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An Invitro study to evaluate the *Krimighna Karma* (Antimicrobial Activity) of *Dugdhika Patra* (*Euphorbia hirta* Linn.) against *Staphyloccus aureus*

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ABSTRACT

Background: Today's world's mortality rate depends on the infectious diseases and accounts for about 50% of all deaths. Deadly diseases of this century were mainly caused by bacterial infections. Biologically important plants were discovered by evaluation of ethnopharmacological data and they are locally populated with immediate therapeutic action. The recognition of any of the *Dravya* is through its *Karma* (Action). The *Karma* is mainly explained with the help of *Rasa Panchaka* in science of *Ayurveda*. *Krimighna Karma* is one such *Karma* which is responsible for the destruction of the *Krimi.* The plant *Dugdhika* is widely used in traditional medicine to cure various diseases, especially gastrointestinal disorders, respiratory disorders and skin disorders. **Aim:** To evaluate the *Krimighna Karma* of *Dugdhika Patra Swarasa* against *Staphylococcus aureus* bacteria. **Materials and Methods:** In this study, *Dugdhika Patra (Euphorbia hirta* Linn.) *Swarasa* was evaluated for its antibacterial activity against *Staphylococcus aureus* bacteria using well diffusion method. **Observations and Results:** The zone of inhibition was observed through agar well diffusion method. *Swarasa* had shown maximum Zone of inhibition (9mm) for *Staphylococcus aureus* bacteria when dispensed directly and moderate zone of inhibition (6mm) with diluted concentration after 24 hours of incubation period when compared to control group but not as that of standard group. These findings established the potential of *Dugdhika Patra Swarasa* has effective antibacterial agent against *Staphylococcus aureus*. However, further studies are needed to evaluate the active compounds and probable medicinal benefits in humans by clinical trials.

Key words: *Dugdhika, Krimighna, Swarasa, Staphylococcus aureus, Zone of inhibition, Antibacterial.*

INTRODUCTION

Almost every plant has some medicinal value but herbs have innumerable applications in human health. Many plants natural products have served as a major source of medicine for centuries and about quarter of today's drugs are derived from medicinal plants. The World Health Organization estimated that 75 to 80% of the world's population used plant medicines either in part or entirely for health care.[1] India is the largest producer of medicinal herbs and is appropriately called the botanical garden of the world.[2] With the changing lifestyle, the resistance of pathogenic bacteria to various antibiotics has been reported.[3] Most important resistant bacteria include both Gram-positive (*Staphylococcus aureus*), Gram-negative (Pseudomonas aeruginosa) and Mycobacterium tuberculosis.[4] The increasing rate of resistance to commonly used antibiotics have led to search for newer, more effective, affordable and easily available drugs.[5]

Staphylococci are common commensals and parasites of human and animals.[6] Staphylococci are gram-positive cocci which are present in the skin, nasal vestibule, stool etc., Most staphylococcal infections are caused by *Staphylococcus aureus*. Staphylococcal infections of the skin are quite common. The infection begins from lodgement of cocci in the hair root due to

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ISSN: 2456-3110 ORIGINAL ARTICLE October 2024

poor hygiene and results in obstruction of sweat or sebaceous gland duct. Impetigo is a staphylococcal skin infection common in school children in which there are multiple pustular lesions on face forming honey-yellow crusts.[7] Acne may appear in adolescence, and it persists through the early thirties. Urban populations are more affected than rural populations. About 20% of the affected individuals develop severe acne, which results in scarring.[8] *Staphylococcus aureus* also produces heat-stable enterotoxins that cause a rather severe and common food poisoning.[9] Antibiotic resistance is a growing problem in the treatment of *Staphylococcus aureus* infections.[10]

Our *Ayurvedic* literatures have explained the concept of *Krimi* presenting with different symptoms involving various systems. Though many drugs have been explained as *Krimighna*, there are only few works done to prove the efficacy of specific drug activity on specific micro-organism causing skin infections. *Dugdhika* (*Euphorbia hirta* Linn.) belongs to family Euphorbiaceae is a pan tropical annual weed found especially on roadsides and wastelands. The plant is commonly known as Kempu Nene Hakki in Kannada and also called as snake weed and asthma weed. The plant is widely used in traditional medicine to cure various diseases, especially in Asthma, Bronchitis, Diarrhoea, Conjunctivitis, Worm infestation, Ringworm, Skin diseases and Leprosy. The latex is said to be useful in acne vulgaris.[11]

As *Dugdhika (Euphorbia hirta* Linn.) is easily available throughout India and leaf is the part which is present in all seasons, this drug has been taken up for the study. In *Kaiyadeva Nighantu, Dugdhika (Euphorbia hirta* Linn.) is attributed with *Krimighna* and *Kushtaghna Karma.*[12] Hence, the present study is an attempt to evaluate the antibacterial activity of the *Swarasa* of *Euphorbia hirta* Linn. leaves against on one of the skin infections causing organism *Staphylococcus aureus*.

Materials and Methods

Collection of plant material

The fresh plant of *Euphorbia hirta* Linn. was collected from campus of SDM College of Ayurveda and Hospital, Udupi, Udupi district, Karnataka State, India.

Dugdhika botanically identified as *Euphorbia hirta* Linn. is a prostrate annual herb spreading on the ground belonging to Euphorbiaceae family. Leaves opposite, obliquely oblong, small, unequal sided at the base, entire or crenulate, glabrous, and green or reddish above, glaucous and pubescent beneath. Cyathia axillary, 1-3, in short cymes. Fruits ovoid-globose, 1-1.5mm in diameter. Seeds obtusely quadrangular, up to 1mm long, pale brown, transversely rugose.[13]

Preparation of *Swarasa*

The *Swarasa* (fresh juice) was prepared using the standard method.

Fresh leaves of *Dugdhika* which are devoid of any insects were collected and washed thoroughly, then shifted to *Khalva Yantra* and pounded without adding water till a bolus is formed. Then it was taken in a clean cloth and squeezed to get *Swarasa*. It was measured using a measuring jar and stored in a clean container.

Fig 1: A) Plant of *Dugdhika*

Fig 1: B) Collection of leaves

ISSN: 2456-3110 ORIGINAL ARTICLE October 2024

Fig 1: C) Preparing *Swarasa* through *Khalva Yantra*

Fig 1: D) *Swarasa* of *Dugdhika Patra*

Agar well diffusion method was selected for conducting the antibacterial study.

Preparation of Nutrient agar media

Requirements

1. Beef extract (1g)
2. Yeast extract (2g)
3. Peptone (5g)
4. Sodium Chloride (5g)
5. Distilled water - 1000ml
6. Agar -15g

Method of preparation

The first 4 ingredients are weighed using digital balance. These extracts were dissolved in 900 ml of distilled water. The pH was adjusted to 7.2 and the volume was made up to 1000 ml. Finally, 15g agar was added to the media and autoclaved at 121°C for 20 minutes.

Preparation of the inoculum

Staphylococcus aureus (MTCC 3160) was procured from Microbial Type Culture Collection and Gene Bank (MTCC), IMTECH, Chandigarh. They were supplied in frozen form in sealed glass vials.

Optimum care was taken in opening the vials. The work place was cleaned in laminar air flow using 70% ethyl alcohol and UV for 20 minutes. Loopful of 48h old culture of *Staphylococcus aureus* from the slants was transferred to 5 ml of sterile saline and mixed well to prepare a homogenous inoculum.

Preparation of Agar plates

Purpose of preparing the Agar plate is to provide a larger surface area for the growth of micro-organisms. The autoclaved sterile petri plates were taken and labelled with the name of the organism and the drug along with the date of preparation. Then, the different concentrations of the drug were also labelled on the backside of the culture plates.

Well Diffusion Method

The nutrient agar media was cooled to around 45-55°C, around 20 ml of the media was poured into each sterile Petri plate.

One ml of the inoculum was immediately added to the plate, swirled for uniform distribution.

The air bubbles were removed using heat from the Bunsen burner.

A sterile environment was achieved by placing two Bunsen burners.

The plates were left undisturbed, cooled down and solidified to look opaque and this media is autoclaved at 121°C for 20 minutes.

Once it solidifies, equidistant wells were bored using a sterile borer.

In the above said concentrations of test sample, standard drug and the control were dispensed into the bored well. These culture plates were incubated overnight at 37°C and observed after 48 hours. Then, it was taken out and observed for the zone of inhibition.

ISSN: 2456-3110 ORIGINAL ARTICLE October 2024

Fig 2: A) Preparation of the inoculum

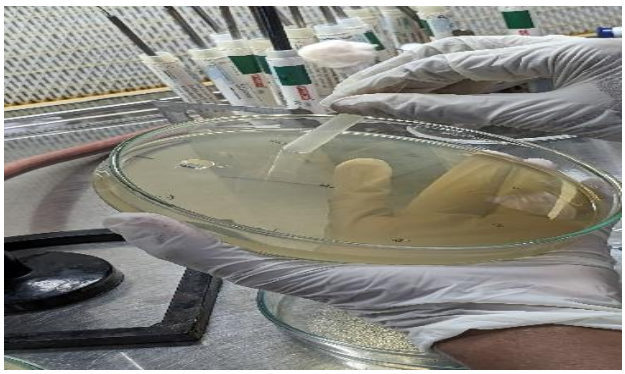

Fig 2: B) Opening wells with upside-down plastic tips

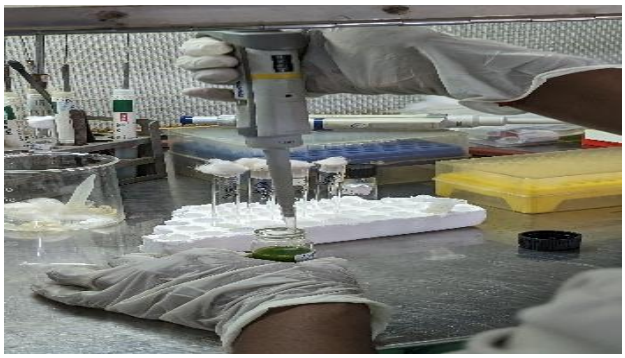

Fig 2: C) Introduction of Sample in the Well

Fig 2: C) Introduction of Sample in the Well

Zone of inhibition (The diameter) was measured for each concentration using a scale and noted for each concentration of *Swarasa* extracts.

OBSERVATIONS

The difference in the zone of inhibition between the control and the test groups were calculated for each concentration and was noted.

Average ZOI of each control group and the test drug group was calculated and compared to the standard group.

Table 1: ZOI of *Swarasa* of *Dugdhika patra* (*E.hirta* Linn.) against *Staphylococcus aureus*

	Volume	Zone of	Inhibition (Radius in mm)
Swarasa of *Dugdhika Patra* (*Swarasa* of *E.hirta* L. Leaves)	10+90µL	0	0
	25+75µL	6	6
	50µL+50µL	7	7
	75µL+25µL	8	8
	50µL	8	8
	100µL	8	8
	150µL	8	9
Control (Double Distilled water)	150µL	0	0
Standard (Ampicillin) 1mg/ml	50µL	16	17

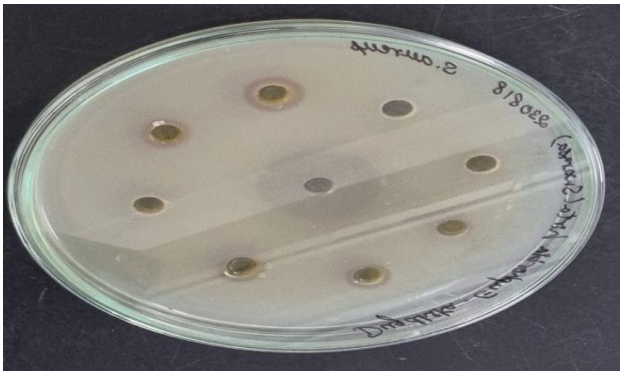

Fig. 3: Agar well plate showing zone of inhibition with the test sample, control and the standard drug.

ISSN: 2456-3110 ORIGINAL ARTICLE October 2024

Table 2: Average ZOI of *Dugdhika Patra Swarasa*

Test Sample	Average
Swarasa (Direct)	9mm
Swarasa with dilutions	7mm
Control (Distilled water)	0
Standard (Ampicillin)	17 mm

The average ZOI of *Dugdhika Patra Swarasa* group was more than the control group but not as that of standard group.

RESULTS

The *Swarasa* of *Dugdhika Patra* (*Euphorbia hirta L)* with and without dilutions had shown positive antibacterial effect against *Staphylococcus aureus* in comparison with the control group but not as much as the standard group.

By all the above observations, we can analyze that the *Swarasa* of *Dugdhika Patra* (*Euphorbia hirta* L*)* without dilution had shown maximum antibacterial effect when compared to different diluted concentrations of *Dugdhika Patra Swarasa.*

DISCUSSION

Based on the selection of the drug

The plant *Dugdhika* is a drug which is abundantly available and *Krimighna Karma* is specifically mentioned for its leaf in Kaiyadeva Nighantu.[12] The leaf is selected because it is the part which can be easily collected and used. Saponins, phenolics, flavonoids, anthraquinones and alkaloids which are secondary metabolites of plants are actually the defensive mechanisms of the plants against pathogens. It has been extensively studied for antibacterial activity against various strains of bacteria. It has demonstrated a profound degree of inhibition on growth of *Staphylococcus aureus* bacteria. The study shows that Swarasa extracts of *E. hirta L* leaves exhibited the antibacterial activity against S. aureus bacteria.

Based on the selection of the form of the drug

As the *Swarasa* is considered as the most potent *Kalpana* among *Panchavidha Kashaya Kalpana* and it is easy to prepare from the leaves of the plant, this form was selected for the study.

Invitro antimicrobial study

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plant extracts. This method is simple, qualitative and easy to perform. Hence, this method was selected to evaluate the antibacterial activity of *Dugdhika* (*Euphorbia hirta* Linn.) *Patra*.

In this study, it was observed that, as the volume of the concentrations of the *Swarasa* is increased, there was a subsequent increase in the zone of inhibition also.

As the concentration decreases, the active molecule content has also decreased which might not be capable to destroy the strains of bacteria.

Based on *Rasa Panchaka*[14-16]

Rasa - Tikta Rasa. *Tikta Rasa* is '*Krimin Jayeth'*, which means destroys or kills the *Krimis*. It can be taken as, by virtue of its *Tikta Rasa*, it helps in destroying the *Krimis* or helps in stopping the growth of the organisms.

Guna - Ruksha, Laghu, Teekshna Guna. Ruksha Guna - The main action of *Ruksha Guna* is *Shoshana, Sthambhana* and also *Kaphahara* which helps in drying or shrinks the size of the cells. Thus, it will act as *Krimi Shoshaka* and it stops the growth of the bacteria. *Laghu Guna* has *Kaphahara* and *Lekhana* property which helps in removing the excess *Kleda* (moisture), thereby inhibits the growth of bacteria. *Teekshna Guna* has *Lekhana, Kaphahara, Shodhana* property which may cause irritation to the *Krimis* and evacuate them through *Sravana* property.

Veerya - Ushna Veerya causes destruction of the cell components of the bacteria because of its *Pachana* property. Thus, it helps in bactericidal action.

Doshaghnata - Kaphavatashamaka

Growth of the *Krimi* is mainly due to *Vata* and *Kapha Dosha*. *Dugdhika Patra* having *Kapha* and *Vatahara* property which inhibits the growth of *Krimi.*

Based on the phytoconstituents

Euphorbia hirta Linn. is reported to contain triterpenes, phytosterols, tannins, polyphenols, and flavonoids.[17]

ISSN: 2456-3110 ORIGINAL ARTICLE October 2024

All these phytoconstituents are proven for antimicrobial activity.[18-21] Hence these help in preventing the growth of the microbes.

Conclusion

Dugdika Patra (*Euphorbia hirta* L.) *Swarasa* possess antimicrobial activity against *Staphylococcus aureus*, although its efficacy is not on par with standard antibiotics. This plant holds potential as a complementary or alternative treatment, particularly in contexts where conventional antibiotics are less effective or where resistance is a concern. Further research is essential to refine its preparation, optimize dosages, and explore synergistic effects with existing treatments, thereby enhancing its practical application in modern healthcare.

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ISSN: 2456-3110 **ORIGINAL ARTICLE** October 2024

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