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Research Article

EVALUATION OF *VANADHANYAKA* (*ERYNGIUM FOETIDUM* LINN.), A FOLKLORE DRUG ON *MUKHADUSHIKA* (ACNE VULGARIS) - AN IN-VITRO STUDY

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ABSTRACT

Face is the index of body and mind. It is the general opinion that smooth and glowing skin of face not only enhances the beauty of a person but it also adds self-confidence. In Ayurvedic classics in the context of *Kshudra rogas, Mukhadushika* is mentioned. Its signs and symptoms are similar to that of acne vulgaris. One of the causative bacteria is Staphylococcus epidermidis. But at present effective medication or preventive measures with less side effects are very few. So, there is a need for one. *Vanadhanyaka* (*Eryngium foetidum Linn*.) is one such drug, which is endowed with a property of antibacterial, anti-inflammatory, analgesic and antioxidant. Phytochemical screening of the leaves indicated the presence of flavonoids, polyphenolic compounds, saponin, phytosteroids, triterpenoids, alkaloids, tannins anthraquinones, cardiac glycosides and terpenes. Eryngium foetidum Linn extracts were carried out. The whole plant was used for the in vitro experiment. The aqueous, alcoholic and hexane extract of the trial drug was tested against the Staphylococcus epidermidis bacteria MTCC (Microbial Type Culture Collection and Gene Bank)435. The in-vitro studies to determine the minimum inhibitory concentration was done by micro-broth dilution method and zone of inhibition by Agar well diffusion method. Hexane extract showed better results compare to alcoholic and aqueous extracts.

INTRODUCTION

Face is the mirror of an individual personality and any least mark can result into a larger impact on the individual.

Mukhadhusshika also called as *Yauvanapidika*, It corelates with Acne vulgaries. It is an inflammatory disease. The primary areas affected are face and rarely on the back and chest. Individually one may suffer from pain, in the acute stage of acne, which later results in permanent scar, thus in turn affects the beauty, which may induce in individual inferiority complex, depression and sometimes isolation from the social life.

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The treatment consists of *Shodhana*, *Lepa* and *Shamanoushadis*. In modern science treatment exists for acne vulgaris like topical therapies, antimicrobials, hormones, surgery, U.V radiations, Intra lesion injections etc. But these treatments have their own side effects like, skin irritation, contact dermatitis, photosensitivity by topical application, gastro-intestinal disturbance and other systemic disorders.

So, there is an intense need for potential, well tolerated treatment which can limit the disease without affecting the beauty and reduce its psychological impact.

Hence this is an attempt to study the antibacterial effect of *Vanadhanyaka* on Staphylococcus epidermidis.

Objectives of the study

1. To evaluate the anti-bacterial activity of *Eryngium foetidum* Linn. on Staphylococcus epidermidis by agar well diffusion method done in triplicate method.
2. MIC (Minimum Inhibition Concentration) determination against Staphylococcus epidermidis

by micro broth dilution technique in triplicate method.

MATERIALS AND METHODS

Study was designed under two headings:

1. Collection and Preparation of the trial drug
2. In -vitro study

Procurement of plant material

The *Eryngium foetidum* Linn. Was procured from Antravalli, Kumuta taluk, Uttara Kannada District. Karnataka.

Drug authentication

The genuinity of the sample was confirmed by Dr.N.M.Ganesh Babu, PhD, Head, Centre for herbal gardens, F.R.L.H.T., Yelahanka, Bengaluru.

Preparation of trial drug

2.8kgs of *Eryngium foetidum* Linn. Was cleaned thoroughly and shade dried. *Panchanga* was used for the study. The drug was then coarsely powdered and stored in airtight container.

Place of preparation of extracts

- The aqueous and alcoholic (methanol) extracts of *Vanadhanyaka* was prepared at 'Green chem', Herbal extracts and formulations, Domlur, 2nd Stage, 3rd Phase, Bangalore 71.
- Hexane extract was prepared at Skanda Lifesciences Pvt. Ltd., # 10, 11, 12, Sri Shaila Bramara complex, Chandana Layout, Srigandadakaval, Nagarbhavi, Bangalore.

***Eryngium Foetidum* Linn**

Place of In-vitro study

Anti-Bacterial activity was carried out in Skanda Life sciences. Bengaluru.

Materials required

Isolated strain of the selected microbe, selective growth agar media, disposable petriplates, micropipettes, incubator, glassware, DMSO, inoculation loop, cork borer, weighing machine, Bunsen burner, disinfectants like dettol, ethanol and spirit.

Place of purchase of required materials

- The isolated strain of Staphylococcus epidermidis (MTCC-435) was obtained from Microbial type Cell Culture and Gene Bank, Chandigarh.
- Nutrient agar, dimethyl sulphoxide, distilled water and Ethyl alcohol was procured from Bharath Scientifics Center, Bengaluru.

Standard

Ciprofloxacin is a standard antibiotic used to treat a number of bacterial infections especially skin related.[1]

Determination of zone of inhibition by agar well diffusion method[2,3]

Test organism: Staphylococcus epidermidis

Test compounds: *Eryngium foetidum* Linn. extracts in aqueous, methanol and hexane.

Ciprofloxacin – Standard.

Inoculum: Cell suspension prepared from cultures grown on Trypsin broth adjusted to 1-2x105cells/ml.

Test concentrations: Drug concentration prepared

- *Eryngium foetidum* Linn. extract 100mg/ml in DMSO (Dimethoxy sulphoxide)
- Ciprofloxacin- 1 mg/ml in DMSO
- Control- DMSO

Procedure

Determination of Antimicrobial activity

- Inoculum of test cultures was inoculated on nutrient agar plates (90mm).
- Test compounds (10µl& 25µl) & Ciprofloxacin (10µl) were impregnated on 5mm wells.
- The plates were Incubated @ 37^0C for 24-48 hours and observe for zone of inhibition around the well.

MIC determination against Staphylococcus epidermidis by micro broth dilution technique as per NCCLS method[2,3]

Test organisms: Staphylococcus epidemidis

Test compounds: *Eryngium foetidum* Linn. aqueous, methanol and hexane extract ciprofloxacin -standard

Inoculum: Cell suspension prepared from bacterial cultures grown on trypsin broth adjusted to 1-2 x 105cells/ml.

Drug concentrations: drug concentration prepared

- Ciproflaxacin: 0.25-16µg/ml (Two-fold dilutions) in Trypsin Broth.
- Test compounds: 16-1024µg/ml (Twofold dilutions) in trypsin broth for bacterial test cultures.
- Control: Trypsin broth inoculated with culture and without test compounds.

Procedure

- Mix 100µl drug/test compounds of different test concentration with 10µl Inoculum in 96 well plate in triplicate
- Control: Mix 100µl Trypsin broth without drug with 10µl Inoculum
- Add 10 µl Alamar blue (0.7mg/ml) to all the well.
- Treated bacterial cultures are incubated at 37°C. The bacterial test plates were observed after 24-48 hours and O.D @ 590nm is measured in Tecan plate reader.
- Determine MIC as Minimum concentration of drug giving 50% inhibition of OD as compared with control.

RESULTS

Table 1: Zone of Inhibition

Test Compound		Concentration (mg/Well)	Zone of Inhibition (in mm) Mean
Eryngium foetidum Linn.	Aqueous Extract (Aq.E)	1	-
		2.5	5.5± 0.0
	Methanolic Extract (Al.E)	1	-
		2.5	7.00 ± 0.5
	Hexane Extract (H.E)	1	6.00 ± 0.0
		2.5	7.00 ± 0.0
Ciprofloxacin		0.01	35.00 ± 1.00
DMSO		2.5	-

- 1mg of hexane extract showed 6mm zone of inhibition but aqueous and alcoholic extracts did not showed zone of inhibition.
- Hexane and alcoholic extract showed 7mm zone of inhibition at the concentration of 2.5mg.
- Aqueous extract at 2.5mg showed 5.5mm of zone of inhibition.

Plate no. 1: inhibitory activity of aqueous and alcoholic extracts

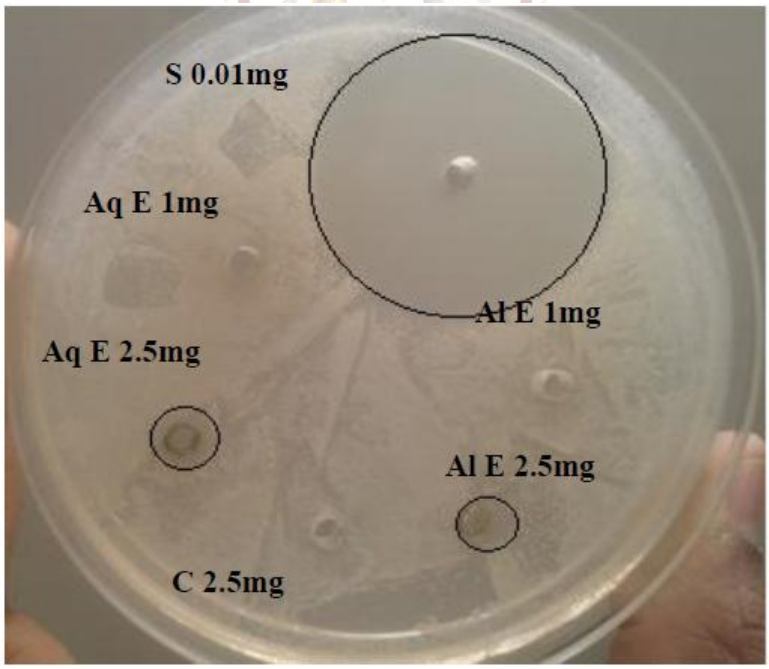


Plate No.2: Inhibitory activity of Hexane extract

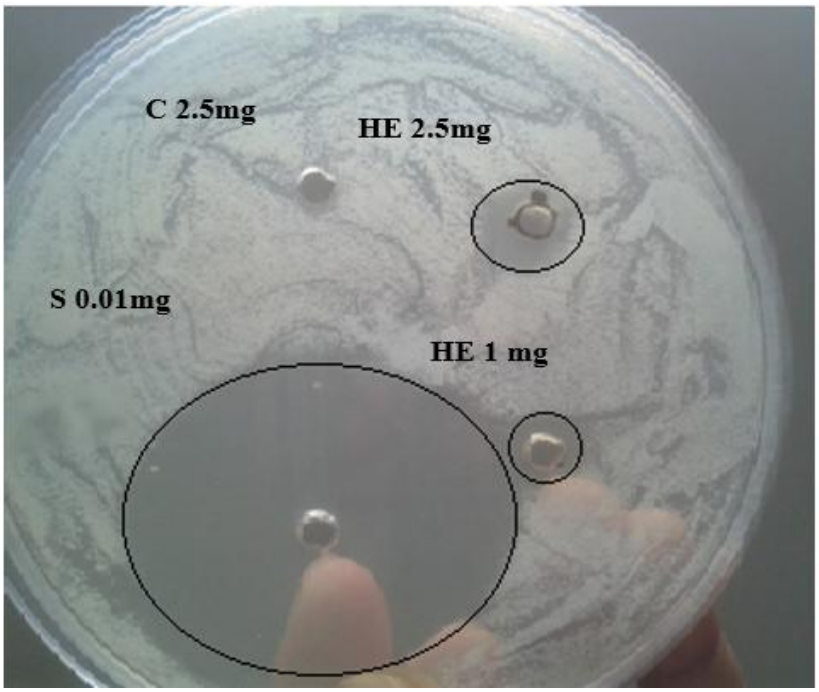
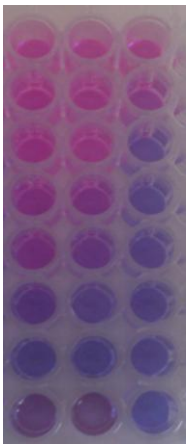
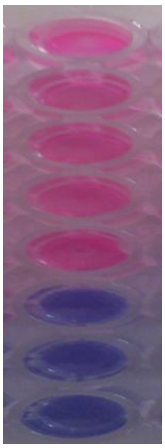
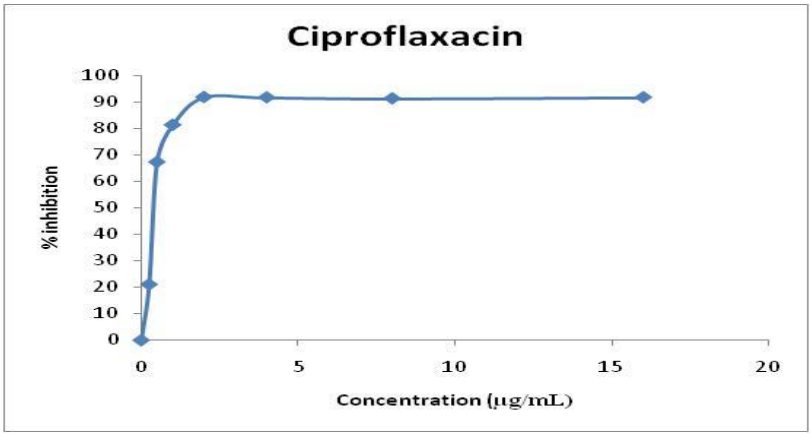


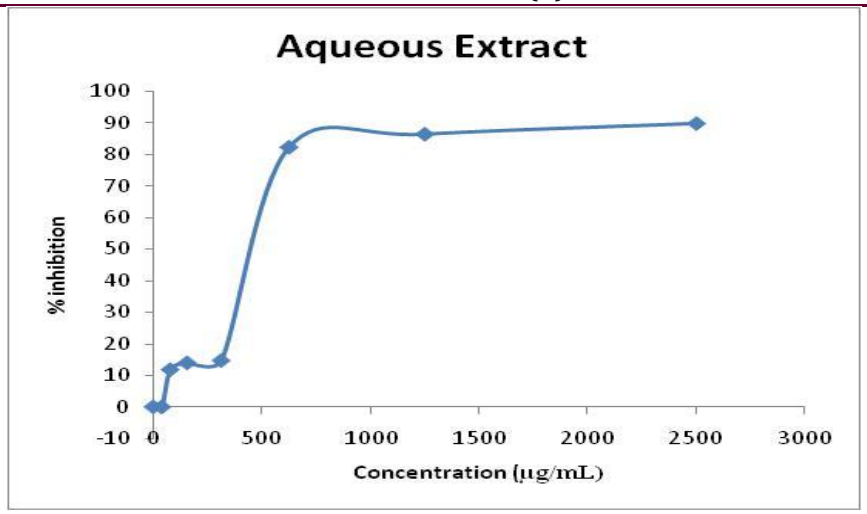
Table 2: MIC of Standard, Aqueous, Methanolic and Hexane extracts

Ciproflaxacin Concentration (nµg/mL)	% inhibition	Aq.E Conc (µg/mL)	% inhibition	Al.E Conc (µg/mL)	% inhibition	H.E (µg/mL)	% inhibition	Microtest plate of *E.foetidum*, Aq, M.E and Ciproflaxacin	Micro test plate of H. E
0.00	0.00	0.00	0.00	0.00	0.00	0	0.00		
0.25	21.09	39.062	11.79	39.0625	2.25	16	5.25		
0.5	67.25	78.125	13.94	78.125	5.25	32	8.00		
1.0	81.25	156.25	14.67	156.25	21.25	64	14.67		
2.0	91.73	312.50	11.02	312.50	28.25	128	48.25		
4.0	91.62	625.00	89.73	625.00	82.25	256	89.73		
8.0	91.22	1250.00	86.34	1250.00	90.25	512	86.34		
16.00	91.65	2500.00	82.22	2500.00	92.25	1024	82.22		
MIC **(µg/mL)**	**0.5**	**MIC(µg/mL)**	**625**	**MIC(µg/mL)**	**625**	**MIC**	**256**		

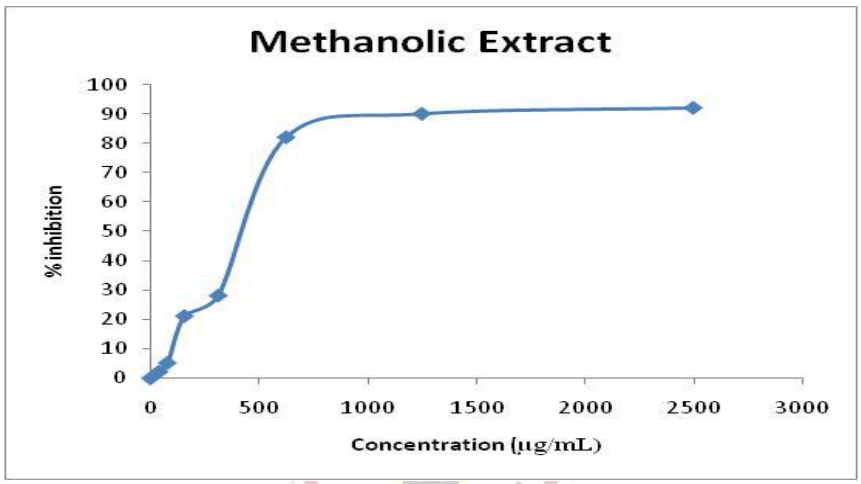
Hexane extract showed M.I.C at the concentration of 256 µg/ml, aqueous and alcoholic extracts showed at the concentration of 625 µg/ml.



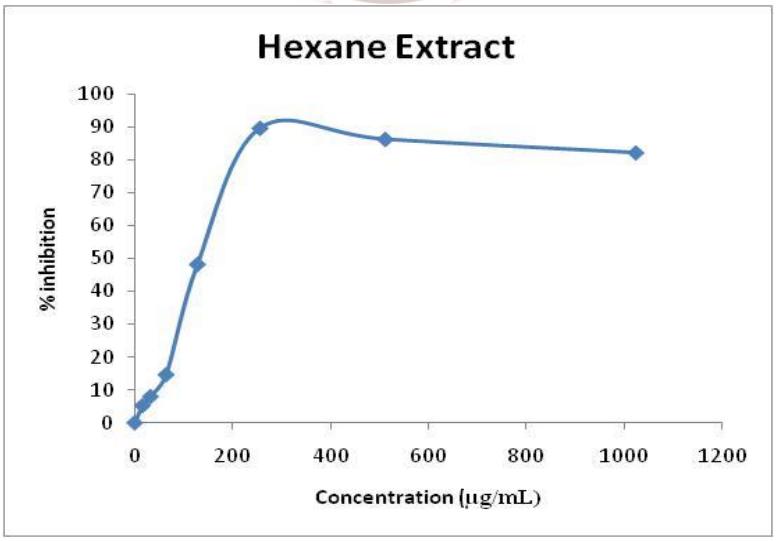
Graph 1: Showing Inhibitory activity of Ciproflaxacin against *Staphylococcus epidemidis*



Graph 2: Showing inhibitory activity of *E. foetidum* Aq extract against *Staphylococcus epidemidis*



Graph 3: Showing inhibitory activity of E. Foetidum Methanolic extract against *Staphylococcus epidemidis*



Graph 4: Showing inhibitory activity of *E. foetidum* Hexane extract against *Staphylococcus epidemidis*

DISCUSSION

M.I.C

Hexane extract showed better M.I.C compared to aqueous and alcoholic extracts.

Zone of inhibition

The drug exhibited dose dependant zones.

- Hexane extract exhibited maximum zone of inhibition, alcoholic extract showed moderate and aqueous extract exhibited least.

• 1mg of hexane extract showed 6mm zone of inhibition but alcoholic and aqueous extracts did not show any activity.

• However at 2.5mg of hexane and alcoholic extracts showed 7mm zone of inhibition, indicating that both the extracts have equal strength. 2.5 mg of aqueous extract exhibited 5.5mm zone of inhibition.

By this it can infer that constituents responsible for antimicrobial activity are more in hexane, alcohol and aqueous extracts respectively.

Probable mode of action of trial drug

Eryngium foetidum linn. Is a exotic plant, when mexicans invaded India, this plant was brought to India in 18th century. Therefore details of *Rasapanchaka* is not available.

A phytochemical study confirmed the presence of alkaloids, flavonoids, glycosides, steroids, triterpenoids, saponins, resins, tannins, phenols, carbohydrates, and proteins.

Organoleptic study showed *Kashaya rasa. Kashaya rasa* has the properties like *Shodhana, Samshamana, Lekhana, Peedana, Ropana, Shleshma Rakta pitta prashamana*[4,5].

• Its *Shodhana* property purifies thoroughly[6].

• Lekhana does the *Vishoshana* of dhatu and mala. Thus purulent condition of inflammation is targeted[7].

• *Peedana* helps in removing localised doshas.

• *Samshamana* subdues the aggravated *doshas*[8].

• *Ropana* helps in healing

In the *Samprapti* of *Mukhadushika kapha*, *Pitta dosha* and *Rakta dushya* is mainly involved. *Kashaya rasa* is *Shleshma rakta pitta Prashamaka*[4] .

Eryngium foetidum linn. Is an antibacterial drug, evidence-based researches have proved for its antibacterial activity against staphylococcus aureus, streptococcus pyogenes. But its efficacy on staphylococcus epidermidis is not evaluated [9].

The main constituent is essential oil known as eryngial. Eryngial is known to be antibacterial.

Essential oils improve the pheripheral circulation. Improved peripheral circulation helps in healing.

Eryngium foetidum linn. Has secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids, steroids, resin, phenols, saponins and glycosides.

• Terpenoids, phenols, alkaloids, and flavonoids are compounds whose hydrophobicity induces partition in the lipids of the bacterial cell membrane and mitochondria, disturbing the structures and rendering them more permeable.

• Flavonoids form the complexes with the cell wall protein and disrupt the microbial membranes, the main function of the cell membrane is to protect the bacteria, so the protecting function is inhibited. Thus proving to be cytotoxic.

• Flavonoids may act through inhibiting cytoplasmic membrane function as well as by inhibition of DNA gyrase and β-hydroxyacyl-acyl carrier protein dehydratase activities.

• Alkaloids directly acts on CNS and has activities like analgesic, anti-inflammatory. Analgesic and anti-inflammatory properties helps in reduction of inflammation and pain.

• Saponins are commonly toxic substances and their special nature is to cause toxicity to the cell as such. Therefore destruction of the cell resulting in death of the cell.

• Steroids are the universal medicine for any given condition. Resistant wound needs a steroidal intervention. As this plant has phytosterols, any chronic condition of acne can be tackled.

• Tannin has the property of precipitating the protein layer through adsorption, makes a protective layer which helps in healing.

• Resins are usually demulcents, they have a soothing action over the skin. Thus promoting softening. Classically mentioned sign like ghanavat(hardness) is taken care of.

• Glycosides are the hydrolyzed products of flavonoids. They helps in strengthening of the blood vessels and improved in circulation.

• Terpenes promote membrane disruption. Thus proving its antibacterial activity.

Thus over all view reveals that constituents of *Eryngium foetidum linn*. Has targeted action towards preventing and resolving pathogenesis of acne vulgaris, thus proving its potent antimicrobial action towards Mukhadushika.

CONCLUSION

Hexane extract comprises of essential oil and triterpenoids proving reason for the efficacy. *Eryngium foetidum* Linn. Is effective against staphylococcus epidermidis bacteria. When compared to standard, hexane extract had showed better results than alcoholic and aqueous extracts. The antibacterial activity exhibited by *Eryngium foetidum* Linn. Through in-vitro method can be revalidated by conducting clinical trials.

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