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Efficacy of Alcoholic Extract of *Tambulapatra*(Piper betle Linn.) against Staphylococcus aureus in *Kaphajakasa* (Acute Bronchitis)

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ABSTRACT

Kaphajakasa is one of the most prevalent disease of the *pranavahasrotas*. The *lakshanas* mentioned in the description of *kaphajakasa* can be correlated with most of the symptoms of acute bronchitis. The notable cause of acute bronchitis is thesecondary bronchi infections produced by an account of gram positive bacteria. Among that, the prior position is taken by *Staphylococcus aureus*.*Tambula* is indicated in *kaphajakasa* and is*janthujith*. Apart from this,*tambula* is cost effective and easily available drug which possess numerous compounds which has higher medicinal values. Present in -vitro study was done to evaluate the efficacy of alcoholic extract of *tambulapatra* against *Staphylococcus aureus*in patients of kaphajakasa, to provide an evidence based approach to the *kaphakasahara* and *janthujith karma* of *tambulapatra*. From the observation and result,it is evident from the study that the alcoholic extract of *tambulapatra* has antimicrobial activity against *Staphylococcus aureus* in *kaphajakasa*.

Key Words*Kaphajakasa, Acute Bronchitis, Tambulapatra, Staphylococcus aureus*

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INTRODUCTION

Sensitivity test for existing *Ayurveda* drugs are very important as it directs the use of these drugs within a narrow spectrum of activity.Itis also done in order to find out the anti-microbial activity of a drug which posses *Krimighna* property against a particular microorganism and to define the anti-microbial property of that particular drug for known concentrations. Before the drug is used clinically on patients, its activity needs to be checked on causative microorganisms in vitro and confirm whether the drug shows sensitivity and hence preliminary evidence can be generated scientifically, so that drug can later be used in patients as *Upashaya*.

Kaphajakasa is one of the commonest disease affecting the *pranavahasrotas*.Most of the *lakshanas* mentioned in the classics like *kasa*,

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kaphashteevana and *bahalam kapham*[1] can be correlated with the symptoms of acute bronchitis[2]. *Staphylococcus aureus* plays a notable role in the pathogenesis of acute bronchitis[3]. *Tambula* is indicated in *kaphaja kasa*[4] and is *janthujith*[5]. Apart from this, *tambula* is cost effective and easily available drug which possess numerous compounds which has higher medicinal values due to *katutikta rasa and ushna veerya*[6]. Present study was planned to provide an evidence based approach to the *kaphakasahara* and *janthujith karma* of *tambulapatra* and to evaluate various attributes of *Staphylococcus aureus* by laboratory diagnosis followed by culture and sensitivity against *tambula* by sputum culture and sensitivity method from patients suffering from *kaphajakasa* with special reference to acute bronchitis.

AIMS AND OBJECTIVES

To evaluate the sensitivity of alcoholic extract of *tambulapatra (Piper betle* Linn.) against *Staphylococcus aureus* from sputum in *kaphajakasa*(Acute bronchitis) subjects by culture and sensitivity in vitro.

MATERIALS AND METHODS

A minimum of 30 subjects aged between 18-60 years, presenting with *kaphajakasalakshanas* within 3 weeks duration was selected for the study.

Diagnostic criteria

Patients complaining of productive cough with thick, dense expectorate associated with two or more of the following symptoms classically explained in *kaphajakasa*.

- *Bahalamkapham*(expectorate profuse sputum)
- *Sandramkapham*(viscid sputum)
- *Ghana kapham*(thick sputum)
- *Vakshasampurnaevamanyate*(feeling of chest filled with sputum)
- *Utklesha* (nausea)
- *Peenasa* (runny/stuffy nose)
- *Mukhenalipyamana*(stickiness in mouth)
- *Sirasoola* (headache)

a) Inclusion criteria

Patients between the age of 18 -60 years

Patients fulfilling the diagnostic criteria

Patients having cough with expectoration within 3 weeks

b) Exclusion criteria

Diagnosed cases of tuberculosis

Sashonithakapha(Reddish brown sputum)

Organisms other than *Staphylococcus aureus*

Table 1 Preperation of alcoholic extract of *tambulapatra*

Drug	Day	Content	Time	Temperature	Quantity obtained
Tambulapatra	13/2/2020 Thursday	Alcoholic filtrate of *tambulapatra*-390ml*(tambula patra*-100gms,ethanol-500ml	10.45 am-2.45 pm	60^{o}C	3.1 gm

METHODOLOGY

Alcoholic extract of *tambulapatra* was prepared by cold maceration method using 100gm of fresh

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and clean *tambula*leaves (table 1). Crushed *tambula* leaves were then added to 500ml ethanol taken in a 1000ml capacity conical flask. This was plugged with cotton and sealed. The conical flask was shaken manually for 10-15 min at an interval of every 3 hours .The procedure was repeated for 7 days during day time. On 7th day the content in the conical flask was filtered, that yielded 390ml of filtrate. This filtrate was kept over water bath at 60^{o}C. Alcoholic extract, 3.1g, was obtained by this process.

Early morning thick sputum sample from the subjects of *kaphajakasa* was collected. A loop full of inoculum was transferred to MacConkey agar plates and culturing was done by streak culture method. The plates were then kept for 24-48 hour culture in incubator at 37^{o}C. Identification of bacteria was done by studying the colony morphology and microscopic examination by gram staining. Further coagulase test was also performed for confirming the presence of coagulase positive *Staphylococcus aureus*.

Different concentrations of aqueous extract of *tambulapatra* were prepared by dissolving 3g of alcoholic extract in 6ml of ethanol that gave a stock solution containing 3000µg/ml of drug concentration. From the stock solution, different concentrations like 2000 µg/ml,1000 µg/ml,900 µg/ml,800 µg/ml and 700 µg/ml of the alcoholic extracts were prepared.

Muller Hinton agar plates were uniformly swabbed with McFarland inoculums. The different concentrations of drug were subjected to antibacterial sensitivity test by Agar well diffusion method. Six equidistant wells were made on the plates with the help of sterile cork baurer. 100 µl of alcoholic extract of different concentrations were poured into labelled wells on different plates. All the plates were incubated at 37^{o}C for 24-48 hours after which zone of inhibition was measured with a ruler in mm

Assessment criteria

If a drug is sensitive, a clear circular 'halo'(zone of inhibition) appears around the well that indicates the absence of bacterial growth that in turn proves the efficacy of the drug against that bacterium.

OBSERVATION AND RESULTS

In vitro antibacterial activity of alcoholic extract of *tambulapatra* was evaluated by agar well diffusion method and mean zone of inhibitionwas measured as shown in table 2.

Table 2 Mean values of zone of inhibition at different concentrations of alcoholic extract of *tambulapatra*

Different concentrations of alcoholic extract of *tambulapatra*	**3000 µg/ml**	**2000 µg/ml**	**1000 µg/ml**	**900 µg/ml**	**800 µg/ml**	**700 µg/ml**
Total number of patients(N)	30	30	30	30	30	30
Mean (mm)	19.30	18.57	18.23	13.00	8.40	4.53

The in- vitro study showed that the susceptibility of *Staphylococcus aureus* against alcoholic extracts of *tambulapatra* was fairly evident between 20-16 mm zone of inhibition, hence it was considered as sensitive, 15-10mm was intermediate, hence moderately sensitive

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and below 10 mm as resistant. In the present study, *Staphylococcus aureus* is sensitive to 3000 µg/ml,2000µg/ml and 1000 µg/ml, and moderately sensitive to 900 µg/ml and resistant to 800 µg/ml and 700 µg/ml.

DISCUSSION

In the present study, 59 subjects with *kaphajakasa*(acute bronchitis) were screened. Among them 30 subjects fulfilled the diagnostic and inclusion criteria and remaining 29 subjects were excluded. Among the excluded, 5were 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 *amraadivarga* and is said to possess *kaphakasahara* effect according to *raja nighantu*[7]. At the same time, *priyaNighantu* mentioned the drug under *pippalyadivarga* and possess the *janthujith karma*[8]. *Tambula* possess *katu, tikta, kasaya rasa, tikshna, ushna,ksharaguna, ushnaveerya* and is *kaphavatashamaka* and has *deepana, pachana*[9] and *janthujith karma*.

PROBABLE MODE OF ACTION OF *TAMBULA*

Active phytochemical compounds in *tambulapatra* extract shows antibacterial action against *Staphylococcus aureus* by changing the surface tension of extracellular medium of organism cell, complexing with extracellular and soluble proteins etc. 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].

CONCLUSION

From this study, it is clear from the observation and result of mean zone of inhibition that the alcoholic extract of *tambulapatra (*Piper betleLinn.) has antimicrobial action against *Staphylococcus aureus* from sputum sample of *kaphajakasa*(acute bronchitis). Further it is also evident that as the concentration of the alcoholic extract of *tambulapatra(piper betl*eLinn.) increases, the zone of inhibition for *staphylococcusaureus* also increases.

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