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Research Article

IN-VITRO STUDY OF *VASA* AND *KANTAKARI* AGAINST *STAPHYLOCOCCUS AUREUS* BY SPUTUM CULTURE AND SENSITIVITY IN *KAPHAJA KASA* (CHRONIC BRONCHITIS)

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ABSTRACT

Now a day clinical conditions pop up relating with Respiratory tract infections is at peak level; use of diagnostic tools like culture and sensitivity, to identify causative microorganism, its characteristics, culture these organism *in vitro* and check sensitivity against *Vasa* and *Kantakari*. *Vasa* (*Adhatoda vasica*) and *Kantakari* (*Solanum xanthocarpum*) are indicated in *kasa* and said to possess *krimighna* property. Hence, adoption of new approaches like Culture and sensitivity methods would strengthen existing *Ayurvedic* knowledge and help in achieving improved diagnostic and curative abilities. Therefore, present study is undertaken to study different features of the micro-organism *Staphylococcus aureus*, its laboratory diagnosis, its culture and assess *Upashaya* capability *in vitro* by sensitivity with south Indian grown *Vasa* and *Kantakari*. Preliminary phytochemical assay of drugs revealed that the presence of various chemical constituent which creates defence mechanism against *Staphylococcus aureus*. The present study aims to compare antibacterial activity of *vasa* and *kantakari* against *staphylococcus aureus* by sputum culture and sensitivity method in *kaphajakasa* (chronic bronchitis). Patients of *kaphajakasa* were subjected for urine culture and those with positive result for *Staphylococcus aureus were* further used. Alcoholic extract of *Vasa* and *Kantakari* was prepared by Soxhlet method further sensitivity test was performed by Agar well diffusion method and zone of inhibition was measured. On comparing the same concentrations of alcoholic extracts of *Vasa* and *Kantakari*, it was observed that *Vasa* is having better antibacterial action than *Kantakari* against *Staphylococcus aureus* in *Kaphaja Kasa* (Chronic Bronchitis).

Keywords: *Kaphaja Kasa*, Chronic bronchitis, *Staphylococcus aureus*, Sputum culture and sensitivity, Alcoholic extract of *Vasa*, Alcoholic extract of *Kantakari*

INTRODUCTION

Kaphaja Kasa, characterized by expulsion of large content of sputum, with very little effort of cough to expectorate, vomiting of thick dense mucus, nasal discharge and tendency of vomiting due to excessive accumulation of *kapha* in *Srotas* are amassed in the concern condition[1]. The description of chronic bronchitis simulates with the description of *Lakshanas* of *Kaphaja Kasa*[2]. Now a day, clinical conditions pop up relating with Respiratory tract infections is at peak level. The foremost cause of bronchi infections arises by an account of gram-positive bacteria. In that, prior position is carried up by *Staphylococcus aureus.*

Vasa[3] (*Adhatoda vasica*) and *Kantakari*[4] (*Solanum xanthocarpum*) are indicated in *kasa* and said to possess *krimighna* property. Hence, adoption of new approaches like Culture and sensitivity methods would strengthen existing *Ayurvedic* knowledge and help in achieving improved diagnostic and curative abilities. Therefore, present study is undertaken to study various attributes of the micro-organism *Staphylococcus aureus*, its laboratory diagnosis, its culture and evaluate *Upashaya* capability *in vitro* by sensitivity with south Indian grown *Vasa* and *Kantakari.*

Objectives of the study

To compare the sensitivity of *Vasa* and *Kantakari* against *Staphylococcus aureus* by sputum culture and sensitivity method in *Kaphaja Kasa* (Chronic Bronchitis)

MATERIALS AND METHODS

A minimum of 30 sputum samples from patients fulfilling diagnostic and inclusion criteria was included for study from OPD and IPD of Sri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, Hassan; other referrals and special camps. Study was approved by Institutional ethical committee and study was carried out as per the ethical standards approved in study.

Methods of collection of data

Early morning thick sputum samples were collected from the subjects fulfilling the diagnostic and inclusion criteria suffering from *Kaphaja Kasa.*

Diagnostic criteria

Patients complaining of productive cough with thick, dense expectorate associated with one or more symptoms of *Kaphaja Kasa*[5].

1. *Bahalammadhuramkapham*
2. *Saandhra, Ghana kapham*
3. *Vakshasampurnaevamanyate*
4. *Utklesha*
5. No pain in chest while coughing

Inclusion criteria

1. Patients between the age of 16 – 60 years
2. Patients fulfilling the diagnostic criteria
3. Patients Irrespective Of Gender, Caste

Exclusion criteria

1. Patients suffering from other systemic illness
2. Other types of *Kasa*

Research design

An observational experimental study

Methodology

Early morning thick sputum sample was collected. Transferred the inoculum on MacConkey's and Blood agar plate and culturing was done by Streak culture method. Then subjected to gram staining and further serological and biochemical test were performed for the identification of *Staphylococcus aureus*. Then antibacterial assay of *Vasa* and *Kantakari* against *S. Aureus* were carried out by Agar well diffusion method.

Drug collection

Vasa leaf and *Kantakari* whole plant was collected from an authenticated shop. It was cleaned and dried and powdered, a coarse powder was prepared. It was stored in a clean and air tight container.

Authentication of the drug

The authentication of the all the raw drugs was done at the Department of *Dravyaguna*, in Shri Dharmasthala Manjunatheshwara College of Ayurveda, Hassan

Extract preparation

Hot extraction by Soxhlet method is adopted for the preparation of both extract.

Agar well diffusion method

Antibacterial Sensitivity study was done by cork borer well diffusion method. 2 Petri dishes are separately used for *Vasa* and *Kantakari*. The bacterial inoculum was swabbed by lawn culture technique and bore was made with the help of sterilized cork borer, 7 wells are created for different concentrations of alcoholic extracts of *Vasa* and *Kantakari* (20, 10, 5, 2.5, 1.25 and 0.625 µl). Ampicillin (10 µg) was used as standard. Six wells were charged with different concentrations of drug extract and one filled with standard, incubated at 37ºC in an incubator for 24 hours. After incubation, zone of inhibition is measured with ruler and the results will be tabulated.[6]

Analytical parameter

The disc diffusion study will be measured by following zones.

a. Sensitive (S) zone
b. Moderately sensitive (MS) zone
c. Resistant (R) zone

Observation and Result

Pharmaceutical study

- Preparation of Alcoholic extract of Vasa
- Preparation of Alcoholic extract of *Kanatakari*

Table 1: Preparation of Alcoholic extracts of *Vasa* and *Kantakari*

Drug	Day	Content	Time	Temperature	Quantity obtained
Vasa	18-06-2018 /Friday	Coarse powder of *Vasa* leaf – 50 gm Ethanol – 500 ml	9. 30 am – 04:00 pm	60◦C-90◦C	4.45gm
Kantakari	19-06-2018 / Saturday	Coarse powder of dried *Kantakari*– 50 gm, Ethanol – 500 ml	09:55 am – 04:00 pm	60◦C-90◦C	4.45gm

Table 2: Details of organisms screened

Particulars	No. of patients
Included	30 (*Staphylococcus aureus*)
Excluded	20 (*Escherichia coli, Streptococcus, pseudomonas* and age above 60 years)
Total (Screened)	50

In the present study, on the basis of diagnostic and inclusion criteria 30 patients of *Kaphaja Kasa* were selected. The sputum samples were collected and subjected to culture for the isolation of *Staphylococcus aureus* and was subjected to antibacterial assay with 6 different concentrations of alcoholic extract of both *Vasa* and *Kantakari*. All the data was recorded in well-designed case proforma. Among 50 patients of *Kaphaja Kasa* 30 samples contained *Staphylococcus aureus* thereby included for the study and 20 samples contained *Escherichia coli, Streptococcus, Pseudomonas* and age above 60 years there by excluded.

Table 3: Observation on sensitivity shown by *Staphylococcus aureus* to different concentrations of alcoholic extract of Vasa

Extract	ZOI in mm against *Staphylococcus aureus*	20 µl N = 30		10 µl N = 30		5 µl N = 30		2.5 µl N = 30		1.25 µl N = 30		0.625 µl N = 30	
		F	%	F	%	F	%	F	%	F	%	F	%
Alcoholic extract of Vasa	0	21	70	18	60	18	60	13	43.3	12	40	14	46.7
	10	-	-			1	3.3						
	12												
	14												
	16			1	3.3			2	6.7	2	6.7		
	18	2	6.7	2	6.7	1	3.3	1	3.3	1	3.3	1	3.3
	20			4	13.3	3	10	4	13.3	5	16.7	3	10
	22	1	3.3	2	6.7	1	3.3	3	10	4	13.3	4	13.3
	24	2	6.7	2	6.7	3	10	4	13.3	3	10	6	20
	26	3	10	1	3.3	1	3.3	3	10	1	3.3	2	6.7
	28					1	3.3						
	30												
	32	1	3.3			1	3.3						
	34									2	6.7		

***** ZOI = Zone Of Inhibition, * F= Frequency

Table 4: Observation on sensitivity shown by *Staphylococcus aureus* to different concentrations of alcoholic extract of Kantakari

Extract	ZOI in mm against *Staphylococcus aureus*	20 µl N = 30		10 µl N = 30		5 µl N = 30		2.5 µl N = 30		1.25 µl N = 30		0.625 µl N = 30	
		F	%	F	%	F	%	F	%	F	%	F	%
Alcoholic extract of kantakari	0	21	70	20	66.7	23	76.7	21	70	15	50	13	43.3
	10												
	12												
	14												
	16					1	3.3	2	6.7	1	3.3		
	18					1	3.3			1	3.3	3	10
	20	1	3.3	3	10	1	3.3	1	3.3	4	13.3	3	10
	22	2	6.7	2	6.7	1	3.3	2	6.7	2	6.7	3	10
	24	1	3.3	2	6.7			2	6.7	3	10	2	6.7
	26					1	3.3			1	3.3	3	10
	28	1	3.3	2	6.7	1	3.3					1	3.3
	30	4	13.3	1	3.3	1	3.3	2	6.7			1	3.3
	32												
	34											1	3.3
	40									3	10		

* ZOI = Zone Of Inhibition, * F= Frequency

In vitro antibacterial activity of alcoholic extract of *Vasa* and *Kantakari* was evaluated by agar well diffusion method. The zones of inhibition of bacterial growth due to antibacterial activities of alcoholic extracts of various concentrations of *Vasa* and *Kantakari* were tabulated. From the data it is evident that the alcoholic extracts of *Vasa* and *Kantakari* showed good antimicrobial activity against *Staphylococcus aureus.* Based on *in-vitro* study susceptibility of *Staphylococcus aureus* against *Vasa* and *Kantakari* is fairly evident between 24- 22 mm zone of inhibition hence it is considered sensitive, 20-18 is intermediate hence moderately sensitive, below 18 is resistant. Here **S** – Sensitive, **M**- Moderately sensitive, **R**- Resistant.

Alcoholic extract of *Vasa*

Alcoholic extract of *Vasa* had shown various zones of inhibition against *Staphylococcus aureus* ranging from 34 mm to 10 mm against various concentrations (20 µl to 0.625 µl). Maximum zone of inhibition (34 mm) was recorded for alcoholic extract of *Vasa* at 1.25 µl and minimum zone of inhibition (10 mm) was recorded at 5 µl concentrations.

Table 5: Mean values of zone of inhibition at different concentration of alcoholic extract of *Vasa*

Different concentrations of alcoholic extract of *Vasa*	20 µl	10 µl	5 µl	2.5 µl	1.25 µl	0.625 µl
N	30	30	30	30	30	30
Mean	7.20	8.33	8.93	12.33	13.47	12.07

Alcoholic extract of *Kantakari*

Alcoholic extract of *Kantakari* had shown various zones of inhibition against *Staphylococcus aureus* ranging from 40 mm to 16 mm against various concentrations (20 µl to 0.625 µl). Maximum zone of inhibition (40 mm) was recorded for alcoholic extract of *Kantakari* at 1.25 µl and minimum zone of inhibition (16 mm) was recorded at 5 µl, 2.5 µl, 1.25 µl concentration.

Table 6: Mean values of zone of inhibition at different concentration of alcoholic extract of *Kantakari*

Different concentrations of alcoholic extract of *Kantakari*	20 µl	10 µl	5 µl	2.5 µl	1.25 µl	0.625 µl
N	30	30	30	30	30	30
Mean	7.87	7.93	5.33	6.80	12.53	13.27

Table 7: Comparing antibacterial action of alcoholic extract of *Vasa* and *Kantakari*

Different concentrations of alcoholic extract	20 µl	10 µl	5 µl	2.5 µl	1.25 µl	0.625 µl
N (total samples)	30	30	30	30	30	30
Mean values of zone of inhibition at different concentration of alcoholic extract of *Vasa* (mm)	7.20	8.33	8.93	12.33	13.47	12.07
Mean values of zone of inhibition at different concentration of alcoholic extract of *Kantakari* (mm)	7.87	7.93	5.33	6.80	12.53	13.27
Difference of mean in mm	0.67	0.4	3.6	5.53	0.94	1.2

Table 8: Sensitivity test for alcoholic extract of different concentrations of *Vasa*

Concentrations	20 µl			10 µl			5 µl			2.5 µl			1.25 µl			0.625 µl		
	S	M	R	S	M	R	S	M	R	S	M	R	S	M	R	S	M	R
No. Of samples	7	2	21	5	6	19	7	4	19	10	5	15	10	6	14	13	4	13

Table 9: Sensitivity test for alcoholic extract of different concentrations of *Kantakari*

Concentrations	20 µl			10 µl			5 µl			2.5 µl			1.25 µl			0.625 µl		
	S	M	R	S	M	R	S	M	R	S	M	R	S	M	R	S	M	R
No. Of samples	8	1	21	7	3	20	4	1	25	6	1	23	9	5	16	11	6	13

Table 10: Comparing the zone of inhibition of alcoholic extracts of *Vasa* and *Kantakari*

Concentrations	20 µl			10 µl			5 µl			2.5 µl			1.25 µl			0.625 µl		
	S	M	R	S	M	R	S	M	R	S	M	R	S	M	R	S	M	R
No. Of samples (*Vasa*)	**7**	**2**	21	**5**	**6**	19	**7**	**4**	19	**10**	**5**	15	**10**	**6**	14	**13**	**4**	13
No. Of samples (*Kantakari*)	**8**	**1**	21	**7**	**3**	20	**4**	**1**	25	**6**	**1**	23	**9**	**5**	16	**11**	**6**	13

Table 11: Statistics

		Mean						
Concentration	N	*Vasa*	*Kantakari*	Mean Difference	SE	f- value	P value	interpretation
20 µl	30	7.20	7.87	**0.67**	1.527	.047	.829	NS
10 µl	30	8.33	7.93	**0.4**	1.418	.020	.889	NS
5 µl	30	8.93	5.33	**3.6**	1.415	1.635	.206	NS
2.5 µl	30	12.33	6.80	**5.53**	1.442	6.432	.014	S
1.25 µl	30	13.47	12.53	**0.94**	1.655	0.78	.781	NS
0.625 µl	30	12.07	13.27	**1.2**	1.530	.152	.0698	NS

Statistical analysis of the data was performed using SPSS 23.0 (IBM corp). The means were compared using One-Way ANOVA test. P = 0.01- 0.001 is considered as statistically highly significant, P = 0.01-0.05 is considered as statistically significant and P > 0.05 is considered as not significant.

On comparing the same concentration of alcoholic extract of *Vasa* and *Kantakari*, it was observed that 2.5 µl was found to be statistically significant and in other concentration (20, 10, 5, 1.25 and 0.625 µl) though not statistically significant, the mean difference seem to be very less. On comparing the same concentration of alcoholic extract of *Vasa* and *Kantakari*, it was observed that 2.5 µl was found to be statistically significant and in other concentration (20, 10, 5, 1.25 and 0.625 µl) though not statistically significant, the mean difference seem to be very less. Zone of inhibition was observed as increasing in lower concentration for alcoholic extracts of both *Vasa* and *Kantakari.*

RESULT AND DISCUSSION

Vasa (*Adhatoda vasica*) and *Kantakari* (*Solanum xanthocarpum*) are indicated in *Kasa* and said to possess *krimighna* property. Apart from this, two research works on Antimicrobial activity of *Adhatoda vasica* against clinical pathogens[7] and Antibacterial Activity of *Solanum xanthocarpum* leaf extract[8] pointed out promising results. In this study alcoholic extraction of *Vasa* and *Kantakari* was extracted. The active chemical constituents of the plants are contained within the cells of the plant. Alcohol provides a particularly effective way of maximizing the bioavailability of the active principles extracted from the plant. Ethanol is a molecule with both a polar and a non-polar end. Many taste molecules are polar whereas most aroma molecules are non-polar and the good thing is that ethanol can be used to extract both groups of compounds. The advantages of conventional Soxhlet extraction include the displacement of transfer equilibrium by repeatedly bringing fresh solvent into contact with the solid matrix, maintaining a relatively high extraction temperature with heat from the distillation flask and no filtration requirement after leaching. Soxhlet method is very simple and cheap. Hence Soxhlet apparatus was used for extraction. *Vasa* (*Adhatoda vasica*) was selected because Acharya *Charaka* has included *Vasa* in *Kasaghna varga* and said to have antibacterial activity.

Preliminary phytochemical assay of drugs revealed that the presence of alkaloid, tannins, flavonoids, coumarins and resin present in the extract serves a defence mechanism against *Staphylococcus aureus*. Alkaloids from plant are commonly found to have antimicrobial properties. The prominent alkaloid found in Adhatoda leaves is quinazoline alkaloid known as vasicine. In addition to vasicine, the leaves and roots of Adhatoda contain the alkaloids l-vasicinone, deoxyvasicine, maiontone, vasicinolone and vasicinol[9,10]. Flavonoids are phenolic structures possessing antimicrobial activity is due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls. Hence based on these merits plant has been selected for the study.

Kantakari (*Solanum xanthocarpum*) is one of the drug which process *K*asaghana and *Krimighna* property and belonging to the family solanaceae[11]. The extract of the plant contains alkaloids, steroids, tannins, flavonoids, saponins, terpenoids and coumarins. The whole plant yield an alkaloid called solasonine. Roots leaves and fruits yield coumarins, scopolin, scopoletin, esculin and esculetin. Fruits yield carpesteral, gluco-alkaloid, solanocarpine, solasodine, solamargine, stigma sterol, campesterol and beta solamargine[12]. The drugs prepared by the Soxhlet extraction contain number of chemical compounds responsible for medicinal activity of drugs. Phytochemical and HPTLC was conducted for the both Vasa and *Kantakari* extract.

Result of preliminary phytochemical test and HPTLC of Alcoholic extract of *Vasa* and *Kantakari*

Part A: Particulars of sample submitted

Test requested by: Dr. Vishnu, SDM College of Ayurveda Hassan
Requested on: 26-09-18
Investigation to be performed: HPTLC
Sample coded as: 18092607
Sample details: Kantakari extract

HPTLC

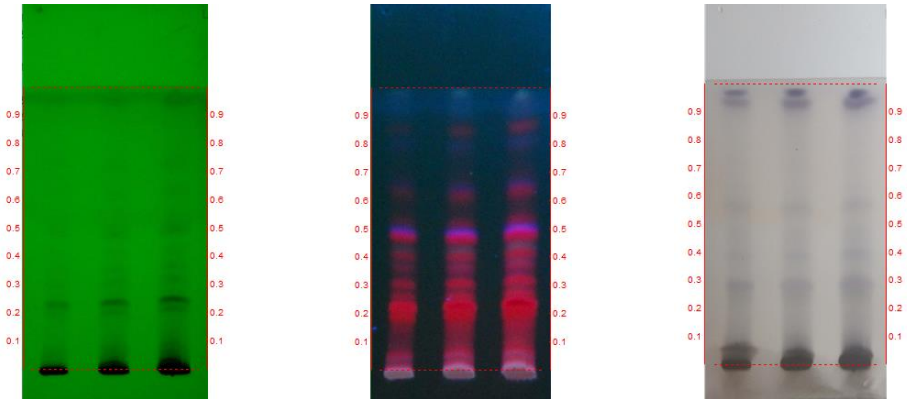
100 mg of Kantakari extract was dissolved in 1.0 ml of alcohol. 4, 8, 12 µl of the above extract was applied on a pre-coated silica gel F254 on aluminium plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl acetate: Diethyl amine (6.0: 0.5: 0.5). The developed plates were visualized in under short UV, long UV and then derivatized with vanillin sulphuric acid and scanned under UV 254 nm, 366 nm. R_f, colour of the spots and densitometry scan were recorded.

Preliminary phytochemical tests

Table 12: Results of preliminary phytochemical screening of Alcoholic extract of Kantakari

Test	**Inference**	
	Kantakari	
Alkaloid	+	
Steroid	+	
Carbohydrate	+	
Tannin	+	
Flavonoids	+	
Saponins	+	
Terpenoid	+	
Coumarins	+	
Phenols	-	
Carboxylic acid	-	
Amino acids	-	
Resin	+	
Quinone	-	
Tests	**Colour if positive**	**Alcoholic extract of Kantakari**
Alkaloids		
Dragendorff's test	Orange red precipitate	Orange red precipitate
Wagners test	Reddish brown precipitate	Reddish brown precipitate
Mayers test	Dull white precipitate	Dull white precipitate
Hagers test	Yellow precipitate	Yellow precipitate
Steroids		
Liebermann- burchard test	Bluish green color	Bluish green color
Salkowski test	Bluish red to cherry red color in chloroform layer and green fluorescence in acid layer	Bluish red to cherry red color in chloroform layer and green fluorescence in acid layer
Carbohydrate		
Molisch test	Violet ring	Violet ring
Fehlings test	Brick red precipitate	Brick red precipitate
Benedicts test	Red precipitate	Red precipitate
Tannin		
With $FeCl_3$	Dark blue or green or brown	Green color
Flavonoids		
Shinoda's test	Red or pink	Red color
Saponins		
With $NaHCO_3$	Stable froth	Stable froth
Triterpenoids		
Tin and thionyl chloride test	Pink	Pink
Coumarins		
With 2 N NaOH	Yellow	Yellow color

Phenols		
With alcoholic ferric chloride	Blue to blue black	Green color
Carboxylic acid		
With water and $NaHCO_3$	Brisk effervescence	No brisk effervescence
Amino acid		
With ninhydrin reagent	Purple color	No Purple color
Resin		
With aqueous acetone	Turbidity	Turbidity
Quinone		
Conc. sulphuric acid	Pink/purple/red	Yellow color



Short UV **Long UV** **After derivatisation**

Figure 1: HPTLC Photo documentation of sample of Alcoholic extract of *Kantakari*

Part A: Particulars of sample submitted
Test requested by: Dr. Vishnu, SDM College of Ayurveda Hassan
Requested on: 26-09-18
Investigation to be performed: HPTLC
Sample coded as: 18092606
Sample details: Vasa extract

HPTLC

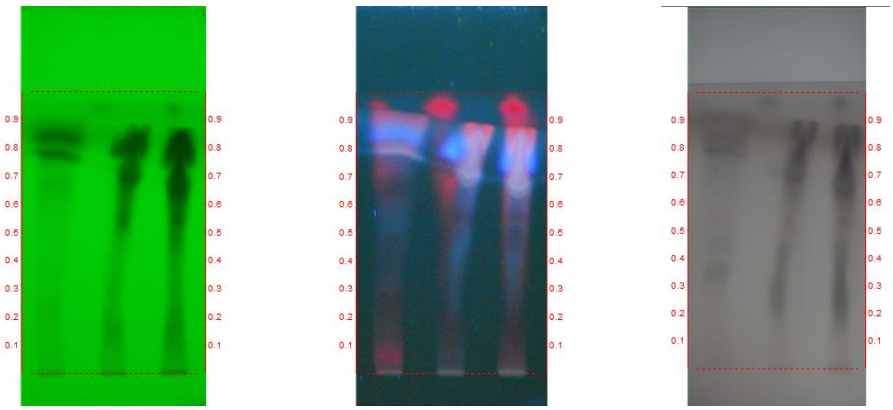
100 mg of Vasa extract was dissolved in 1.0 ml of alcohol. 4, 8, 12 µl of the above extract was applied on a pre-coated silica gel F254 on aluminium plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Methanol: Ammonia (8.0: 2.0: 0.2). The developed plates were visualized in under short UV, long UV and then derivatised with vanillin sulphuric acid and scanned under UV 254 nm, 366 nm. R_f, colour of the spots and densitometric scan were recorded.

Preliminary phytochemical tests

Table 13: Results of preliminary phytochemical screening of Alcoholic extract of vasa

Test	**Inference**	
	Vasa	
Alkaloid	+	
Steroid	-	
Carbohydrate	+	
Tannin	+	
Flavonoids	+	
Saponins	-	
Terpenoid	-	
Coumarins	+	
Phenols	-	
Carboxylic acid	-	
Amino acids	-	
Resin	+	
Quinone	-	
Tests	**Color if positive**	**Alcoholic extract of *Vasa***
Alkaloids		
Dragendroff's test	Orange red precipitate	Orange red precipitate
Wagners test	Reddish brown precipitate	Reddish brown precipitate
Mayers test	Dull white precipitate	Dull white precipitate
Hagers test	Yellow precipitate	Yellow precipitate
Steroids		
Liebermann- burchard test	Bluish green color	No bluish green color
Salkowski test	Bluish red to cherry red color in chloroform layer and green fluorescence in acid layer	No bluish red to cherry red color in chloroform layer and green fluorescence in acid layer

Carbohydrate		
Molisch test	Violet ring	Violet ring
Fehling's test	Brick red precipitate	Brick red precipitate
Benedicts test	Red precipitate	Red precipitate
Tannin		
With $FeCl_3$	Dark blue or green or brown	Green color
Flavonoids		
Shinoda's test	Red or pink	Red color
Saponins		
With $NaHCO_3$	Stable froth	No stable froth
Triterpenoids		
Tin and thionyl chloride test	Pink	Green color
Coumarins		
With 2 N NaOH	Yellow	Green color
Phenols		
With alcoholic ferric chloride	Blue to blue black	Green color
Carboxylic acid		
With water and $NaHCO_3$	Brisk effervescence	No brisk effervescence
Amino acid		
With ninhydrin reagent	Purple color	No purple color
Resin		
With aqueous acetone	Turbidity	Turbidity
Quinone		
Conc. sulphuric acid	Pink/purple/red	Green color



Short UV **Long UV** **After derivatisation**

Figure 2: HPTLC Photo documentation of sample of Alcoholic extract of *Vasa*

Alcoholic extracts of drugs are enriched with antimicrobial components such as Terpenoids, Alkaloids, Saponins, Flavonoids and Phenolic compounds. Comparatively alcoholic extracts are shown more significant zone of inhibition than aqueous and chloroform extracts because alcohol provides effective way of maximizing the bioavailability of the active principles from the plant. Ethanol is a molecule with both the polar and non-polar ends. So it can be used to extract both groups of compounds of drug. So that the saturation level of these phytochemical compounds is at maximum level. While carrying out sensitivity, phytochemical constituents interact with enzymes and proteins of cell membrane causing its disruption to disperse a flux of protons towards cell exterior which will cause cell death or inhibit amino acid biosynthesis of microbial cell[13] and in other hands, hydrophobic characters of these extracts enable to react with protein of microbial cell membrane and mitochondria to disturbing their cell structures and permeability. Likewise for different strains of bacteria it has been proposed that the mechanism of the antimicrobial effects involves the inhibition of various cellular processes followed by an increase in plasma membrane permeability and finally ion leakage from the cells. Saponins have been reported to possess a wide range of biological activities including antifungal, antiviral, and antibacterial activities also. Altering the surface tension of the extra cellular medium of cell is key characteristic feature Flavonoids are phenolic structures possessing antimicrobial activity is due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls. Alkaloids from plant are commonly found to have antimicrobial properties. Meantime for different concentrations of same drug, it may show different zone of inhibition. Because, the different components diffuse at different rates may have been responsible for the varying zone of inhibition against microorganisms. In lower concentrations, the molecular size of the active components will be too small via complete dissolution and thereby it can penetrate easily through cell membrane of microorganism. So it will show maximum zone of inhibition than other higher concentrations. For higher concentrations, as the drug content is more, it may not show significant zone of inhibition. While diluting the concentrations, the active components completely will dissolve into that solution. So the drug will be incapable to forward with antibacterial action even it would reach and set at cell membrane. The variation of susceptibility of the microorganisms could be attributed to their intrinsic properties and permeability of their cell surface to the

extracts. Meantime active phytochemical contents fail to thrive with antimicrobial action depends on cytological characteristics of organism. Porosity of cell membrane varies cell to cell via different conditions and the membrane inhibits cell structure perturbations because of its defiance to phytochemical components. The antibacterial assay was done at 6 different concentrations of alcoholic extracts of both *Vasa* and *Kantakari* to understand their effective activity. Here anti – microbial study is done by Agar well diffusion method. Both the extracts were used for checking activity against *Staphylococcus aureus*.

In order to evaluate efficacy and conclude efficacy of alcoholic extract of Vasa and *Kantakari* zone of inhibition between 24 - 22 mm was considered as sensitive zone, 20 – 18 mm as moderately sensitive and below 18 mm as resistant.

Comparing the same concentrations of alcoholic extracts of *Vasa* and *Kantakari* by One-Way ANOVA test it was observed that 2.5 µl was found to be statistically significant. But the other concentrations (20, 10, 5, 1.25 and 0.625 µl), though not statistically significant, the mean difference seem to be very less. Further on comparing the mean values, mean value of vasa (12.33 mm) was greater than mean value of *Kantakari* (6.80 mm) at 2.5 µl concentrations, hence it can be concluded that Vasa is having better antibacterial action than *Kantakari* against *Staphylococcus aureus* in the present study

CONCLUSION

In the present study, on comparing the same concentrations of alcoholic extracts of *Vasa* and *Kantakari*, it was observed that 2.5 µl was found to be statistically significant. But the other concentrations (20, 10, 5, 1.25 and 0.625 µl), though not statistically significant, the mean difference seem to be very less. Further on comparing the mean values, mean value of *Vasa* (12.33 mm) was greater than mean value of *Kantakari* (6.80 mm) at 2.5 µl concentrations, hence it can be concluded that *Vasa* is having better antibacterial action than *Kantakari* against *Staphylococcus aureus* in the present study

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