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Antibacterial Effect of *Karna Dhoopana Yoga* Against *Staphylococcus aureus* in the Management of *Puti Karna* (CSOM)

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ABSTRACT

Pūti Karna is a Chronic Disease caused by vitiated Kapha and Pitta dosha with its characteristic features of profuse thick Pūti (foul odor), pūya (pus) discharge from the ear with or without pain. This entity can be correlated with the disease specified in modern ENT (ear, nose and throat) known as Chronic Suppurative Otitis Media (CSOM). This disease entity in turn leads to severe complications as facial paralysis, lateral sinus thrombosis, labyrinthitis, meningitis and brain abscess. According to the Ayurveda system of medicine Pūti Karna can be effectively managed by a very effective local modality known as a Karna Dhoopana. This study was carried out at the central laboratory of Gampaha Wickramarachchi University of Indigenous Medicine, Sri Lanka, using specially prepared fumigation instrument. This Karna Dhoopana Churna has mentioned in "Hasthasara Aushada Yoga Samgraha". According to the observation, results of the study confirmed the fumigation for 30 minutes has a bactericidal effect on Staphylococcus aureus in vitro. This could be due to the bactericidal components present in the fumes of dhupana yoga. Further, this study confirmed that duration of Karna Dhoopana is an important factor. Its clinical effectiveness was confirmed without any reported complications after Karna Dhoopana treatment. Further, the study proved that the temperature of the fumes does not produce any side effects in the ear. Therefore, this study endeavored to validate the clinical data scientifically.

Keywords: Karna Dhoopana; Chronic Suppurative Otitis Media; Puti Karna

INTRODUCTION

 $P\bar{u}ti \, Karna$ is a Chronic Disease caused by vitiated Kapha and $Pitta \, dosha$ with its characteristic features of profuse thick $p\bar{u}ti$ (foul odor), $p\bar{u}ya$ (pus) discharge from the ear with or without pain [1]. This entity can be correlated with the disease described in modern ENT (ear, nose and throat) known as Chronic Suppurative Otitis Media (CSOM). CSOM is a chronic inflammation of the middle ear and mastoid cavity which presents recurrent foul smelling suppurative discharge through a tympanic perforation [2]. It is a major cause of acquired hearing impairment in children as well as adults, especially in developing countries.

Over 90% of the burden is borne by countries in the South-East Asia and Western Pacific regions, Africa and several ethnic minorities in the Pacific Rim. Global burden of illness from CSOM involves 65 – 330 million individuals with draining ears, 60% of whom (39 – 200million) suffer from significant hearing impairment [3]. CSOM accounts for 28000 deaths and a disease burden of over 2million. This disease entity in turn leads to severe complications such as facial paralysis, lateral sinus thrombosis, labyrinthitis, meningitis and brain abscess [4]. While considering its management, most approaches to its treatment have been unsatisfactory or are very expensive and difficult.

According to the Ayurveda system of medicine, *Pūti Karna* can be effectively managed by a very beneficial local modality known as a *Karna Dhoopana*^[5]. This study was designed to determin the anti-bacterial activity of each samples on *Karna Dhoopana* yoga by using the spread plate method (Qualitative) against the *Staphylococcus aureus* (ATCC 25923).

RESEARCH METHODOLOGY

This study was carried out at the central laboratory of Gampaha Wickramarachchi University of Indigenous Medicine (GWUIM), Sri Lanka by using specially prepared fumigation instrument. This *Karna Dhoopna Churna* was mentioned in "*Hasthasara Aushada Yoga Samgraha*". This recipe belongs to *Godamunne* Tradition of Sri Lankan traditional medicine.

Drug preparation:

All the ingredients were identified and authenticated by the Department of *Dravyaguna*, Faculty of Indigenous Medicine, GWUIM, Sri Lanka. It was prepared according to *Churna Paribhasha* ^[6].

Sanskrit Name	Scientific Name	Part used
Punarnava	Boerhavia diffusa	Roots
Apamarga	Achyranthes aspera	Roots
Devadara	Cendrus deodare	Stem
Vacha	Acorus calamus	Rhizome
Sarshapa	Brassica campestris	Seeds
Nimba	Azadirachta indica	Tender leaves
Rasona	Allium sativum	Dried cover of fruit

TABLE 1: Ingredients of *Dhoopana Yoga*

Equal quantity of each ingredient in fine powders have been mixed well and prepared.

Microbial culture preparation

The antibacterial activity of each samples was tested against *Staphylococcus aureus* (ATCC 25923) using the spread plate method ^[7] (Qualitative). *Staphylococcus aureus* (ATCC 25923) was grown in a separate plate from a stock culture. A single colony was drawn with a sterile wire loop and dissolved in sterile nutrient broth. This was incubated overnight at 37°C to prepare a broth culture of *Staphylococcus aureus*.

After incubation, the culture was adjusted by adding sterilized peptone water until the turbidity matched that of a McFarland $0.5~(10^6-10^8~cells/ml)$ standard $^{[8]}$.

Antibacterial susceptibility testing

The agar well diffusion method was performed to assess the susceptibility of bacteria against *karna dhoopana churna*. The turbidity adjusted bacterial culture ($100\mu L$) was pipetted out onto Mueller-Hinton Agar plates ^[9]. Then the inoculum was spread over the agar surface evenly using sterile cotton swab.

Karna Dhoopana Procedure

• Method of Fumigation

Test 01

The culture plate was kept in a fumigation box and fumigated by using $Karna\ Dhoopana$ drug until 10 minutes. Then the plate was incubated at $37^{\circ}C/24$ hours.

Control 01

The pure culture plate was kept in the incubator (at $37^{\circ}C/24$ hours) without smoke.

Test 02

The procedure was adopted as in the test 01 and fumigation time was extended up to 30 minutes.

Control 02

The culture plate was kept in fumigation box and fumigated by using only charcoal fumes until 30 minutes. (Without *Karna Dhoopana Churna*). Each test was triplicated.

Karna Dhoopana Procedure



In vitro study



In vitro study

FIGURE 1

RESULTS

Test	Procedure	Bacterial Growth (+/-)
Control 01	Culture without fumigation	Positive (+)
Control 02	Culture + Charcoal fumigation – 30 min	Positive (+)
Test 01	Culture + Charcoal + Drug fumigation - 10 min	Positive (+)
Test 02	Culture + Charcoal + Drug fumigation - 30 min	Negative (-)

TABLE 2: Observation & Results

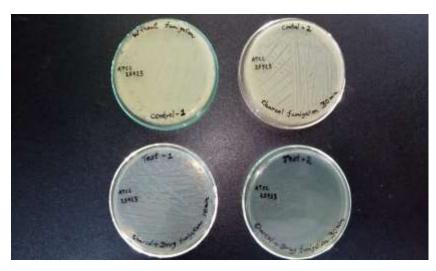


FIGURE 2

EVIDENCES FROM MODERN LITERATURE TO SUPPORT THE CLAIM OF ANTI-BACTERIAL ACTIVITY OF KARNA DHOOPANA YOGA AGAINST STAPHYLOCOCCUS AUREUS

Priya et.al. [10] confirmed that methanol and aqueous extracts of Boerhavia diffusa stem and root showed high therapeutic activity due to the presence of potential phytochemicals such as flavonoids, diterpenoids, alkaloids, tannin and saponins and also its' anti- inflammatory potential was observed through several studies. Boerhavia diffusa is an important plant in an inflammatory process therefore, in an indigenous literature it is called "Sothaghni", which means alleviation of inflammation. Therefore, it is widely included in several Ayurveda formulations as an ingredient. Several studies have been reported in which leaves and roots were either used locally or orally to produce promising results such as wound healing and anti-inflammatory potentials due to the presence of liridendrin, quercetin and kaempferol [11]. Further studies done by Umamaheswari et al. [12] on various extracts of Boerhavia diffusa against gram positive and gram-negative microbes confirmed its' highest antimicrobial activity.

Naidu et al. [13] work also confirmed the largest zone of inhibition by methanol, chloroform and n-hexane extracts of whole plant of *Achyranthes aspera* to be against *Staphylococcus aureus, Bacillus subtilis, Salmonella typhi* and *Escherichia coli* while compared with standard positive control of ciprofloxacin and Clotrimaxazole. In another study, water extract of *Cedrus deodara* showed sensitive inhibition against *Staphylococcus aureus*, reportedly proposed as an alternative source for the treatment of bacterial infections [14].

The phytochemical screening of *Cedrus deodara* leaf parts showed the presence of flavonoids, alkaloids, tannins and saponins, while the stem wood of the plant lignin mixture consisted of wokstromal, matairesinol, benzylbutyrolactol, cedeodarin, dihydromyricetin, cedrin, cedrinoside and isohimachalone^[15].

Dalal et al. ^[16] investigated the antibacterial susceptibility of *S. aureus*, *S. epidermidis* and *Pseudomonas aeruginosa* to the *Brassica campestris* plant extracts using agar well diffusion method. The overall results confirmed that *B. campestris* demonstrated an appreciable antibacterial activity ^[16]. The phytochemical screening identified the presence of saponins (12.82%), alkaloids (20.58%), flavonoids (6.57%), glycosides (20.01%), reducing sugar (5.56%), phlobatanins (15.05%) and volatile oil (25.13%) in the aqueous extract of *Brassica* ^[17].

Several studies have reported the antimicrobial activity of *Acorus calamus* roots, rhizome and essential oils; with rhizome and leaf extracts predominantly possessing antifungal and anti-yeast activities except against E. coli [18]. MacGaw et al. [19] reported that ethyl acetate was the best solvent for extraction of active ingredients (α and β -asarone) from rhizome and leaves of *Acorus calamus*. This study further established that the α and β - asarones found in leaf, roots and rhizome tissues are responsible for all the antimicrobial activities of *Acorus calamus*. In addition, Kumar et al. [20] in their study on *Acorus calamus* revealed 10 different active phytocomponents. Among these, alkaloids, Palmitic and Linoleic acids are the three important components identified as responsible for antimicrobial, anti-inflammatory and antioxidant activities.

Vinoth et al. [21] identified the antibacterial activity of ethanol, acetone and methanol extracts of *Azadirachta indica*. It was observed that ethanol extract exerted 19mm of maximum zone of inhibition, while acetone and methanol extracts exerted 18mm and 12mm, respectively against *Staphylococcus aureus*. Phytochemical analysis of same study investigated and confirmed the presence of alkaloids, tannins, flavonoids, terpenoids, saponins and glycosides, which are responsible for antimicrobial, anti-inflammatory and wound healing activities.

EL- Mahmood [22] observed in his study *Staphylococcus aureus* was most susceptible to the active ingredients present in garlic. It had zones of growth inhibition diameters of 28mm in water extract, 24mm in ethanol and 23mm in chloroform extracts.

Phytochemical studies of *Allium sativum* [23] showed the presence of carbohydrates, reducing sugars, lipids, flavonoids, ketones, alkaloid, steroids and triterpenes under water and ethanol extraction, while acetone extraction did not yield alkaloids, steroids and triterpenes. Alkaloids possess analgesic, antibacterial and antispasmodic effects.

All these scientific evidences supported and confirmed each of these actions of individual herbs synergistically leading to potential antibacterial activity of *karna dhoopana* yoga against *Staphylococcus aureus*.

DISCUSSION

The disease *Puti karna* can be correlated with CSOM due to the similarities of its symptoms. CSOM is commonly seen in the society as an exigent problem due to reappearance and resistance to therapy. Treatment recommendations have included local debridement, local and systemic antimicrobial agents.

Sometimes, it is a challenging disease because of its longterm treatment and follow up, yet its recurrence rate remains high.

While considering the pharmacodynamics of *karna dhoopana churna*, it is predominant with *katu, tikta* rasa, *laghu, tikshna, ruksha* guna, *ushna virya, katu vipaka* with *tridosa shamaka* especially *kapha pitta samana* properties. In addition to that, ingredients of *karna dhoopana* yoga has *shothahara* (anti-inflammatory), *krimighna* (anti-microbial), *vrana shodhana* (wound cleansing), *vrana ropana* (wound healing) and *putihara* (pus removal) actions make the middle ear cavity and mastoid structure aseptic and dry. The scientific evidences confirmed by previous studies significantly proved the pharmacological actions mentioned in Ayurveda texts on ingredients of *Karna Dhoopana Yoga*.

CONCLUSION

Puti karna is a chronic condition which causes a long-term suffering to the individual. This chronicity can be broken down through the dhoopana karma. In the present era, dhoopana karma can occupy a major position as a disinfectant as it is eco-friendly and comparatively cheaper [24]. This local treatment is more successful than systemic administration as it treats the disease effectively. Therefore, in vitro antimicrobial study proved that the fumigation for 30 minutes has a bactericidal effect on Staphylococcus aureus (ACTT 25923). This could be due to the bactericidal components present in the medicinal fumes. Also, according to this study, duration of Karna Dhoopana is an important factor, as well as the temperature of the fumes. This did not produce any side effects in the ear as the temperature is approximately equal to the room temperature.

Finally, the clinical effectiveness of *Karna Dhoopana* without any of the reported complications after treatment further confirmed its scientific basis. *Karna dhoopana* is one of the best local therapies, which is beneficial for discharging ear cases like *puti karna*, *karna srava* and *krimi karna* [25].

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None declared

CONFLICT OF INTEREST

None declared

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