

SCREENING FOR ANTIMICROBIAL AND SELECTED MINERAL COMPONENTS IN THE STEAM DISTILLATE OF *CARUM COPTICUM*

V.SATHIYASEELAN*K.NIRANJAN**K.VELAUTHAMURTY***

Introduction

Herbal based medicines are mainly used in the system of medicine. There are 64 varieties of medicinal preparations that are practiced in Siddha Ayurvedic system of medicine. Steam distillation is one of important method of medicinal plant preparation. This is called as "Theeneer" as mentioned in Siddha system of medicine. This preparation method is practiced by the Siddha Ayurvedic graduates and also by the traditional practitioners. According to the concept of "Food is medicine; medicine is food" non-toxic herbal based products can be used to treat patients aging from childhood to senile.

Justification of the study

Until now there has been no scientific screening of antimicrobial and mineral components steam distillate of *Carum copticum*. Since *carum copticum* is used as a home remedy and as food supplement there is no proven side effects.

Literature review

Carum copticum belongs to umbelliferae family.

Tamil name- Omam .

Sinhala name- Asamodagam

English name- lovage

A minutely pubescent herb with a fusiform root, stem 30-90 cm tall erect, branched leafy, leaves pinnate, ultimate segments of all leaves linear, bracts several linear, bracteoles 3-5 enlarged or irregular petals, calyx-tube adnate to ovary, calyx teeth small, petals 5, emarginated, stamens 5, epigynous, ovary inferior, 2-locular, disc 2-lobed, styles 2, stigmas capitate, ovules one in each loculus, pendulous, fruit ovoid, 2mm long, carpels subpentagonous, dorsally compressed, ridges distinct, vittae solitary, small.

The dry fruits possess carminative, stomachic, tonic, aromatic, antispasmodic, and antiseptic properties. The Siddha quotation consists of abdominal pain, flatulence, indigestion, diarrhea, cholera, asthma, and skin disorders can be cured by *Carum copticum*. – K.S. Murugasamudhaliyar Gunapadam (Mooligai Vakuppu).

Unit of Siddha Medicin*, Department of Botany** and Department of Chemistr***
University of Jaffna, Sri Lanka



Materials and Methods

Preparation of Steam distillate

Omaththeeneer(distillate of *Carum copticum*)

Distilled water, *Carum copticum* dry fruits, and traditional steam distillatory apparatus are required for the preparation. This preparation was carried out in the pharmacy laboratory at the unit of Siddha medicine. *Carum copticum* and water were mixed in the ratio of 1:6:20. The mixture was kept for 24 hours. Then the traditional steam distillatory apparatus was prepared and heated to obtain the final product. Seven hundred and fifty ml omaththeeneer was collected and bottled in a sterilized container according to the Siddha text ThiyagarajaMudhaliyar-GunapadamThaathujeevam.

Study 1

Antibacterial screening of steam distillate of *Carumcopticum*

This was kept in refrigerator until it is used for the screening of antibacterial effect and This distillate kept in refrigerator until it is used for the screening of antibacterial effect and minerals components. In-vitro screening of the above distillate for antibacterial activity was carried out with different bacteria *Staphylococcus aureus*, *Proteus vulgaris*, *Bacillus sp.*, *E.coli*, *Serratia sp.*, *Pseudomonas aerogenosa* and *Klesiella sp.* by agar well diffusion method. 10^{-6} cells/ml bacterial suspensions were prepared by ten fold dilution method. Haemocytometer was used to count the bacterial cells. Spread plates of bacteria were prepared by adding 0.1 ml of bacterial suspension onto freshly prepared solid Nutrient Agar (NA) medium in a petridish and spreaded with the help of sterilized glass spreader to ensure the uniform distribution of bacterial cells and then two wells were made in each plate with the help of 6 mm diameter cork borer and 100 μ l of distillate and Streptomycin (1mg/1ml) were dispensed separately into the wells of each bacteria spreaded plates with the help of micro pipette (triplicate plates). Streptomycin and distilled water were used as (+) ve and (-)_ve control respectively. These plates were incubated at 37°C for 24 hours and the

inhibition zones were recorded. The results were subjected to one- way ANOVA followed by LSD test.

Bacteria	Diameter of clear zone(mm)#	
	Streptomycin	Distillate
<i>Staphylococcus aureus</i>	22.0 ± 0.01 ^{ef}	16.0 ± 0.01 ^a
<i>Proteus vulgaris</i>	35.0 ± 0.01 ^a	12.0 ± 0.01 ^d
<i>Bacillus sp.</i>	22.5 ± 0.01 ^c	12.0 ± 0.01 ^d
<i>E. coli</i>	28.5 ± 0.01 ^d	14.0 ± 0.01 ^b
<i>Serratia sp.</i>	30.0 ± 0.01 ^b	13.0 ± 0.01 ^c
<i>Pseudomonas aerogenosa</i>	29.7 ± 0.06 ^{bc}	13.0 ± 0.01 ^c
<i>Klebsiella sp.</i>	30.3 ± 0.07 ^b	—

— No activity

Zone of inhibition including the diameter of the well (6mm)

Values are mean ± SD and values with different superscript on the same column are significantly (p<0.05) different

The standard antibiotic Streptomycin shows higher inhibition than the distillate for all tested bacteria.

Distillate also shows remarkable antibacterial effect to all tested bacteria except *Klebsiella sp.*. Inhibition effect on *Proteus vulgaris* is equal to the effect on *Bacillus sp.* and the effect on *Serratia sp.* is equal to the effect on *Pseudomonas aerogenosa*. There is significant difference between these two pairs. Also they significantly differs from *Staphylococcus aureus* and *E.coli*. As the tested bacteria are infectious agents of common cold, fever, food borne diseases and wound infections. Therefore we can use this *Carum copticum* distillate ('omaththeener') as a medicated water to get relief from these problems.

Study 2

Screening for selected mineral components in the steam distillate of *Carum copticum*

Determining of the total amount of calcium and magnesium

Procedure

10.0ml of aliquots of the test solution was pipette into the titration flask. 2ml of buffer solution (pH=10), 2 drops of Erichrome Black T indicator and 1ml of masking reagent were added to the above solution. Then the resulting solution was titrated against standard EDTA solution. At the endpoint colour of the solution changed from wine red to blue.

Determining the amount of calcium in the presence of magnesium

Procedure

10.0ml of aliquots of test solution was pipette into the titration flask. Then 1ml of KOH(8M) was added to it, the resulting solution was allowed to stand for 5 minutes, with occasional

shaking. Then 1ml of masking reagent and 2 crystals of Patton and Reeder's were added to the above solution. Then the resulting solution was titrated against the standard EDTA.

Determining the amount of Sulphate

The amount of sulphate present in the water samples was determined by an indirect titration procedure.

Procedure

50.0ml of water sample was concentrated and made up to 25ml in a volumetric flask and used as the test solution. 10.0ml of test solution was pipette and excess of known volume of BaCl_2 (10ml of 0.01M) was added. (The sulphate will be precipitated as barium sulphate). The excess barium ions present in the solution was determined by titrating the resulting solution with standard EDTA solution. Magnesium chloride (0.01M, 5.0ml) was added near the end point to detect the end point clearly and titrated against the same standard EDTA solution.

Determining of amount of the nitrate

Procedure

Initially program number was 355 was entered in DR 2400 Spectrophotometer for nitrate. Then wave length was adjusted to 500nm. Then enter key was pressed. 25ml sample was measured into dry sample cell. 25ml demineralized water was measured into a dry sample cell. Nitrate 5 nitrate powder pellet was added into each cell and it was shaken vigorously. It was allowed for 2 minutes for reaction period. Then the cell was placed into the cell holder. The sample was checked. Zero key was pressed, and after the sample was placed into the cell holder. Finally reading was recorded.

Results

Ca^{2+} - 8.48ppm

Mg^{2+} - 26.42ppm

SO_4^{2-} - 10.84ppm

NO_3^- - 14.6ppm

Discussion and Conclusion

It is showed better anti microbial activity. Therefore we can use this distillate as a medicated water to cure urinary tract infection and fever and also to some symptoms due to the consumption of spoiled foods. The distillate of *Carum copticum* found to be chemically safe for drinking purposes and it should be tested for the presence of microorganism.

The above results shows that the calcium, magnesium, sulphate, nitrate levels are found to be within the Sri Lankan standard level in order to use this as medicated mineral waters we should study about its microbiological quality and storage life. The standard antibiotic Streptomycin shows higher inhibition than the distillate for all tested bacteria. Distillate also shows remarkable anti bacterial effect to all tested bacteria except *Klebsiella sp.* inhibition

effect on *Proteus vulgaris* is equal to the effect on *Bacillus sp.* and the effect on *Serratia sp.* is equal to the effect on *Pseudomonas aerogenosa*. There is significant difference between these two pairs. Also they significantly differ from *Staphylococcus aureus* and *E. coli*. As the tested bacteria are infectious against respiratory diseases, skin diseases, and urinary infection. Therefore we can use this *Carum copticum* (Omatheeneer) as a medicated water to get relief from these problems. Since the plant raw material is easily available, flavourable, and has rich medicinal values we suggest that steam distillate of *Carum copticum* is marketable for herbal mineral water.

References

- Murugesamuthaliyar, K.S Gunapadam Part I Mooligaivagupu 7th edition Indian medicine & homeopathy dept Chennai.
 - Jeyaweera, D. M.A ; Medicinal plants used in Ceylon Part V National Science Council of Sri Lanka.
 - Nadkarni K.M; Indian Material Medicavol I reprint 2002.
 - The wealth of India Raw materials Vol VIII :(Ph-Re)A reprint 2003 National institute of science communication.
 - Kirtikar.K.R&Basu B.D Indian Medicinal plants volume II 2nd edition,1993.
 - E.ChristyJeyaseelan ,S.Tharmila, V.Sathiyaseelan and K.Niranjan. Antibacterial activity of various solvent extracts of some selected medicinal plants present in Jaffna peninsula. International Journal of Pharmaceutical and Biological Archives 2012; 3(4)
 - Christy Jeyaseelan E, Tharmila S, Kavitha R, Niranjan K and Thavaranjit AC. Antibacterial activity and phytochemical analysis of different aqueous extracts of *Sennaalata*. Vavuniya campus annual research session, 2011.
 - R.Mageswaran, K.Velauthamurty, Some quality parameters of ground water in Jaffna peninsula, 2003, 3, Jaffna Science Association.
- Text book of Vogel's quantitative analysis, 5th Edition.