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Efficacy of aqueous extract of *Tambula patra* (*piper betle* Linn.) against *Staphylococcus aureus* in *kaphaja kasa* (Acute bronchitis)

RADHIKA RANI V[1*], GOPIKRISHNA.S[2] AND SHASHIREKHA K S[3]

1: P G Scholar, Department of Roga Nidana Evam Vikrti Vijnana, SDM College of Ayurveda and Hospital, BM Road, Thanniruhalla, Hassan, Karnataka, India

2: Professor, Department of Roga Nidana Evam Vikrti Vijnana, SDM College of Ayurveda and Hospital, BM Road, Thanniruhalla, Hassan, Karnataka, India

3: Microbiologist, Department of Roganidana and Vikrti Vijnana, SDM College of Ayurveda and Hospital, BM Road, Thanniruhalla, Hassan, Karnataka, India

***Corresponding Author: Dr. Radhika Rani V: E Mail: rr180031@gmail.com**

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ABSTRACT

Kaphaja kasa is one of the most prevalent disease of the *pranavaha srotas*. The *lakshanas* mentioned in the description of *kaphaja kasa* can be correlated with most of the symptoms of acute bronchitis. The notable cause of acute bronchitis is the bronchi infections arised by an account of gram positive bacteria. Among that, the prior position is taken by *Staphylococcus aureus*. *Tambula* is indicated in *kaphaja kasa* 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 aqueous extract of *tambula patra* against staphylococcus aureus in patients of kaphaja kasa, 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 aqueous extract of *tambula patra* has antimicrobial activity against *Staphylococcus aureus* in *kaphaja kasa*.

Keywords: *kaphaja kasa*, acute bronchitis, *tambula patra, Staphylococcus aureus*

INTRODUCTION

Ayurveda gives explanations to various drugs possessing *krimigna* action, but indication of specific drug on specific causative microorganism is missing. Therefore, this type of diagnostic tool like culture and sensitivity method along with identification of microorganism and its characteristics is to be incorporated and the drug sensitivity is to be analysed before clinical administration of the drug. Sensitivity test for existing *Ayurveda* drugs are very important as it directs the use of these drugs within a narrow spectrum of activity, thus specific indication. It is also done in order to find out the anti-microbial activity of a drug which possess *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*.

Kaphaja kasa is one of the commonest disease affecting the *pranavaha srotas*. Most of the *lakshanas* mentioned in the classics like *kasa, kapha shteevana* 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 *katu tikta 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 *kaphaja kasa* with special reference to acute bronchitis.

AIMS AND OBJECTIVES:

To evaluate the sensitivity of aqueous extract of *tambula patra (piper betle* Linn.) against *Staphylococcus aureus* from sputum in *kaphaja kasa*(Acute bronchitis) subjects by culture and sensitivity in vitro.

MATERIALS AND METHODS

A minimum of 30 subjects aged between 18-60 years, presenting with classically mentioned *kaphaja kasa lakshanas* 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 *kaphaja kasa*.

- *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)
- *Sirasoola* (headache)

a) Inclusion crieteria

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

Sashonitha kapaha (Reddish brown sputum)

Organisms other than *Staphylococcus aureus*

METHODOLOGY

Aqueous extract of *tambula patra* was prepared by cold maceration method using 100gm of fresh and clean *tambula* leaves. Crushed *tambula* leaves were then added to 500ml distilled water 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 7^{th} day the content in the conical flask was filtered, that yielded 420ml of filtrate. This filtrate was kept over water bath at 60^{o}C. 3.06 gms of aqueous extract was obtained by this process.

Early morning thick sputum sample from the subjects of *kaphaja kasa* 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 *tambula patra* were prepared by dissolving 3g of aqueous extract in 6ml of distilled water that gave a stock solution containing 3000µl/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 aqueous 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 aqueous 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 aqueous extract of *tambula patra* was evaluated by agar well diffusion method and mean zone of inhibition was measured as shown in **Table 1**.

Table 1: Mean values of zone of inhibition at different concentrations of aqueous extract of *tambula patra*

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

The in- vitro study showed that the susceptibility of *Staphylococcus aureus* against aqueous extracts of *tambula patra* was fairly evident between 20-16 mm zone of inhibition, hence it was considered as sensitive, 15-10 mm was intermediate, hence moderately sensitive and below 10 mm as resistant. In the present study, *Staphylococcus aureus* is sensitive to 3000 µg/ml, 2000µg/ml and 1000µg/ml, moderately sensitive to 900µg/ml, and resistant to 800 µg/ml and 700 µg/ml.

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 effect according to *raja nighantu* **[7]**. At the same time, *priya Nighantu* mentioned the drug under *pippalyadi varga* and possess the *janthujith karma* **[8]**. *Tambula* possess *katu, tikta, kasaya rasa, tikshna, ushna, kshara guna, ushna veerya* and is *kapha vata shamaka* and has *deepana, pachana* **[9]** and *janthujith karma*.

PROBABLE MODE OF ACTION OF TAMBULA

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. 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 aqueous extract of *tambula patra (piper betle* Linn.) has antimicrobial action against *Staphylococcus aureus* from sputum sample of *kaphaja kasa* (acute bronchitis). Further it is also evident that as the concentration of the aqueous extract of *tambula patra (piper betle* Linn.) increases, the zone of inhibition for *Staphylococcus aureus* also increases.

REFERENCES

[1] Agnivesha, Caraka, Dridabala, Acharya YT. Caraka Samhita with Ayurveda Deepika commentary of Chakrapanidatta on Carakasamhita by Agnivesha .Chiktsasthana; kasa chiktsa adhyaya; chapter 18: verse 4. Varanasi, Chaukhamba Vishwa Bharati Academy; 2008.

[2] Naidu ET, Sitaram B.A comparative clinical study of aswagandha kshara in the management of *kaphaja kasa*. An international journal of research in AYUSH and allied Systems, AYUSHDHARA, may-june 2018, vol 5, issue 3, p1721

[3] MacSween NMR, Whaley K, Muir's Text book of pathology, ELBS, 1992.p 531

[4] Dwivedi V. Commentary on Raja nighantu by *narahari; amradi varga*, verse 246-47,p 390

[5] Acharya PS. Priya Nighantu; chaukambha surabhi prakasan

Varanasi, pippalyadi varga;verse26-28,p 62

[6] Agnivesha, Caraka, Dridabala, Acharya YT. Caraka samhitha with Ayurveda Deepika commentary of chakrapanidutta on caraka samhitha by agnivesha. Sutrastana; Matrasitiya adhyaya: chapter 5,verse 78-80.

[7] Dwivedi V. Commentary on Raja nighantu by narahari; amradi varga, verse 246-47,p 390

[8] Acharya PS. Priya nighantu; chaukambha surabhi prakasan varanasi pippalyadi varga; verse26-28, p 62

[9] Sushruta. Su 46/279-280. In: Y T Acharya (eds.) Sushruta Samhita. Varanasi: Chaukhambha Publications; 2017. P.234

[10] Pelczar JM, Chan ECS, Krieg RN, Microbiology, Mcgraw hill education 2018, chapter 24, p.517.