



COMPARATIVE EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF FRESH AND DRY *Vasa (Adhatoda vasica* Nees.) LEAVES AGAINST *Staphylococcus aureus*- IN-VITRO STUDY

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ABSTRACT

INTRODUCTION: Acharya Sharangadhara has mentioned the rule of taking the Dravyas in fresh or dry form. *Vasa* is one such drug advocated to be used in fresh form. So, to revalidate this concept Fresh and Dry *Vasa (Adhatoda vasica* Nees.) leaves were tested for antimicrobial activity against the *Staphylococcus aureus*.

METHODS: The coarse powder of fresh and dry *Vasa* leaves were prepared and both the samples were subjected and compared for Physico-chemical analysis and Hydro-alcoholic extracts (50% distilled water and 50% ethanol) of leaves were prepared, subjected and compared for Phytochemical screening with the standard Biochemical assays and antimicrobial assay against *Staphylococcus aureus* using Agar well diffusion method.

RESULTS & DISCUSSION: Both samples exhibited characteristic Pharmacognostic features. Loss on drying was 73.1% in fresh *Vasa* and 8.3% in dry *Vasa*. Water-soluble extractive values were 6% and 26%, while alcohol-soluble extractive values were 5% and 4%, respectively. The Hydro-alcoholic extract of both Fresh and Dry *Vasa* leaves showed the presence of Carbohydrates, Alkaloids, Tannins and Amino acids. Dry *Vasa* leaves showed the maximum zone of inhibition of average 10mm at 100 μ L concentration comparing to fresh *Vasa* leaves (6.4mm) in its antimicrobial activity against *Staphylococcus aureus*.

CONCLUSION: Dry *Vasa* leaves are more efficacious than the Fresh *Vasa* leaves in its antimicrobial activity against *Staphylococcus aureus* through Agar-well diffusion method.

KEYWORDS: Antimicrobial activity, Hydro-alcoholic extract, *Staphylococcus aureus*, *Vasa (Adhatoda vasica* Nees.).

INTRODUCTION

The understanding of the state of the *Dravya* (drug) being used is vital since it plays a major role in the biochemical composition and medicinal properties of a drug, thereby influencing the quality of the product. Acharya Sharangadhara mentions the principles for the selection of *Dravyas* (drugs) in either fresh or dry form. *Vasa (Adhatoda vasica* Nees.) is one among the *Dravya* mentioned by Acharya Sharangadhara, which should always be used in a fresh state, and the quantity should not be doubled. In any preparation there is a rule of taking fresh drugs in double the quantity if there is an unavailability of dry drugs. This rule is also an exception for this drug.⁽¹⁾

Adhatoda vasica Nees, commonly referred to as *Vasa* or *Vasaka*, belonging to the family *Acanthaceae* holds a prominent place as an herbal remedy in the *Ayurvedic* and Unani medicinal systems.⁽²⁾ *Vasa* is an evergreen under shrub and been used to treat various conditions such as coughs, colds, pneumonia, fevers, jaundice, and asthma due to its expectorant and antispasmodic actions. The plant's vital role in treating respiratory disorders is emphasized by an old Indian saying: "As long as the *Vasa* plant exists, no person suffering from phthisis should lose hope".⁽³⁾

Staphylococcus aureus is a Gram-positive, spherically shaped bacterium, a member of the family *Bacillota* and is a usual member of the microbiota of the body.⁽⁴⁾ It is detected in 30% of the population, mostly as a nasal commensal, but also in the throat, on the skin and in the gastrointestinal tract. In addition to these innocuous interactions with the human host, *Staphylococcus aureus* has the potential to develop a wide range of diseases in humans, from mild infections of skin and soft tissues to severe and fatal infections, such as bacteraemia and pneumonia.⁽⁵⁾ *Staphylococcus aureus* expresses a wide repertoire of surface proteins that recognize cellular adhesive molecules and it is therefore able to adhere and internalize in lung epithelial cells, which protects the bacteria from host immune system and facilitate chronic infections of respiratory system.⁽⁶⁾

Although, *Vasa* is traditionally recommended in fresh form according to Acharya Sharangadhara, comparative experimental evidence evaluating the antimicrobial activity of fresh and dried *Vasa* leaves were carried out under standardized conditions

MATERIALS & METHODS

STUDY AREA:

The study was conducted at Sri Dharmasthala Manjunatheshwara Centre for Research in Ayurveda and Allied Sciences, Kuthpady, Udipi, Karnataka.



PLANT MATERIAL

Collection of Leaves

The Dry sample of Vasa patra was collected from the Herbal garden of Sri Dharmasthala Manjunateshwara College of Ayurveda and Hospital, Hassan on 24th April 2025. The Fresh sample of Vasa patra was collected from the Herbal garden of Sri Dharmasthala Manjunateshwara College of Ayurveda, Udupi on 3rd August 2025.

Preparation of Crude Extracts

Fresh and dried *Vasa* leaves were separately processed and reduced to coarse powders. For each sample, an accurately weighed quantity of 5 g of the respective coarse powder was transferred into separate conical flasks. Hydro-alcoholic extraction was carried out using a solvent system consisting of distilled water and ethanol in equal proportions (25 mL each). The mixtures were subjected to continuous agitation on a Spinex orbital shaker at 16 rpm for 24 h at room temperature. After completion of the extraction period, the contents were filtered through Whatman filter paper, and the filtrates were collected in pre-weighed china dishes. The solvents were evaporated on a hot water bath maintained at 105°C until complete dryness. The dried extracts were reweighed to determine the extractive yield, transferred into appropriately labelled vials, and stored under suitable conditions for subsequent experimental analysis.

PRELIMINARY PHYTOCHEMICAL ANALYSIS: ⁽⁷⁾

Tests for Carbohydrates

1. **Fehling's test:** Mix 1ml Fehling's A and Fehling's B solutions, boil for 1 min. Add equal volume of test solution. Heat in boiling water bath for 5-10mins. First yellow, then brick red ppt is observed.
2. **Benedict's test:** Mix equal volume of Benedict's reagent and test solution in Test tube. Heat in boiling water bath for 5 mins. Solution appears green, yellow or red depending on amount of reducing sugar present in test solution.

Tests for Steroids

1. **Salkowski reaction:** To 2ml of extract, add 2ml chloroform and 2ml conc. H_2SO_4 . Shake well. Chloroform layer appears red and acid layer shows greenish yellow fluorescence.
2. **Liebermann's reaction:** Mix 3ml extract with 3ml acetic anhydride. Heat and cool. Add few drops of conc. H_2SO_4 . Blue colour appears.

Tests for Glycosides

1. **Borntragers's test for anthraquinone glycosides:** To 3ml extract. Add dil. H_2SO_4 , Boil and filter. To cold filtrate, add equal volume benzene or chloroform. Shake well. Separate the organic solvent. Add ammonia, ammoniacal layer turns pink or red.

Tests for Saponin

1. **Foam Test:** Shake the drug extract or dry powder vigorously with water. Persistent stable foam observed.

Tests for Flavonoids

1. **H_2SO_4 Test:** To small quantity of residue, add lead acetate solution. Yellow coloured precipitate formed.

Tests for Tannins

To 2-3ml of aqueous or alcoholic extract, add few drops of following reagents:

1. **5% $FeCl_3$ solution:** Deep blue-black colour.
2. **Lead acetate solution:** White precipitate.

Tests for Alkaloids

Evaporate the aqueous, alcoholic and chloroform extracts separately. To residue, add dilute HCl, Shake well and filter. With filtrate, perform following tests:

1. **Drangendroff's test:** To 2-3ml filtrate, add few drops of Drangendroff's reagent. Orange brown precipitate is formed.
2. **Mayer's test:** To 2-3ml filtrate, add few drops of Mayer's reagent, gives Cream/White precipitate.
3. **Wagner's test:** To 2-3ml filtrate, add few drops of Wagner's reagent, gives reddish brown precipitate.

Amino Acids

1. **Ninhydrin Test:** Heat 3ml test solution and 3 drops 5% Ninhydrin solution in boiling water bath for 10min. Purple or bluish colour appears.



ANTIMICROBIAL ASSAY

Test Organism

Standard strains of *Staphylococcus aureus* (MTCC 3160) were procured from Microbial Type Culture Collection and Gene Bank (MTCC 3160), IMTECH, Chandigarh. A loopful of organisms from the slants was transferred to nutrient agar slant for further use.

Standard Antibiotics

Ampicillin (5mg/500µl) having a broad-spectrum property was used as a Standard drug.

Inoculum Preparation

The work place cleaned in laminar airflow using 70% ethyl alcohol and UV for 20 minutes. Loopful of 24hrs old culture from the slants was transferred to sterile saline and mixed well to prepare a homogenous inoculum.

Agar-Well Diffusion Method

Susceptibility tests were performed by agar well diffusion method using nutrient agar media. Initially nutrient agar media is prepared by adding Beef extract (1 g), Yeast extract (2 g), Peptone (5 g) and Sodium Chloride (5 g) 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, 15 g agar was added to the media and autoclaved at 121°C for 15 minutes. Then the media was cooled to around 45-55°C, around 20ml each was poured into sterile petriplates. One ml of the inoculum was immediately added to the plate, swirled for uniform distribution. Wells were bored using a sterile borer. On each plate, six equidistant wells (one in the centre and five wells at the corner) were made. Four of the holes were aseptically filled with 100 µL of different concentrations of each plant extracts (i.e. 25 mg/ mL, 50 mg/mL, 75 mg/mL and 100 mg/mL) sequentially, and at the centre, Ampicillin was added as a standard drug. The Hydro-alcohol (50% Distilled water & 50% Ethanol) was taken as a control and amount of control was same as the tested sample on the wells. The petriplates were incubated overnight at 37°C and observed after 24 h. At the end the inhibition zones formed were measured to the nearest millimetres and the experiment was performed in duplicate.

Statistical Analysis

Descriptive statistics was used. The data was represented as Mean and Compared.

RESULTS

PHYSICO-CHEMICAL ANALYSIS

Table no.1: Physico-Chemical Analysis of Fresh and Dry *Vasa* leaves

Qualitative tests	Fresh <i>Vasa</i> leaves	Dry <i>Vasa</i> leaves	API Standards
Loss on Drying	73.1%	8.3%	-
Foreign matter	0%	0%	Not more than 2%
Total Ash	9%	9%	Not more than 21%
Acid insoluble Ash	0%	0%	Not more than 1%
Water extractive	6%	26%	Not less than 22%
Alcohol extractive	5%	4%	Not less than 3%

PRELIMINARY PHYTOCHEMICAL ANALYSIS

The Hydro-alcoholic extract of both Fresh and Dry *Vasa* leaves showed the presence of Carbohydrates, Alkaloids, Tannins and Amino acids, And were absent for Saponin, Flavonoids, Steroids and Glycosides.

Table no.2: Phytochemical analysis of Hydro-alcoholic extract of Fresh and Dry *Vasa* leaves

Tests	Fresh <i>Vasa</i> Leaves	Dry <i>Vasa</i> Leaves	Positive Observations
1) Carbohydrates			
• Fehling's test	+	+	Brick red ppt
• Benedict's Test	+	+	Green
2) Alkaloids			
• Dragendorff's Test	+	+	Orange brown
• Mayer's Test	-	-	-
• Wagner's Test	-	-	-
3) Tannins			
• 5% FeCl ₃ solution	+	+	Blue / black
• Lead Acetate Solution	+	+	White ppt

4) Steroids			
• Libermann's Reaction	-	-	-
• Salkowski Reaction	-	-	-
5) Saponins			
• Foam Test	-	-	-
6) Flavonoids			
• H ₂ SO ₄ Test	-	-	-
7) Glycosides			
• Borntrager's Test	-	-	-
8) Amino acids			
• Ninhydrin solution	+	+	Purple

+ :Present; - :Absent

ANTIMICROBIAL ASSAY

The hydro-alcoholic extract of fresh *Vasa* leaves demonstrated antimicrobial activity only at the highest tested concentration, producing a mean zone of inhibition of 6.46 mm at 100 µL, while no inhibitory zones were observed at 25, 50, and 75 µL. In contrast, the hydro-alcoholic extract of dried *Vasa* leaves exhibited concentration-dependent antimicrobial activity at higher doses, showing a mean zone of inhibition of 9 mm at 75 µL and a maximum mean zone of inhibition of 10 mm at 100 µL. No zone of inhibition was detected at 25 and 50 µL concentrations for the dried sample. The hydro-alcoholic solvent control did not exhibit any zone of inhibition, whereas the standard drug, ampicillin, produced a mean zone of inhibition of 8.47 mm. On comparing antimicrobial activity of both Fresh and Dry *Vasa* leaves, Dry showed maximum zone of inhibition of organism.

Table no.3: Zone of inhibition against *Staphylococcus aureus* of Dry and Fresh *Vasa* leaves extract

Sample	Volumes	Zone of inhibition (Radius in mm)		Average
Hydro-alcoholic extract of Dry <i>Vasa</i> leaves(100 mg/ml)	25 µl	0	0	0
	50 µl	0	0	0
	75 µl	9	9	9
	100 µl	10	10	10
Hydro-alcoholic extract of Fresh <i>Vasa</i> leaves(100 mg/ml)	25 µl	0	0	0
	50 µl	0	0	0
	75 µl	0	0	0
	100 µl	6	7	6.5
Internal Control (Hydro-alcohol)	50 µl	0	0	0
Standard (Ampicillin) 5 mg/500µl	25 µl	9	8	8.5

Data are expressed as mean.

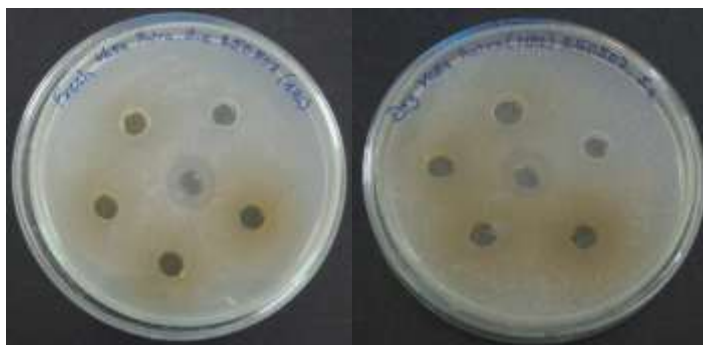


Figure 1: Petri plates of Hydro-alcoholic extract of Fresh and Dry *Vasa patra* showing Zone of Inhibition of *S. aureus*

Statistical Analysis

The data was represented as Mean and Compared. Descriptive statistics was used.

**Table No- 4: Result of Comparative Antimicrobial Activity of Hydro-alcohol extract of Fresh and Dry *Vasa* leaves against *Staphylococcus aureus*.**

Concentration (in μL)	Fresh <i>Vasa</i> leaves (Radius in mm)	Dry <i>Vasa</i> leaves (Radius in mm)	Interpretation
25	0	0	No Activity
50	0	0	No Activity
75	0	9	Activity Showed only in Dry
100	6.5	10	Higher Activity in Dry compared to Fresh
Control-Hydro-alcohol (Ethanol+ D.W)-50 μL	0	0	No Activity
Standard- Ampicillin (5mg/500 μL)-25 μL	8.5	8.5	Equal Activity in both sample

DISCUSSION

The present study was undertaken to examine an important practical question in *Ayurvedic* pharmacology: whether the fresh and dry forms of *Vasa* exhibit comparable biological activity. The rationale arises from the classical instruction of *Sharangadhara* that *Vasa* is among the drugs that should be used in the fresh state. The present experimental findings provide an opportunity to examine this classical drug-form principle through a modern anti-microbial model.

The physicochemical findings demonstrated a substantial difference in loss on drying between fresh and dry samples. Fresh *Vasa* showed 73.1% loss on drying, whereas dry *Vasa* showed only 8.3%. This difference primarily reflects the moisture content of the respective plant materials. The lower moisture content of dried plant material can also influence the relative concentration of extractable constituents and extraction efficiency. Also, there is a marked difference between the water-soluble extractive values. Dry *Vasa* demonstrated 26% water-soluble extractive compared with 6% in fresh *Vasa*. The phytochemical screening demonstrated carbohydrates, alkaloids, tannins and amino acids in both extracts.

The antimicrobial assay revealed that Fresh *Vasa* showed measurable activity only at 100 μL , whereas dry *Vasa* demonstrated activity at both 75 and 100 μL . At 100 μL , dry *Vasa* produced a 10-mm zone compared with 6.5 mm for fresh *Vasa*. The difference in anti-microbial activity of fresh and dry *Vasa* is may be due to the moisture content and solubility of phytoconstituents in different forms. As moisture content is less & extractive value is high in dry sample attributed for its higher anti-microbial activity compared to fresh form.

The probable antimicrobial action of *Vasa* (*Krimighna karma*) may be attributed to its *Tikta rasa*, *Laghu guna*, and *Kapha-pitta shamaka* properties. *Tikta rasa* and *Laghu guna* help inhibit *Krimi* growth, while *Kapha shamaka* action further contributes to controlling microbial proliferation.⁽⁸⁾⁽⁹⁾⁽¹⁰⁾ Phytoconstituents such as alkaloids, tannins, and amino acids may support this activity by disrupting microbial enzymes and cell membranes, inhibiting protein synthesis, and inducing reactive oxygen species (ROS).⁽¹¹⁾⁽¹²⁾

CONCLUSION

The present comparative in-vitro investigation demonstrated that both fresh and dry *Vasa* (*Adhatoda vasica* Nees.) leaves possess detectable antimicrobial potential against *Staphylococcus aureus* under the tested conditions. while preliminary phytochemical screening demonstrated carbohydrates, alkaloids, tannins and amino acids in both hydro-alcoholic extracts.

The principal finding was that the dry *Vasa* extract exhibited greater antimicrobial activity than the fresh extract. At 100 μL , the dry extract produced a mean inhibition zone of 10 mm compared with 6.5 mm for the fresh extract, while inhibition was detectable with dry *Vasa* even at 75 μL . The negative control produced no inhibition.

Thus, although classical *Ayurveda* recommends *Vasa* in the fresh state, the present experimental findings indicate that the dried leaf form retains antimicrobial activity and, under the specific conditions of this study, demonstrated greater inhibition of *Staphylococcus aureus*. This observation does not invalidate the classical recommendation for fresh *Vasa*, because the classical rule encompasses broader therapeutic considerations than the single antimicrobial endpoint investigated here. Rather, the study suggests that the biological activity of a medicinal plant may vary according to its physical form, time & place of collection and extraction methods.

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