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GCMS Analysis of Aqueous Extract of Tambula Patra (Piper betle Linn) and its Efficacy against Staphylococcus Aureus in *Kaphaja Kasa* (Acute Bronchitis)

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ABSTRACT

Acute Bronchitis is an airway inflammatory disease. One of the causes for the progression of the disease is the secondary bronchi infections caused by the bacteria's like *Staphylococcus aureus. Kaphaja kasa* is one of the most common disease affecting *pranavaha srotas*, and if mismanaged leads to *swasa*, *kshaya* etc. *Tambula (piper betle*.Linn) has numerous traditional uses for different ailments like oliguria, nervous disorders, headache, respiratory disorders, constipation, sore throat, wounds and boils. In the present study, GCMS analysis and action of aqueous extract of *Tambula patra (piper betle*.Linn) against *Staphylococcus aureus* derived from sputum of *kaphaja kasa*(Acute bronchitis) subjected to culture and sensitivity is evaluated. From the mean zone of inhibition, it is evident that aqueous extract of *Tambula patra* is having antimicrobial action against *Staphylococcus aureus*. Further it is evident that better sensitivity is observed in higher concentrations of aqueous extract of *Tambula patra*.

Key Words *Acute bronchitis*, *Kaphaja kasa*, *Staphylococcus aureus*, *Tambula*, GCMS

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INTRODUCTION

Acute bronchitis, if mismanaged can lead to the onset of major morbidities like pneumonia, chronic bronchitis, sinusitis and asthma. Most common primary causative agents would be viruses,that paves the way for the secondary bronchi infections, caused by bacterias. *Staphylococcus aureus* also plays a major role in the causation[1]. Indeed many drugs are derived from chemicals found in plants[2]. Plants are the oldest source of medicine. Clues about these have been obtained from traditional systems of medicine prevalent in various parts of the world[3]. According to WHO (1993) almost 80% of the world's population is reliant on traditional medicine[4] and a main division of traditional therapies involve the use of plant extracts or their active constituents. The traditional medicinal

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methods, particularly the use of medicinal plants still play a crucial role to cover the fundamental health needs in developing countries.

Therapeutic action is rendered on most of the plant drugs by their *krimighna* (anti microbial) property. Sensitivity test for existing Ayurveda drugs is important because it directs the use of these drugs within a narrow spectrum of activity, thus specific indication. Thereby preliminary evidence invitro study is generated scientifically, so that drug can be later used in patients as *upashaya*.

AIMS & OBJECTIVES

GCMS analysis and sensitivity of aqueous extract of *Tambula patra (piper betle.*Linn*)* on *Staphylococcus aureus* from sputum of *kaphaja kasa*(Acute bronchitis) subjects.

MATERIALS AND METHODS

Present study included 30 subjects between 18-60 years age, of either gender irrespective of caste and religion. Subjects presenting productive cough with thick,dense expectorate associated with two or more *kaphaja kasa lakshanas* like *bahalam kapham*(expectorate profuse sputum), *Sandram kapham*(viscid sputum), *Ghana kapham*(thick sputum), *Vaksha sampurna eva manyate*(feeling of chest filled with sputum), *Utklesha* (nausea), *Peenasa* (runny/stuffy nose), *Mukhena lipyamana*(stickiness in mouth) and *Sirasoola* (headache).

Inclusion crieteria

- Patients between the age of 18 -60 years
- Patients fulfilling the diagnostic crieteria
- Patients having cough with expectoration within 3 weeks

Exclusion criteria

- Diagnosed cases of tuberculosis
- *Sashonitha kapaha*(Reddish brown sputum)
- Organisms other than *Staphylococcus aureus*

RESEARCH DESIGN

Observational experimental study

Methodology

Plant collection and authentication was carried out. Cold maceration method[5] was used for preparing alcoholic extract of *Tambula patra* as it is very easy to perform, cost effective and simple without using any complex instruments but yields highly potent extract with many bio active principles. Fresh and clean *Tambula* leaves about 100 grams was weighed using a weighing balance.

Using a clean mortar and pestle, these leaves were crushed coarsely without adding water to facilitate easy release of active principles on addition of extracting solvent ethanol. Crushed *Tambula* leaves were added to 500ml distilled water in a 1000ml capacity conical flask. Further plugged tightly with cotton and then sealed with tape. The conical flask was shaken manually for 10-15minutes, once in every 2-3 hours as shown in table 1.

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Table 1 Frequency and duration of shaking in cold maceration process.

Date	Time	Duration (min)
19/12/20	02.00pm	15
	05.00pm	15
21/12/20	09.30am	15
	12.30pm	10
	03.30pm	15
	05.30pm	10
22/12/20	09.30am	10
	12.00pm	10
	02.00pm	15
	05.00pm	15
23/12/20	09.30am	15
	12.00pm	15
	02.30pm	15
	05.00pm	15
24/12/20	09.30am	15
	12.30pm	10
	03.30pm	15
	05.30pm	15
25/12/20	09.30am	15
	12.30pm	15
	03.30pm	10
	05.30pm	15

The procedure was repeated for 7 days. On the 7th day 420ml of filtrate was obtained by filtering the content of conical flask. Further, 3.06g of aqueous extract of *Tambula patra* was obtained by keeping the filtrate over water bath in a petri dish at 60°C.

Early morning sputum sample was collected from kaphaja kasa subjects and streaking was done on Mc Conkey and blood agar plates. The plates were then kept for 24 hour culture in incubator at 37°C. Identification of bacteria was done by studying the colony morphology and microscopic examination by gram staining. Further coagulase test was done with each *Staphylococcus aureus* culture to confirm the organism as coagulase positive. Different concentrations of aqueous extract of *Tambula patra* were prepared by dissolving 3g of aqueous extract in 6ml of distilled water that gave a stock solution carrying 3000µg/ml of drug concentration. From the stock solution different concentrations like 2000µg/ml, 1000µg/ml, 900µg/ml, 800µg/ml and 700µg/ml of the alcoholic extract were prepared.

Muller Hinton agar plates were uniformly swabbed with standard Mc Farland inoculums. Different drug concentrations were then subjected to anti bacterial sensitivity by well diffusion method. Six equidistant wells were made on the plates with the help of sterile cork baurer. 100µl each of different concentrations of aqueous extract were poured into labeled wells on different plates. All the plates were incubated at 37°C for 24 hours. Further zone of inhibition was measured with a ruler in mm.

Assessment criteria

Sensitivity of drug to a bacterium is assessed by a clear circular 'halo' (zone of inhibition) that appears around the well denoting the absence of organism. This indicates that, the particular drug is effective against that bacterium.

Present in vitro study revealed that susceptibility of *Staphylococcus aureus* against aqueous extract of *Tamula patra* is quite marked between 22-20mm and is considered as sensitive, 18-16mm is moderately sensitive and 14-12mm as resistant.

RESULTS

GCMS analysis of aqueous extract of *Tambula patra* revealed the presence of the following compounds as shown in table 2, possibly

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contributing to antimicrobial activity against *Staphylococcus aureus* found in *Kaphaja kasa* (Acute bronchitis).

Table 2 Compounds observed on GCMS analysis of *Tambula patra*

COMPOUND	AREA%
Pentanoic acid	1.44
Cycloheptasiloxane	0.49
Phenol	1.75
Dodecanoic acid	6.44
Cyclo octasiloxane	0.46
Methyl tetra decanoate	3.29
Cyclononasiloxane	0.34
Tetradecanoid acid	2.40
Pentacosane	0.33
Cyclodecasiloxane	0.28
Hexa decanoic acid	18.6
n-Hexadecanoic acid	6.67
Hepta decanoic acid	0.28
Octa decadienoic acid	25.1
Furanone	1.06
Oleic acid	25.1
Octadecanoic acid	4.71
Octadecamethyl-Benzene ethanamine	0.32
Eicosanoic acid	0.42
Docosanoic acid	0.78
Benzeneethanamine	0.27
Tetracosanoic acid	0.24
Pentacosanoic	0.24

The concentration of the compound observed in the extract is identified by the peaks observed in the graph as shown in table 3, figure 1.

Table 3 Peaks observed in gas chromatography and mass spectrometry of aqueous extract of *Tambula patra*

Peak number	RT Min	First Scan	Max Scan	Last Scan	Peak Height	Corr. % Max	% of Total
1	17.545	1393	1466	1474	450213	5.72	1.437%
2	33.790	3371	3379	3394	1320838	1.97%	0.494%
3	35.276	3543	3554	3571	3809250	6.95%	1.745%
4	35.556	3572	3587	3598	5114969	11.34%	2.846%
5	38.504	3812	3934	3973	1489719	25.66%	6.440%
6	39.176	3997	4013	4026	666333	1.82%	0.457%
7	44.831	4644	4678	4701	2898124	13.12%	3.294%
8	47.390	4963	4980	4991	484474	1.35%	0.338%
9	48.138	4991	5068	5083	912446	9.55%	2.397%
10	51.881	5479	5508	5520	416877	1.31%	0.328%
11	52.376	5548	5566	5574	559085	1.13%	0.284%
12	52.736	5574	5609	5622	15678968	74.24%	18.634%
13	54.335	5732	5797	5801	2749728	18.71%	4.695%
14	54.584	5801	5826	5851	3761581	26.56%	6.666%
15	55.131	5879	5891	5903	623037	1.11%	0.278%
16	55.903	5950	5982	5991	544934	1.12%	0.280%
17	56.593	6054	6063	6074	636757	1.34%	0.337%
18	56.784	6074	6085	6092	2967718	6.09%	1.529%
19	56.916	6092	6101	6114	2236754	4.23%	1.062%
20	57.428	6144	6161	6172	7613685	17.36%	4.358%
21	58.507	6189	6288	6310	6298535	100.00%	25.100%
22	58.756	6310	6318	6322	3025488	9.28%	2.328%
23	58.894	6322	6334	6344	4168505	18.76%	4.710%

Aqueous extract of *Tambula patra* had shown various zones of inhibition by agar well diffusion method against *Staphylococcus aureus* ranging from 26 mm to 2 mm against various concentrations (3000 µg/ml to700 µg/ml) as shown in table 4. Maximum zone of inhibition (26 mm) was recorded for aqueous extract *of Tambula patra* at 3000µg/ml and minimum zone of inhibition (2 mm) was recorded at 2000 µg/ml, 800 µg/ml and 700 µg/ml concentrations.

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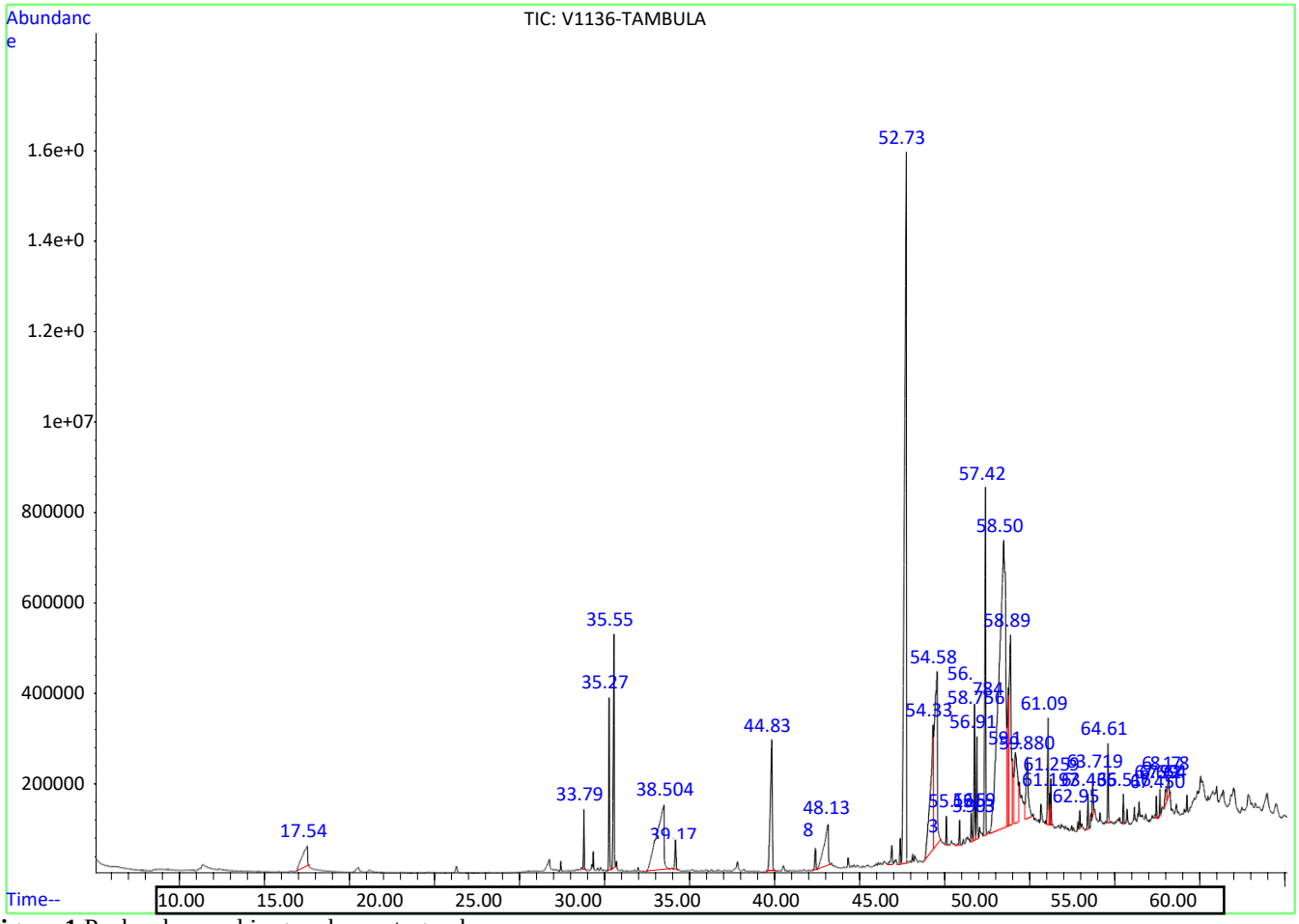

Figure 1 Peaks observed in gas chromatography

Table 4 Distribution based on sensitivity of aqueous extract of different concentrations of *Tambula patra* against *Staphylococcus aureus*

Extract	ZOI in mm against staph.aureus	3000µg/ml N=30		2000µg/ml N=30		1000µg/ml N=30		900µg/ml N=30		800µg/ml N=30		700µg/ml N=30	
		F	%	F	%	F	%	F	%	F	%	F	%
Aqueous extract of *tambula patra*	0	-	-	-	-	3	10	2	6.7	9	30	15	50
	2	-	-	1	3.3	-	-	-	-	3	10	5	16.7
	4	2	6.7	-	-	-	-	-	-	1	3.3	-	-
	6	-	-	2	6.7	1	3.3	-	-	5	16.7	4	13.3
	8	1	3.3	-	-	-	-	-	-	-	-	1	3.3
	9	-	-	-	-	-	-	-	-	1	3.3	-	-
	10	-	-	1	3.3	1	3.3	9	30	2	6.7	3	10
	12	-	-	-	-	-	-	3	10	4	13.3	-	-
	14	1	3.3	-	-	-	-	-	-	2	6.7	-	-
	16	1	3.3	-	-	9	30	2	6.7	2	6.7	-	-
	18	3	10	9	30	8	26.7	7	23.3	1	3.3	2	6.7
	19	4	13.3	7	23.3	3	10	5	16.7	-	-	-	-
	20	10	33.3	8	26.7	3	10	1	3.3	-	-	-	-
	22	6	20	1	3.3	2	6.7	1	3.3	-	-	-	-
	24	1	3.3	1	3.3	-	-	-	-	-	-	-	-
	26	1	3.3	-	-	-	-	-	-	-	-	-	-

* ZOI = Zone of Inhibition
* F = Frequency of samples showing sensitivity against alcoholic extract of *tambula patra*
* N = Total no. of samples

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The mean Zone of Inhibition of the aqueous extract of *Tambula patra*(*Piper betle* Linn) decreases on decreasing the concentration as shown in table 5.

Table 5 Mean values of zone of inhibition at different concentrations of aqueous extract of *Tambula patra* against *Staphylococcus aureus*

Different concentrations of aqueous extract of *Tambula patra*(µg/ml)	**3000**	**2000**	**1000**	**900**	**800**	**700**
Total number of patients	30	30	30	30	30	30
Mean (mm)	18.60	17.50	15.50	14.03	6.50	3.60

Number of sensitive, moderately sensitive and resistant zones for different concentrations of aqueous extractobtained is shown in table 6.

Table 6 Sensitivity test for aqueous extract of different concentrations of *Tambula patra*

Concentrations	**3000 µg/ml**			**2000 µg/ml**			**1000 µg/ml**			**900 µg/ml**			**800 µg/ml**			**700 µg/ml**		
	S	M	R	S	M	R	S	M	R	S	M	R	S	M	R	S	M	R
No. of samples	**22**	**4**	**4**	**17**	**9**	**4**	**8**	**17**	**5**	**7**	**9**	**14**	**0**	**2**	**28**	**0**	**2**	**28**

S – Sensitive, M- Moderately sensitive, R- Resistant

DISCUSSION

In the present study, 59 subjects with *kaphaja kasa*(acute bronchitis) were screened. Among them 30 subjects fulfilled the diagnostic and inclusion criteria and remaining 29 subjects were excluded. Among the excluded, 5 were not in inclusion age group ,5 having cough with expectoration more than 3 weeks,4 with *sashonitha kapham,*13 excluded ,were the organism was other than *staphylococcus aureus* on culture ,2 were coagulase negative *Staphylococcus*.

Tambula is classically categorized under *amraadi varga* and is said to possess *kaphakasa hara* [6]effect according to *raja nighantu*. At the same time, *priya Nighantu* mentioned the drug under *pippalyadi varga* and possess the *janthujith karma*[7]. *Tambula* possess *katu, tikta, kasaya rasa, tikshna, ushna, kshara guna, ushna veerya* and is *kapha vata shamaka* and has *deepana, pachana*[8] and *janthujith karma*.

Active phytochemical compounds in *Tambula patra* extract disturbs the different mechanisms of *Staphylococcus aureus* by changing the surface tension of extracellular medium of organism cell, complexing with extracellular and soluble proteins etc[9]. In higher concentration of the extract, the drug content is more, hence showing noticeable zone of inhibition .On diluting the concentrations, the active components completely dissolve in the solution. So the drug is unable to render the antibacterial action even though it reaches the cell membrane of the organism. Even though the drug has active phytochemical constituents, the variation in susceptibility of the organism can also be attributed to its intrinsic properties, cytological properties and cell wall permeability[10].

In the present study GCMS analysis of the aqueous extract was done. Gas Chromatography and Mass Spectrometry is a modified technique useful to analyse and to separate the volatile

compounds from an organic or inorganic substance[11]. So to know the concentration of different components and the number of peaks present in the *Tambula patra*, this study was conducted. The report of GCMS study of *Tambula patra* conveyed that there were 23 multiple volatile compounds observed out of which octadecadienoic acid and oleic acid were the compounds which covered maximum area of the graph depicting the highest concentration in the *Tambula patra* sample. It was followed by the abundance of hexadecanoic acid, pentadecanoic acid, dodecanoic acid, phenol and pentanoic acid. All these compounds have proven antimicrobial activities and which can be assumed as the components that are involved in the antimicrobial action of the extract.

CONCLUSION

From the zone of inhibition found in this study it is clear that aqueous extract of *Tambula patra* is having antimicrobial action against *Staphylococcus aureus* from *kaphaja kasa*(Acute bronchitis). Further it is evident that better sensitivity is observed with higher concentrations of aqueous extract of *Tambula patra.*

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