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## Research Article

### STANDARDIZATION OF HERBAL DRUGS: VACHA (*ACORUS CALAMUS* LINN.) AND DVIPANTARA VACHA/CHOPCHINI/CHINA ROOT (*SMILAX CHINA* LINN.) WITH THEIR IN-VITRO ANTIMICROBIAL POTENTIAL

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#### ABSTRACT


*Ayurveda* is a part of the "science of life," which is the study of traditional medicinal plants that are used and found worldwide to treat and prevent various illnesses. There is increasing awareness and general acceptability of the use of herbal drugs in today's modern practice. Around 80% of the world's population relies on herbal treatments and products for health, despite their unconventional uses. This growth in the usage; abuse and adulteration of herbal products has also resulted in consumers and manufacturers being disappointed, and in extreme cases, the repercussions are lethal. Standardization of herbal medicines is the process to ensure the efficacy and safety of medicinal plants. *Vacha* (*Acorus calamus* Linn.) and *Dvipantara vacha/ Chopchini/ China root* (*Smilax china* Linn.) are among the four varieties of *Bhavaprakash Nighantu*. Both drugs are used in disorders like epilepsy. Plants contain a huge diversity of chemical compounds known as secondary metabolites. This study investigates the phytochemicals and in-vitro antimicrobial potential of *Vacha* and *Dvipantara vacha*; particularly focusing on their antibacterial and antifungal activities against specified bacteria and fungi namely *Staphylococcus aureus* and *Candida albicans*.

#### INTRODUCTION

Standardization of herbal medicines is the process of prescribing a set of standards or inherent characteristics, constant parameters along with definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility. It is the process of developing and agreeing upon technical standards. Specific standards are worked out by experimentation and observations, which would lead to prescribing a set of characteristics exhibited by the particular herbal medicine. Hence, standardization is a tool in the quality control process.

Several problems not applicable to synthetic drugs often influence the quality of herbal drugs. For instance:

1. Herbal drugs are usually mixtures of many constituents.
2. The active principle(s) is (are), in most cases unknown.
3. Selective analytical methods or reference compounds may not be available commercially.
4. Plant materials are chemically and naturally variable.
5. Chemo varieties and chemo cultivars exist.
6. The source and quality of the raw material are variable.
7. Methods of harvesting, drying, storage, transportation, and processing (for example, mode of extraction and polarity of the extracting solvent, instability of constituents, etc.) also affect herbal quality.<sup>[1]</sup>

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Ayurveda is the world's most comprehensive system of spiritual teachings and the "oldest medical system" that have ever encountered. Over the ages, many people have accumulated priceless information that around 70% of medications come from natural sources. *Vacha* (*Acorus calamus* Linn.) means the drug that improves speech and memory, commonly known as 'sweet flag' of the family Araceae, is a semi-aquatic, perennial, aromatic herb with creeping rhizomes, sword-shaped leaves, spadix inflorescence and grows either as a wild or cultivated crop throughout the India ascending upto 1800m in the Himalayas.<sup>[2]</sup> *Dvipantara vacha* (*Smilax china* Linn.) means the drug that has been imported from *Dvipantara desha* like Indonesian islands, China, and Japan to India which is beneficial in *Phiranga roga* and commonly known as *Chopchini* in Hindi, *Dvipantara vacha* in Sanskrit and China root in English.<sup>[3]</sup> Due to a vast range of chemical compounds and biological activities, pharmaceutical industries rely heavily on plant secondary metabolites to discover new compounds with novel biological characteristics; often obtained from their original plant matrices by phytochemical screening, extraction, isolation, and spectral analysis.<sup>[4]</sup> Micro-organisms are the smallest unit of life and are too small to be seen by the naked eye, which can be seen only with help of magnification especially the microscope. Micro-organisms include bacteria, viruses, protozoa, algae, fungi and other microbes. *Staphylococcus aureus* also known as "golden staph" is a gram-positive bacteria and can cause local infections of the skin, nose and gastrointestinal tract.<sup>[5]</sup> *Candida albicans* is an opportunistic dimorphic fungus that can cause disease in the human body. It is the natural flora of the skin, oral, GIT, vagina, and the urinary tract.<sup>[6]</sup> Phytochemical and antimicrobial analysis in numerous scientific domains particularly in pharmacology and chemistry is required for physiological and biochemical responses. Roots of *Vacha* and *Dvipantara vacha* are utilized in various therapeutic contexts but their comparative phytochemical analysis and antimicrobial assay against *S. aureus* and *C. albicans* relative to reference drugs such as gentamycin and fluconazole respectively have not been thoroughly investigated. This study aims to address these gaps by identifying and quantifying the phytochemical compounds present in root extracts, providing insights into their chemical composition, and assessing the antimicrobial assay to better understand their medicinal efficacy.

## AIM AND OBJECTIVES

1. To evaluate phytochemical standards from the root part of *Vacha* and *Dvipantara vacha* extracts.
2. To evaluate and compare antimicrobial action among *Vacha* and *Dvipantara vacha* extract.

## MATERIALS AND METHODS

### Sample Collection and Authentication

The fresh roots of *Vacha* and *Dvipantara vacha* were cultivated and collected under the supervision of Guide and Co-guide of Dayanand Ayurvedic College, cleaned in a basin with tap water allowed to dry naturally without mold growth, kept out of direct sunlight, and then ground into a powder using a grinder.

### Preparation of Hydro-alcoholic extract<sup>[7]</sup>

100gm. coarsely powdered root samples of *Vacha* and *Dvipantara vacha* each was macerated for 48 hours at room temperature with stirring in the aqueous and ethanol medium as solvent simultaneously known as hydro-alcoholic extract and the supernatant was collected. The remaining residuals were re-soaked and the previous steps were repeated. All the macerates obtained were evaporated in an oven to calculate the yield of each plant extract and was stored in sealed glass vials in a refrigerator at 4-5°C.

**%yield hydro-alcoholic extract = (Weight of extract/ Powder taken for extraction) X 100**

**Methodology:** Screening of the preliminary phytochemicals both qualitatively and quantitatively and the antimicrobial effect of *Vacha* (*Acorus calamus* Linn.) and *Dvipantara vacha* (*Smilax china* Linn.) was done at Drug Testing Laboratory (DTL), dist. Patiala and Herbal Health Research Consortium (HHRC), Amritsar, India.

### A. Phytochemical Analysis

Standard methods were used to identify and measure chemical constituents such as alkaloids, tannins, saponins, phenolic compounds, etc both qualitatively and quantitatively.

### B. Antimicrobial Study

Standard methods were used to identify and measure Zone of Inhibition (ZOI) of antibacterial and antifungal activity against gram-positive bacteria *Staphylococcus aureus* and fungus *Candida albicans* by Agar Diffusion Method further categorized into Cavity pour Method.

**Features of the strains to be studied:** Pathogens came from the CSIR- Institute of Microbial Technology in Chandigarh, India's Microbial Type Culture Collection & Gene Bank (MTCC) i.e., bacteria *S. aureus* (MTCC 902) and fungus *C. albicans* (MTCC 227).



Fig. 1 ZOI showing antibacterial action

Fig. 2 ZOI showing antifungal action

**OBSERVATIONS AND RESULTS**

**Table 1: Percentage yield value of extracts of Vacha and Dvipantara vacha**

S.No.	Sample	Powder taken for extraction (in gm)	Solvent [Water + Ethanol (95%)]	Wt. of petri dish (in gm.)	Wt. of Extract (in gm.)	% yield of Hydro-alcoholic extract
1.	Vacha	100.30	900ml	42.44	33.89	33.788
2.	Dvipantara vacha	100.05	900ml	46.79	36.79	36.771

**Table 2: Phytochemical analysis of Vacha and Dvipantara vacha root qualitatively**

S.No.	Materials	Functional groups	Test Applied	Appearance	Results (Vacha)	Results (Dvipantara vacha)
1.	Phyto-chemical tests performed in Hydro-alcoholic extract	Alkaloids	Mayer's reagent	Creamy or white precipitates	Present	Present
2.		Tannins	Dil. FeCl <sub>3</sub>	Blue color	Present	Present
3.		Glycosides	Keller Killiani test	Red dense precipitates	Present	Present
4.		Flavonoids	HCl test (Shinoda test)	Pink to magenta color	Present	Present
5.		Phenols	FeCl <sub>3</sub>	Dark purple	Present	Present
6.		Steroids	Liebermann-Burchard reaction	Bluish green color	Present	Present
7.		Saponin	Foam test	honeycomb like froth	Present	Present
8.		Proteins	Biuret test	Violet color	Present	Present
9.		Carbohydrates	Molish Test	Red-violet ring	Present	Present
10.		Reducing sugar	Fehling test	Brick red precipitate	Present	Present
11.		Amino acids	Ninhydrin test	Purple or bluish color	Present	Present
12.		Starch	Iodine Test	Blue color	Present	Present

**Table 3: Phytochemical analysis of *Vacha* and *Dvipantara vacha* root quantitatively**

S.No.	Quantitative Parameters	<i>Vacha</i>	<i>Dvipantara vacha</i>
1.	Alkaloids	0.68%	0.56%
2.	Tannins	0.16%	0.11%
3.	Phenols	0.38%	0.35%
4.	Saponin	12.6%	8.3%
5.	Stigmasterol	0.19%	0.27%

**Table 4: Antibacterial study of *Vacha* and *Dvipantara vacha* root extract**

S. No.	Micro-organisms	Zone of Inhibition (ZOI)		
		Hydroalcoholic extract of <i>Vacha</i>	Hydroalcoholic extract of <i>Dvipantara vacha</i>	Gentamycin
1.	DMSO	6mm	13mm	
2.	<i>Staphylococcus aureus</i>	12mm	14mm	24mm

**Table 5: Antifungal study of *Vacha* and *Dvipantara vacha* root extract**

S.No.	Microorganisms	Zone of Inhibition (ZOI)		
		Hydro-alcoholic extract of <i>Vacha</i>	Hydro-alcoholic extract of <i>Dvipantara vacha</i>	Fluconazole
1.	DMSO	05mm	05mm	
2.	<i>Candida albicans</i>	15mm	13mm	22mm

## DISCUSSION

Phytochemical analysis reveals that root extracts of both plants contain several important functional groups indicating their potential medicinal properties such as alkaloids known for their analgesic, anti-inflammatory and antimicrobial properties. Glycosides mainly are bitter in taste and water-soluble compounds, insoluble in organic solvents and possess cardiac stimulant and antibacterial properties. Flavonoids exhibit anti-inflammatory, antioxidant, and anticancer properties. Phenols are known as immuno-stimulants and tannins help in wound healing and show anti-inflammatory actions. Saponins are known for their therapeutic effects such as immuno-stimulant, cleansing agents and antimicrobial activity. Steroids possess medicinal and pharmaceutical activities such as anti-tumor, immunosuppressive, hepato-protective, antibacterial, anti-helminthic, cytotoxic, and cardio-tonic activity. Quantitatively *Vacha* exhibits higher alkaloids 0.68%, tannin 0.16%, Phenol 0.38%, and Saponin 12.6% suggesting analgesic, anti-inflammatory, antimicrobial, immune-stimulant, wound healing and act as better cleansing agents than *Dvipantara vacha*. Conversely, *Dvipantara vacha* has higher stigmasterol 0.27% than *Vacha* may be beneficial as an anti-tumor, immunosuppressive, anti-inflammatory, hepato-protective, antibacterial, anti-helminthic, cytotoxic, and cardio-tonic properties.

Antimicrobial analysis exhibits that both the root extracts of drugs have positive action against the specified bacteria *Staphylococcus aureus* and fungus *Candida albicans* but in comparison to the zone of inhibition of standard medicines, namely gentamycin (24mm), and fluconazole (22mm), exhibited low grade antibacterial and antifungal activity against *S. aureus* and *C. albicans*. An interpretation was based on the anti-bacterial action of the hydro-alcoholic extract of *Vacha* on *S. aureus* reveal a minimum inhibition zone of 12 mm as compared to *Dvipantara vacha* i.e., 14mm. An anti-fungal action of hydro-alcoholic extract of *Dvipantara vacha* on *C. albicans* showed a minimum inhibition zone of 13mm than *Vacha* i.e., 15mm. It was shown that there were differences in sensitivity based on its zone of inhibition.

## CONCLUSION

Researchers face significant difficulty in developing robust analytical techniques for profiling phytochemical composition including quantitative assessment of markers/bioactive chemicals and other important ingredients. Standardization is an important step in establishing a standard biological activity, chemical profile or quality assurance program for herbal medication manufacture and production. WHO-specific criteria for assessing safety, effectiveness, and quality of herbal medicines are critical for worldwide standardization. In this

paper, the researcher attempted to establish the phyto-pharmacological standardization of the famous Ayurvedic medicinal plant extracts of *Vacha* (*Acorus calamus* Linn.) and *Chopchini/ China root* (*Smilax china* Linn.) with their antibacterial properties. The study is open for further exploration at the clinical level to validate and authenticate the results.

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