

Research Article

Evaluation of Anti-bacterial activity of *Charakokta Vishaghna Gana* tablet against *Cutibacterium acnes*: An In-vitro study

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Abstract

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The present research study aims to assess and authenticate classic recipes with scientific evidence. To evaluate the *in vitro* anti-bacterial potential of *Vishaghna Gana* tablet and its constituents against *Cutibacterium acnes*, a causative organism of *Acne vulgaris*. This study also assessed the morphological and physicochemical parameters of the *Vishaghna Gana* tablet. The results of the morphological and physicochemical tests on the tablets included hardness, weight variation, friability, thickness, disintegration time, ash value, extractive value, and pH, as per the CCRAS pharmacopeial standards. The formulated tablet exhibited a rounded shape with a yellowish-brown color and a typical odor and taste. The antibacterial screening was conducted using disc diffusion and punch well techniques on *Bacillus Blood Agar* under anaerobic conditions, and the results were compared with those of azithromycin as a standard control. Tests were performed in triplicate to ensure reliability. In an *in vitro* antibacterial assay, the zone of inhibition for *Sireesha* and *Kataka* showed the highest activity, with inhibition zones greater than 18 mm. In contrast, *Haridra*, *Manjistha*, *Chandana*, and *Slesmataka* were moderately active, with inhibition zones ranging from 13 to 18 mm. The methanolic extracts of the *Vishaghna Gana* tablet showed remarkable antibacterial activity against *C. acnes*. These findings validate *Sireesha* as an *Agrya aushadha* (best medicine) for *Visha* (poison), as quoted in Ayurveda classics, and corroborate *Kataka*'s folk usage in water purification (*Ambu prasadina*). The results underscore the necessity of optimising extraction techniques and concentrations to improve antibacterial activity.

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Introduction

Acne Vulgaris (Mukha Dooshika) is a common dermatological disease affecting more than 85% adolescents and young Adults worldwide. (1) More than 650 million people are affected globally, and it is said to be the 8th most prevalent disease worldwide. (2) Prevalence varies from country to country, depending on the Age group and Acne severity. It is a skin disorder of the pilo-sebaceous Unit, characterised by Non-inflammatory (Closed, open comedones) and Inflammatory (Pustules, Papules, Nodules) lesions. Bacterial Proliferation of *C. acne*, along with Oxidative stress in cells and excessive sebum production, combined with multiple factors, is responsible for the causation of *Acne Vulgaris*. Exogenous factors, such as incompatible food, Environmental pollution, Mental Stress, and

excessive Cosmetic usage, also trigger such conditions. The incidence of *Mukha Dooshika* is increasing day by day due to the consumption of junk food, fast food & cold drinks, lack of hygiene, increased incidence of PCOD conditions in female adolescents, and hormonal imbalance, which leads to imbalance in *Doshas*. This *dosha vaishamya* can be corrected with Ayurvedic herbs that have *Rakta-shodhana*, *Tridoshaghna*, *Kushthaghna* & *Vishaghna* properties, along with *Sodhana* & *Shamana* therapy. The conventional treatment modalities for acne, including topical applications and systemic drugs such as clindamycin, salicylic acid, isotretinoin, erythromycin, and tetracycline, often come with side effects like skin irritation and dryness, as well as the risk of developing antibiotic resistance due to prolonged use.(3) Moreover, the consumption of foods with a high glycemic index can indirectly stimulate the overproduction of sebum, thereby promoting *Cutibacterium acnes* infection and leading to inflammation. Due to these constraints, interest is increasing in seeking alternative and complementary therapies from natural sources providing similar effectiveness with fewer side effects. Here, the Ayurvedic system of medicine presents a potentially valuable area for research regarding the "*Vishaghna Gana*" compound, as described in the *Charaka Samhita*, an ancient Ayurvedic medical text. Ayurveda has immense significance in

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maintaining good health since ancient times, and it is gaining more importance in today's modernised era. Various aspects of Health have been well explained in the *Ashtangas* (eight branches) of Ayurveda. *Agadatantra* (Ayurvedic Toxicology) is one among the 8 branches of Ayurveda. It deals with the diagnosis & management of both animate & inanimate poisoning, *Dooshi Visha* (cumulative poison), *Gara Visha* (artificial poison) and *Viruddhahara* (incompatible diet). Many formulations mentioned in our classics can be used singly or combined with other drugs. Charaka Acharya has explained 50 *Mahakashayas* in *Shad Virechana Shatashriteeyya Adhyaya* of *Charaka Samhitha, Sutra sthana*. Charaka Acharya mentioned the best available 10 drugs with a common indication at that time, under one Category.(4) *Vishaghna Gana* is one among them. It undoubtedly has good Anti-toxic properties. Drugs which act against Toxins or Toxic substances are said to be *Vishaghna*. This *Vishaghna Gana* may treat many poisonous conditions and chronic ailments, including skin disorders like *Mukha Dooshika*. As per modern literature and research, the ingredients in *Vishaghna Gana* have antibacterial, antiviral, antioxidant, anti-acne, anti-inflammatory and immunomodulator properties.(5-8) *Vishaghna Gana* includes -*Haridra*(*Curcuma longa Linn.*), *Manjishtha*(*Rubia cordifolia Linn.*), *Suvalaa*(*Alpinia galanga(L.) WILLD.*), *Sooksm Ela*(*Ellettaria cardamomum MATON*), *Paalindaa*(*Operculina turpethum (L.) S.MANSO*), *Chandana*(*Santalum album Linn.*), *Kataka*(*Strychnos potatorum Linn.*), *Shireesha*(*Albizia lebbeck(L.) BENTH*, *Sinduvara*(*Vitex negundo Linn.*) and *Shleshmaataka*(*Cordia dichotoma FORST.F.*).

The present research study is focused on the formulation of the *Vishaghna Gana* tablet and an in-vitro antibacterial study of *Vishaghna Gana* & its individual drugs against *Cutibacterium acnes*. This study will give an insight into the new therapeutic options for acne vulgaris, which may diminish the use of conventional antibiotics and their side effects. The skin microbiome is increasingly becoming one of the primary targets for therapeutic intervention, and studies on the microbiome primarily concentrate on adults. (9) With their complex variety of active constituents, plant remedies offer multiple modes of action on androgenicity, sebum function, infection, inflammation, and hyperkeratinisation relevant to acne.(10) Such an approach coincides with growing appreciation for the value of integrating philosophies into resolving multifaceted dermatoses.

Aim of Study

Our classics mention about many drugs which possess potential to cure *Mukha Dooshika*. However, due to the unavailability and high cost of certain herbs, there is a need to explore new readily available, cheaper formulations to meet the demand for combating Acne Vulgaris. Anti-bacterial studies of many Ayurvedic formulations have not been conducted so far, and there is a need to investigate the anti-bacterial potential against specific bacteria for its validation. The conventional treatment therapies for Acne are leading to innumerable adverse effects.(11) Hence finding safe alternatives is need of hour. This study has been conducted for an in-vitro assessment of the anti-bacterial activity of *Vishaghna Gana* Tablet and its constituent drugs against the anaerobic bacterium *Cutibacterium acnes*.

Materials and Methods

Plant collection

All the 10 Drugs of *Vishaghna Gana*, ie. *Haridra*, *Manjista*, *Suvalaa*, *Sukshma Ela*, *Palindi*, *Chandana*, *Kataka*, *Shirisha*, *Sinduvara*, and *Sleshmataka* were purchased from trusted raw

drug sellers of the local market in Kerala. The collected drugs were washed under running tap water, air dried, homogenised to a coarse powder and stored in air-tight bottles. The drugs were authenticated at the Ambuja Institute of Ayurvedic Research and Documentation, Kerala. Coarse powder of individual 10 drugs & tablet was prepared from the pharmacy of Rasashastra and Bhaisajya Kalpana department of MVR Ayurveda Medical College, Kannur.

Preparation of tablets and evaluation

All the ingredients of *Vishaghna Gana* were taken in quantity of 1 kilogram each, thoroughly cleaned, washed & dried in the shade and ground into a powder form. Out of the 10 kg, 5 Kg was made into coarse powder for preparing the Kashaya(decoction). The 5 kg of coarse powder was added with 16 times of water and reduced to one-eighth. The standard kashaya preparation method, as per CCRAS Guidelines, was adopted. The remaining 5kg of fine powder was ground in the Kashaya for a period of 15 days. Finally, 500-milligram tablets were prepared in the Tablet punching machine after adding sufficient excipients. The finished product was analysed for the parameters mentioned as per CCRAS ie., appearance, colour, odour, thickness, hardness, pH value, disintegration time, average weight, friability, loss on drying, Total ash value, acid insoluble ash value, water-soluble extract & alcohol soluble extract. Upon analyzing these parameters, it was proven that the prepared tablet met the standard quality.

Figure 1: Ten Drugs Of *Vishaghna Gana*



A=Haridra, B=Manjistha, C=Suvalaa, D=Ela, E=Palindi, F=Chandana, G=Kataka, H=Shireesha, I=Sinduvara, J=Sleshmataka

Evaluation of morphology characteristics of tablets

The morphological characteristics of tablets, such as shape, size, and surface features (i.e., smooth, rough, chipped, mottled), were recorded for differences. The colour of the tablets against a standard colour chart was recorded for any variations or colour inconsistencies. The smell of tablets describe the characteristic, odourless, or with any distinctive noticeable smell.

Evaluation of thickness, hardness, weight variation, friability, and pH of tablet

To measure the thickness of the tablet, a minimum of 10 tablets were used randomly and the average thickness and the range of variation were measured using the vernier callipers. The hardness of the tablet is needed to determine the force necessary to break. The 10 tablets were taken randomly and tested for hardness using a Monsanto hardness tester. The hardness of the tablet was calculated, and the average hardness was noted. The 6 tablets were taken in the disintegration tester apparatus, with a buffer of pH 7.8, and the time taken to disintegrate at the temperature is entirely according to CCRAS guidelines. The pre-weighed tablets

were tested for friability and run for the mentioned time and rotations according to pharmacopeial specifications. Re-weigh the tablets, determine the weight loss percentage, and compare with the mentioned limits. Prepare a suspension/solution of the tablet according to CCRAS guidelines and determine the pH by using a pH meter that has been calibrated.

Determination of the ash value of the tablet

To determine the total ash value, the 10 pre-weighed tablets are incinerated with a known amount of the powdered tablet at a given temperature (typically 600°C) in a muffle furnace, and later the constant weight of ash is calculated.

To determine the acid-insoluble ash value, the tablets are boiled with dilute hydrochloric acid, filtered, and washed with hot water. Burn the filter paper and the residue in a muffle furnace, weigh the residual ash and calculate the percentage of acid-insoluble ash.

The percentage water-soluble extract value of tablets is macerated, and the powdered tablet is accurately weighed with water as per standard guidelines. Later, the filtration of the extract evaporates to dryness and determines the residue weight. Proceed similarly to the water-soluble extract but with the alcohol indicated as per the standards. Determine the percentage of the alcohol-soluble extract.

Preparation of Extracts of the tablet for in vitro antibacterial test

Both aqueous extract and methanolic extract of the individual 10 drugs & Tablet were prepared, and dilutions using Dimethyl Sulphoxide(DMSO) were done in concentrations of 100% (undiluted), 50%, 25% & 12.5% of all the samples.

Bacterial Cultures

ATCC 11827 *Cutibacterium acnes* bacterial strains were procured from Hi Media Laboratories, Pvt. Ltd, Mumbai.

Method used for evaluating Antibacterial Activity

Antibacterial screening of drugs was done at the Microbiology department of Father Muller's Research Centre, Mangalore, after getting the Ethical Clearance (FMIEC/CCM/079/2025). Both the Disc diffusion & Punch well methods were adopted initially for the samples to determine the antibacterial activity of herbal extracts. Brucella Blood Agar incubation in an anaerobic environment with BD Gaspack for 48-72 hours was maintained. The plates were incubated for 48 hours at 37°C. The anti-bacterial activity was evaluated by measuring the diameter of inhibition zones. The experiment was carried out in triplicate, and the average diameter of the zone of inhibition was recorded. Discs of 6mm diameter, Whatman No.1 Filter paper, were saturated with various dilutions and the Undiluted drug in the Disc-diffusion method. They were applied on Brucella Blood Agar plates pre-streaked with *Cutibacterium acnes*. An azithromycin disc of 15 mcg was used as a positive control.

Punch well Method: As the results showed that the Punch well technique showed good results, further experiments were carried out employing the same technique using various dilutions of the drug. Undiluted, 50%, 25% & 12.5% of concentrations.

Antimicrobial Susceptibility Testing

The Kirby-Bauer method, employing Brucella Blood Agar in an anaerobic environment, tests susceptibility to antibacterial agents. *Cutibacterium acnes* ATCC 11827 was employed in parallel as a control. The antibiogram was reported in comparison

with the Kirby-Bauer chart. *Cutibacterium* were grown in VL broth, and turbidity was adjusted to 0.5 McFarland standards. The bacterial suspension was applied to the Brucella Blood Agar. Filter paper discs made from Whatman No.1 filter paper were impregnated with an appropriate concentration of herbal extracts and placed on lawn culture. The Zone of Inhibition corresponding to the degree of sensitivity of the antimicrobial agent was measured after 48 hours of incubation in an anaerobic environment in a BD Gas pack jar.

Results

Results of Morphology, thickness, hardness, weight variation, friability, pH and ash value of the *Vishaghna Gana* tablet

The hardness is indicated as a range (3-3.5 kg/cm³). It is usually better to indicate hardness as an average with a standard deviation, from several measurements. This gives a better picture of the consistency of tablet hardness. The weight variation is beyond the generally acceptable range of $\pm 5\%$ (as usually laid down in pharmacopeial standards, applicable current standards. Determining the cause of this variation and maintaining uniformity is essential for quality control. A friability of 1.0476% exceeds the acceptable standard of less than 1%. This indicates possible problems with tablet strength during handling and transportation. All the resulted are tabulated in table 1

Table 1: Physico-chemical Analysis of *Vishaghna Gana* Tablet

SL.No.	Test	<i>Vishaghna Gana</i> Tablet
1	Appearance	Round shaped tablet
2	Colour	brown
3	Odour	Characteristic
4	Taste	Characteristic
5	Diameter	13.13 mm
6	Thickness	5.52 mm
7	Hardness	3- 3.5 kg/cm ³
8	pH	5.63
9	Disintegration time	30 min
10	Average weight	0.7232 g
11	Uniformity of weight	+2.434 % -4.065%
12	Friability	1.0476%
13	Loss on Drying	5.1410 % w/w
14	Total ash	5.4489 % w/w
15	Acid insoluble ash	0.6282 % w/w
16	Water-soluble extract	26.5162 % w/w
17	Alcohol soluble extract	4.7269 % w/w

In vitro Antibacterial test

The readings were taken at the end of 48 hours. Bacteria showing a clear zone of inhibition > 8 mm in diameter were considered sensitive. Experiments were performed in triplicate for each extract combination and bacterial strain. The results were expressed in terms of the diameter of the inhibition zone: < 9 mm is inactive; 9-12 mm is partially active; 13-18 mm is active; >18 mm is very active.(12) The antibacterial activity of herbal extracts against *Cutibacterium acnes* is shown in Table 2.

Table 2: The antibacterial activity represented by Zone of Inhibition (mm) produced by methanolic extracts of all the 10 individual drugs and Vishaghna Gana Tab

SL.No .	TEST DRUG	A=100% Conc.	B=50% Conc.	C=25% Conc.	D=12.5% Conc.
1	Haridra	14 mm	10 mm	8 mm	R
2	Manjistha	16 mm	14 mm	12 mm	10 mm
3	Rasna	8 mm	R	R	R
4	Ela	8 mm	R	R	R
5	Trvrt	10 mm	8 mm	R	R
6	Chandana	16 mm	14 mm	12 mm	10 mm
7	Kataka	22 mm	18 mm	16 mm	14 mm
8	Sireesha	29 mm	21 mm	18 mm	17 mm
9	Sindhuvara	12 mm	8 mm	R	R
10	Slesmataka	15 mm	14 mm	12 mm	10 mm
12	VG Tab	8 mm	R	R	R

[Conc.= Concentration of Herbal extract. R=Resistant zone ie.No Zone of Inhibition]

Results showed that amongst the *Vishaghna Gana* drugs, *Sireesha* and *Kataka* possess very strong antibacterial activity against *Cutibacterium acnes* while *Haridra*, *Manjistha*, *Chandana* & *Slesmataka* also possess moderate antibacterial potential against the specific micro-organism. This establishes the fact said in Ayurveda classics about *Sireesha* proving it to be *agrya*(best) *aushadha* for *Visha* proving its' antibacterial potential. In Ancient days, *Kataka* was used for water purification measures due its *ambuprasadana* & *Vishaghna* gunas, thus highlighting its antimicrobial property. This study suggests, that other drugs of *Vishaghna Gana* ie. *Haridra*, *Manjistha*, *Chandana* & *Slesmataka* which are having *krimighna*, *kandughna*, *kusthaghna* & *varnya* gunas can combat the Acne causing bacterium.

Figure 2: Graphical representation of the zone of inhibition and concentration of individual drugs and VG tablets

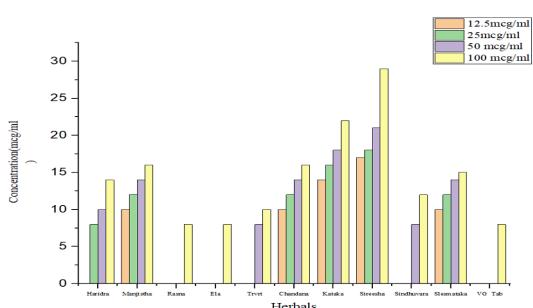


Table 3: Antibacterial activity represented by Zone of Inhibition (mm) produced by & Aqueous (Aq.) extracts of VG Choorna & Meth(methanolic) extract of VG Tab

	Test Drug	100% Conc.	50% Conc.	25% Conc.	12.5% Conc	Azithromycin (Control)
1	Meth. VG Tab	22 mm	15mm	14mm	R	34 mm
2	Aq. VG Tab	11 mm	R	R	R	23 mm

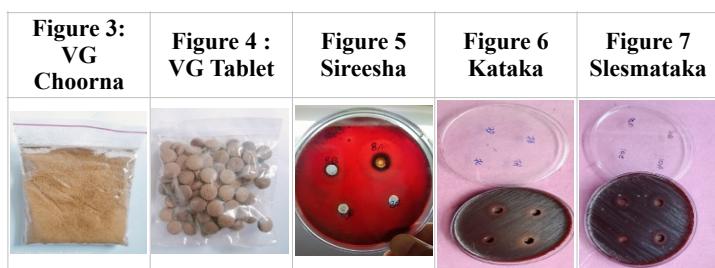
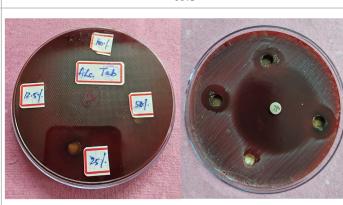


Fig.8 Aqueous extract of VG choorna



Fig.9 Methanolic extract of VG Tab



Discussion

The method that is outlined in the present research of the *Vishaghna Gana* tablets, blends classical Ayurvedic practice and the contemporary tabletting process. Incorporating a decoction (*kashaya*) and powdered drugs(*choorna*) is routine in Ayurveda. The evaluation criteria that is enumerated are in conformance with generic quality control examination for tablets, which include appearance, colour and odour etc. These are fundamental organoleptic attributes assisting with product conformity. The thickness evaluates the uniform thickness that contributes to packaging and dispensing requirements. The hardness of the tablet firmness is an essential factor that impacts disintegration and dissolution. The variation in hardness is reported as a range (3-3.5 kg/cm³). The reported hardness is an average with a standard deviation.

The pH value of the tablet can influence stability and dissolution of the drug. The disintegration time will determine how long it takes for the tablet to break down into smaller particles.(13) The average weight uniformity ensures that each tablet contains the correct drug dose. Weight variation is outside the usual acceptable range of $\pm 5\%$. It is necessary to identify the reason behind this variation and ensure uniformity for quality control. The friability tests the tablet's abrasion and breakage resistance during handling and transportation.(14) A friability of 1.0476% is above the acceptable level of less than 1%, which suggests tablet strength issues during handling and transportation. Total ash value and acid-insoluble ash value will indicate the purity of the herbal materials. Water-soluble and alcohol-soluble extracts determine how much material can be extracted from the tablets with water and alcohol. According to the parameters evaluated, the tablets appear to be of satisfactory quality. Nevertheless, it's imperative to overcome the issue of hardness and weight difference to provide a uniform product quality and comply with pharmacopeial requirements.

The *in vitro* anti-bacterial assay outcomes, indicate the therapeutic potential of various drugs of *Vishaghna Gana* for anti-acne treatments, focusing on *C.acnes*. The differences in the levels of inhibition among the drugs under consideration may be attributed to differences in concentrations of their bioactive compounds or modes of action. As per the present study, most of the individual drugs of *Vishaghna Gana* showed significant anti-bacterial activity against *C.acnes* with zone of inhibition sizes ranging from 10 to 29. They are *Haridra*, *Manjistha*, *Chandana* & *Slesmataka* with an active zone of inhibition. *Sireesha* & *Kataka* have shown astonishing anti-bacterial potential with very active zones of inhibition of 22mm & 29 mm. Because of concentration variations, the polyherbal formulation (VG Tab) exhibited no anti-bacterial activity. Further studies with higher concentrations, different extraction methods, or different solvents might be necessary to determine their anti-bacterial potential. As per research interest, further anti-bacterial assay was repeated with the aqueous as well as methanolic extract of VG Tab. in which,

methanolic extract of VG Tab showed significant zone of Inhibition.

Sireesha and *Kataka* are potent antibacterial drugs which validates their traditional application and presents a potential direction for future research. *Sireesha* is the best aushadha for *Visha*, and *Kataka* was utilised traditionally in water purification because of its antimicrobial activity. The moderate activity of *Haridra*, *Manjistha*, *Chandana*, and *Slesmataka* indicates that these drugs may play a part in an extended therapeutic action, perhaps treating other features of acne pathophysiology based on their *krimighna*, *kandughna*, *kustaghagna* & *varnya* gunas. The lack of activity of the polyherbal tablet formulation initially, when the individual components were active, is intriguing. This may be due to several factors, including the formulation's active compound concentration, which could have been too low to produce any observable effect. The various extracts could have interacted, which might have caused antagonism or decreased bioavailability of the active compounds. The initial extraction method to formulate the tablets may not have been the best to extract the active antibacterial compounds. The following discovery that aqueous extract of VG Choorna(polyherbal formulation) and methanolic extract of VG Tab revealed a large Zone of Inhibition implies that the extraction solvent is vital in getting the active anti-bacterial fractions. This puts in, the perspective for optimisation of extraction protocols to recover bioactive molecules in maximum quantities from herbal preparations. These results give a scientific rationale for the folk use of these medicinal plants in treating acne. Additional studies are needed to identify the bioactive compounds responsible for the anti-bacterial activity and investigate the mode of action of these compounds against *C. acnes*. Optimise the formulation of polyherbal products to maximise their antibacterial activity. Need to assess the *in vivo* efficacy and safety of these herbal extracts and formulations in the treatment of acne. Further investigating the therapeutic potential of these *Vishaghna Gana* medicines could make it feasible to design new, safe, and effective herbal remedies for acne vulgaris.

Conclusion

Our findings suggest that most of the ingredients of *Vishaghna Gana* possess high antibacterial potential against anaerobic microorganisms, *C. acnes*, and they can be used to treat *Mukhadooshika*(Acne vulgaris). The data obtained in these studies justify using such Polyherbal preparations in medical practice to combat Acne conditions. This study has demonstrated and evaluated the effectiveness of *Vishaghna Gana*, which has shown promising results. The findings may conclude that these results can open up new therapeutic options for acne treatment. There is a future scope of opting for *Vishaghna gana* selective drugs for new pharmaceutical preparations.

Conflict of Interest : The authors declare that there is no conflict of interest.

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