteria for the work which is required for regulated and controlled growth.

Macro-Broth dilution assay was performed as disc diffusion method did not work with samples prepared.

Materials:

Test tubes (Borosil, Chennai), Muller Hinton broth (MHB; Himedia, Mumbai), 24 hrs old *Escherichia coli, Staphylococcus aureus, Bacillus subtilis* and *Salmonella typhi*

culture, standard antibiotic Gentamycin (Himedia, Mumbai), *Kevala Madhu*, *Niragni Sadhita Ardra Haridra Swarasa, Sa-agni Sadhita Shushka Haridra Rasa, Madhu Samskarita Ardra Haridra Swarasa* and *Madhu Samskarita Shushka Haridra Rasa*. Triphenyl tetrazolium chloride (TTC, Sigma-Aldrich, US), Micropipette (Tarsons, New-Delhi), Micropipette tips (Tarsons, New-Delhi).

Media Preparation:

Mueller Hinton broth was prepared by dissolving 315 mg in 15 mL of distilled water. 1 ml of prepared media was dispensed into each test tube and kept for autoclaving.

Procedure:

1. After sterilization, test tubes were kept for cooling and labelled as growth control, positive control, 400µg, 20µg, 100µg, and 50µg.
2. Test tube labelled as growth control was inoculated with 50µL of 24hrs old test pathogen.
3. Test tube labelled as Positive control was amended with 30 µg of standard antibiotic Gentamycin and inoculated with a tested pathogen.
4. Serial dilution of sample A was done by adding a 2mL aqueous solution containing 400µg of sample test tube labelled as 200µg and mixed thoroughly.
5. From the test tube labelled as 200µg, 2mL was pipetted out into next test tube labelled as 100µg and properly mixed.
6. The process was repeated till the last test tube was serially diluted.
7. After serial dilution, all the test tubes were inoculated with 50µL of 24hrs old test pathogen and incubated at 37 °C for 24 hours.
8. After the incubation period, 200µL of 100mg/mL TTC solution was added to each test tube and again kept for incubation at room temperature for 2hrs.
9. Same concentrations have been maintained and performed during the experiments in three trials. (Triplicates)

High-performance liquid chromatography:

To evaluate the curcumin concentration in test samples, HPLC was carried out for 3 samples such as;

1. Standard curcumin
2. *Ardra Haridra Samskarita Madhu*
3. *Shushka Haridra Samskarita Madhu*

Curcumin in the concentration of 1mg/mL standard was used, whereas *Haridra Swarasa* and *Haridra Rasa Samskarita Madhu* samples were used in concentration of 250mg/ml so as to detect the negligible amount of curcumin.

RESULTS

Minimum Inhibitory concentration:

→Minimum Inhibitory Concentration of *Kevala Madhu* against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Salmonella typhi* was observed to be 200µg/mL.

→Minimum Inhibitory Concentration of *Niragni Sadhitha Ardra Haridra Swarasa* against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Salmonella typhi* was observed to be 200µg/mL.

→Minimum Inhibitory Concentration of *Sa-agni Sadhita Shushka Haridra Rasa* against *S. aureus*, *E. coli*, and *S. typhi* was observed to be 200µg/mL and against *B. subtilis* it was observed to be 400µg/mL.

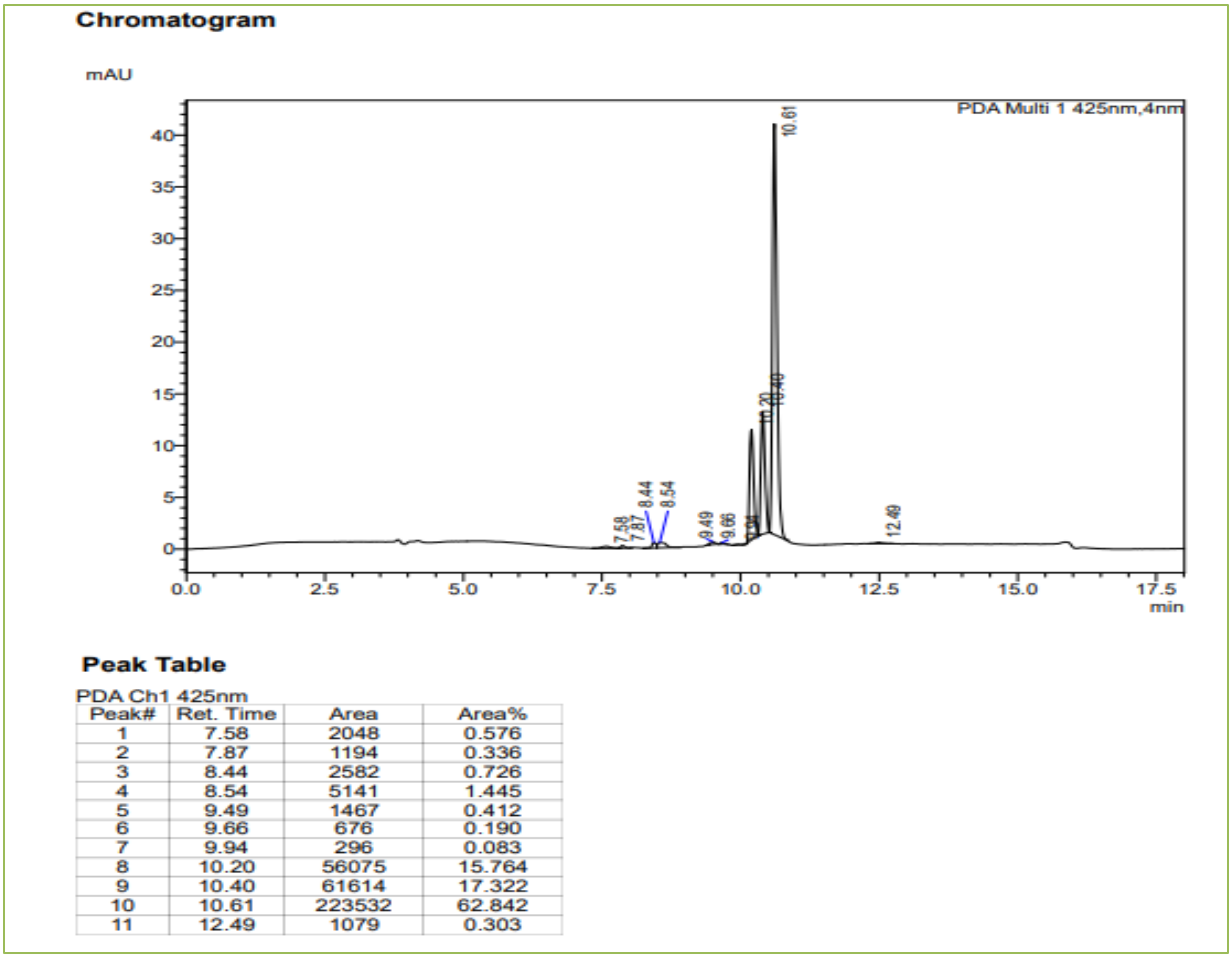
→Minimum Inhibitory Concentration of *Madhu Samskaritha Ardra Haridra Swarasa*

against *S. aureus, E. coli, B. subtilis and S. typhi* was observed to be 100µg/mL.

→Minimum Inhibitory Concentration of *Madhu Samskaritha Shushka Haridra Rasa* against *S. aureus, E. coli, B. subtilis and S. typhi* was observed to be 200µg/mL.

HPLC assay:

Graph No.1: Showing HPLC graph of Ardra Haridra Swarasa samskarita Madhu

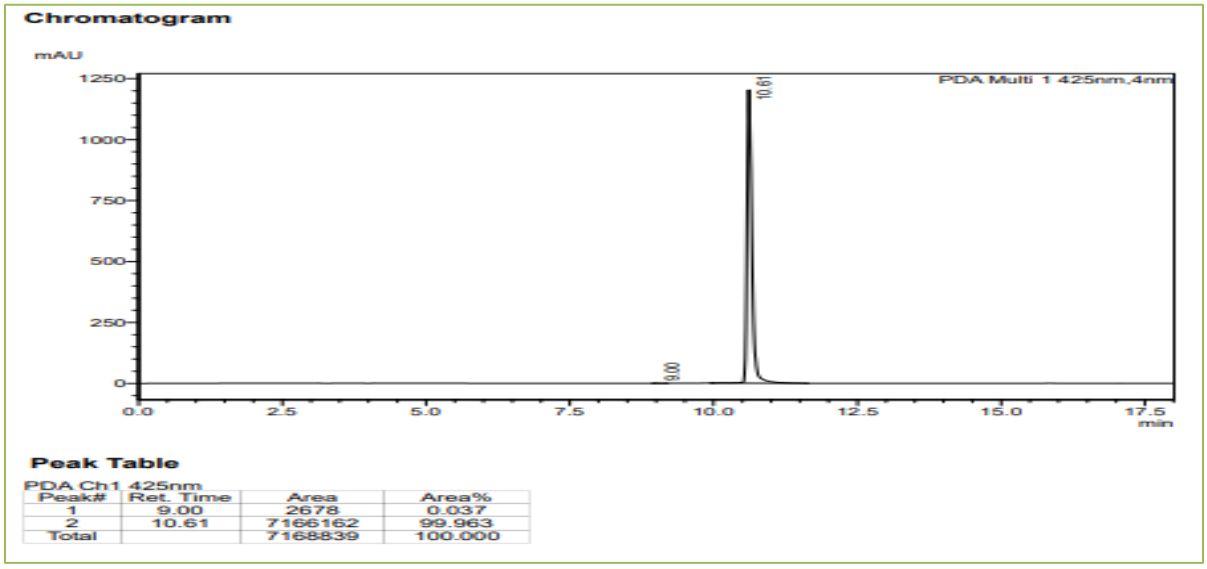


Peak Table

PDA Ch1 425nm

Peak#	Ret. Time	Area	Area%
1	7.58	2048	0.576
2	7.87	1194	0.336
3	8.44	2582	0.726
4	8.54	5141	1.445
5	9.49	1467	0.412
6	9.66	676	0.190
7	9.94	296	0.083
8	10.20	56075	15.764
9	10.40	61614	17.322
10	10.61	223532	62.842
11	12.49	1079	0.303

Graph No.2: Showing HPLC graph of *Standard curcumin*

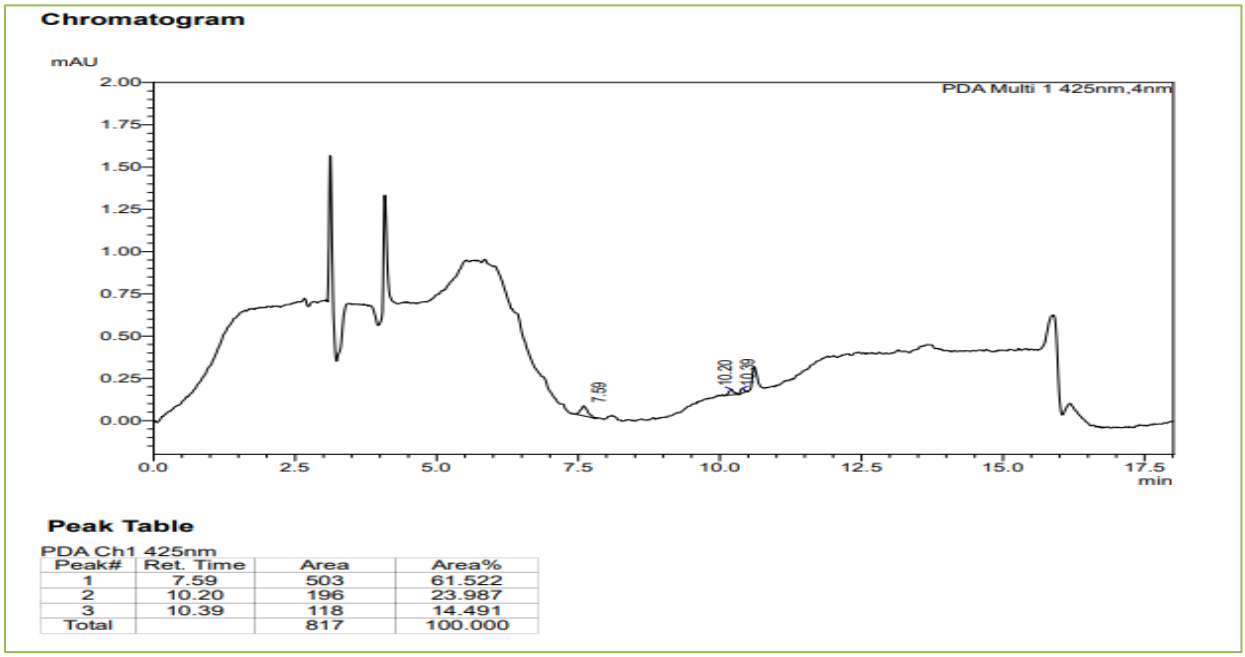


Peak Table

PDA Ch1 425nm

Peak#	Ret. Time	Area	Area%
1	9.00	2678	0.037
2	10.61	7166162	99.963
Total		7168839	100.000

Graph No.3: HPLC graph of *Shushka Haridra rasa Samskarita Madhu*



Peak Table

PDA Ch1 425nm

Peak#	Ret. Time	Area	Area%
1	7.59	503	61.522
2	10.20	196	23.987
3	10.39	118	14.491
Total		817	100.000

Results of HPLC assay:

As per the calculations using the formula, 250mg/ml concentration of test sample contains 31.25µg of Curcumin in *Ardra Haridra swarasa samskarita Madhu* according to HPLC Assay. Second sample (*Shushka Haridra rasa samskarita Madhu*) did not show any traces of curcumin

DISCUSSION:

Ayurveda is regarded an alternative and supplementary medicine, and it is becoming more popular in long-term treatment of diseases and disorders with the

administration of relatively safe herbal drugs. Herbal Ayurvedic preparations and Extracts have a lot of potential as antimicrobials against bacterial infections and can be utilized to treat infectious disorders. *Haridra- Curcuma longa L*., is thought to be one such magical drug. Curcumin, a component of the rhizome of the herb *Curcuma longa L*., has drawn a lot of attention in the last decade.[8]

Microorganisms (micro biota) inhabit plants, animals, and humans and have a significant impact on the development and function of all living systems. They contribute to adaptation and evolution of the host immune system of which. But at times the immune system may fail to fight off microbial infections adequately resulting in prolonged illnesses, infection and delayed wound healing being one of them. Despite pharmacological advancements, the management of persistent non-healing wounds remains a concern for clinicians. *Sushruta* recognized the need of wound management and described the *Shashthi Upakramas (*sixty surgical measures*) for Vrana Ropana (*wound healing), one of which is the use of *Madhu.*[9]

Although Sushruta credits Madhu alone with *Krimighna karma*, described under *Vrana Ropana karma*, processing it with *Dravyas* which have comparable actions may enhance the desired effect. Due to its classically defined "*Yogavahi*" nature, honey is utilized as a "*Anupana*" with a variety of other items. Honey has been used as a synergistic ingredient in various products.[10]

Multidrug-resistant bacteria pose a significant threat to health-care systems in the present day. Despite the introduction of certain novel antimicrobial medicines, the widespread rise of bacterial resistance to a significant number of antimicrobial medications offers major health concerns due to treatment challenges. We investigated the effects of combining Haridra and Madhu against four bacteria types, including *Staphylococcus aureus, Escherichia coli, Bacillus subtilis*, and *Salmonella typhi.*

Curcumin is fragile, reactive and has very low bioavailability due to which some experts opine that further clinical trials are unnecessary. The particularly low bioavailability may be attributed to its poor absorption, hydrophobic nature, quick metabolism, chemical instability, and rapid systemic elimination.[11] In Broth Macro-dilution method, concentrations of samples were added in gradually increased concentrations. At higher concentrations, curcumin distributes evenly and comes in direct contact with the microbial cell, and probably causing cell death.

Although previous studies have proven that curcumin is heat sensitive, it is said to be relatively stable if heated for short duration. Before it can be dried and powdered, fresh turmeric must be cured. Turmeric is boiled before being sun-dried. Boiling the turmeric allows the starch to gelatinize, which helps to deepen the color and distribute it evenly throughout the rhizome. Heat has also been discovered to promote turmeric absorption, providing evidence to the popular practice of blending turmeric in hot milk.[12] Studies have shown that adding sugar improves its solubility in water.

In the present study, *Madhu Samskarita Ardra Haridra Swarasa* has shown noticeable results of MIC with 100µg/ml concentrations. According to previous studies, Curcumin has been demonstrated to

have considerable synergistic activity when combined with other components. On the other hand, *Samskaras* of *Madhu* have been emphasized. When used together, the drugs were not only synergistic but also potent bactericidal and bacteriostatic. It is also observed that after processing with sugar, the aqueous dissolution of curcumin produces solitary amorphous sugar based solid dispersion of curcumin which significantly increases bioavailability.

Even though the effects of curcumin were specifically criticized in this study, some of the studies examined used entire turmeric instead of curcumin alone. As a result, it's feasible and likely that turmeric contains a variety of chemicals with medicinal potential. According to studies, curcumin preparations including turmeric oils had better anti-inflammatory benefits than curcumin alone.

CONCLUSION

Ardra Haridra swarasa Samskarita Madhu is a better anti-microbial formulation as compared to *Madhu* without *Samskara*. *Shushka Haridra* has limited anti-microbial efficacy. Thus present study confirms the bio-availability enhancing activity of *Madhu* on anti-microbial potential of *Haridra Swarasa.*

REFERENCES

1. Cohen M. L. (1992). Epidemiology of drug resistance: implications for a post-antimicrobialera. *Science(NewYork,N.Y.), 257*(5073),1050–1055. https://doi.org/10.1126/science.257.5073.1050

2. Aslam, B., Wang, W., Arshad, M. I., Khurshid, M., Muzammil, S., Rasool, M. H., Nisar, M. A., Alvi, R. F., Aslam, M. A., Qamar, M. U., Salamat, M. K. F., & Baloch, Z. (2018). Antibiotic resistance: a rundown of a global crisis. *Infection and drug resistance*, *11*, 1645–1658. https://doi.org/10.2147/IDR.S173867

2. Cohen M. L. (1992). Epidemiology of drug resistance: implications for a post-antimicrobial era. *Science (New York, N.Y.), 257*(5073), 1050–1055. https://doi.org/10.1126/science.257.5073.1050

3. Aslam, B., Wang, W., Arshad, M. I., Khurshid, M., Muzammil, S., Rasool, M. H., Nisar, M. A., Alvi, R. F., Aslam, M. A., Qamar, M. U., Salamat, M. K. F., & Baloch, Z. (2018). Antibiotic resistance: a rundown of a global crisis. *Infection and drug resistance*, *11*, 1645–1658. https://doi.org/10.2147/IDR.S173867

4. Sharngadhara. Prathama khanda/ 7. In: Adhamalla, Kashirama (eds.) Sharngadhara Samhita with Deepika Commentary. Varanasi: Chaukhambha Orientalia; 2012. p. 103-105.

5. Sharngadhara. Prathama khanda/ 7. In: Adhamalla, Kashirama (eds.) Sharngadhara Samhita with Deepika Commentary. Varanasi: Chaukhambha Orientalia; 2012. p. 103-105.

6. Sharngadhara. Prathama khanda/ 7. In: Adhamalla, Kashirama (eds.) Sharngadhara Samhita with Deepika Commentary. Varanasi: Chaukhambha Orientalia; 2012. p. 103-105.

7. Kunnumakkara, A. B., Bordoloi, D., Padmavathi, G., Monisha, J., Roy, N. K., Prasad, S., & Aggarwal, B. B. (2017). Curcumin, the golden nutraceutical: multitargeting for multiple chronic diseases. *British journal of pharmacology*, *174*(11), 1325–1348. https://doi.org/10.1111/bph.13621

8. Sushruta. Dwivraniyam. In: Shasti, A.D (ed.) Sushruta Samhita. Varanasi: Chaukhambha Sanskrit Sansthan; 2006. p. 1/65.

9. Mishra, B.L. Dravyaguna Hastamalaka. (3rd ed.). Jaipur: Premalatani Publication Scheme; 1995.

10. Anand, P., Kunnumakkara, A. B., Newman, R. A., & Aggarwal, B. B. (2007). Bioavailability

of curcumin: problems and promises. *Molecular pharmaceutics*, *4*(6), 807–818. https://doi.org/10.1021/mp700113r

11. Prakash, M. How to make curcumin cure better. Down to Earth. Weblog. [Online] Available from: https://www.downtoearth.org.in/news/how-to-make-curcumin-cure-better-3821 [Accessed 27 April 2022].

CORRESPONDING AUTHOR

Dr Satish Pai

Reader & Head, Department of PG studies in Dravyaguna Vignana, JSS Ayurveda Medical College, Mysuru, Karnataka, India

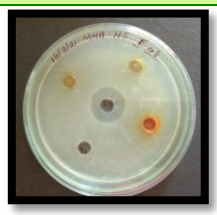
Email: satishayurveda@gmail.com

Source of support: Nil

Conflict of interest: None Declared

Cite this article as

Dr Satish Pai.: Evaluation of Antimicrobial Activity of Haridra (Curcuma Longa) Vis-À-Vis Madhu Samskara; VIII(3): 2291-2298

 Ardra Haridra	 Ardra HaridraSwarasa
 Shushka Haridra	 Shushka Haridra Swrasa
 Madhu Paka vidhi	 Haridra swarasa Samskarita Madhu
 Disc diffusion method	 Broth dilution method