Scope
Volume 14 Number 02 June 2024

Antibacterial, Antifungal Activity and Gas Chromatography Massspectrometry of Coconut Shell Liquid Smoke (Cs-Ls)

Mageshwaran B[1], Bhuvaneshwari Gunasekar[2]*, Jamith Basha[3] & Catherine Thamayendi[4]

[1]M.Sc., student, Molecular Virology, King Institute Of Preventive Medicine and Research , Guindy, Tamilnadu, India.
[2]Assistant Professor, Department of microbiology, Saveetha Medical College, Saveetha Institute of Medical and Technical Sciences, Thandalam, Tamilnadu, India.
[3]Faculty of Applied Medical science, Northern Border University Saudi Arabia.
[4]M.Sc., student, Department of microbiology, Saveetha Medical College, Saveetha Institute of Medical and Technical Sciences, Thandalam, Tamilnadu, India

*Corresponding Author –**G.Bhuvaneshwari**

Abstract: According to World Health Organization (WHO), the prevalence rate of superficial fungal infection and bacterial infection in worldwide has been found to be 20-25%. However, the increase in antibiotic resistant strains together with the lack of and high cost of new generation antibiotics increased woundrelated morbidity and mortality. So far, Herbal medicines are being used by about 80% of the world population mostly in the developing countries for primary health care. These medicines have stood the test of time for their safety, efficacy, cultural acceptability and lesser side effects. Traditionally used coconut shell liquid smoke a natural resources has been used as antibacterial and antifungal agent. The aim of this project is to analysis the antibacterial, antifungal activity and gas chromatography massspectrometry of CS-LS Extract .In this study identification of the organism wea carried using convential method and species were confirmed by biochemical test,most of the orhanism were comes under *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and fungal organism *Aspergillus niger, Aspergillus fumigatus, Candida albicans, Rhizopus* species were done by conventional method. Antibacterial susceptibility was performed by Agar well diffusion method and Its components were identified by Phytochemical analysis using Gas Chromatography Mass Spectrometry for the identification of active compound. CS-LS showed zone of inhibition for bacterial and fungal organism except Rhizopus in the agar cup diffusion method. It was compared with standard drugs and antiseptic and can be used in the treatment of skin related infections. The phytochemical analysis relived the major organic component which can incorporated in disinfectant and antiseptic in future to overcome the hospital acquired infection.

Key words Coconut shell liquid smoke CS-LS, antibiotic, antimicrobial Susceptibility test

Introduction:

Coconut (Cocos nucifera) are found in tropical countries and are considered as important fruit crops. Even though the shell often considered as agro waste we used its leaves for thatching our roofs and its wood to build houses, coir for making ropes, bark as fillers in cement industry and its fruit to quench thirst. The nut contains white meat and sweet water coconut shell contain more antimicrobial activity the extracted liquid from coconut shell are coconut shell liquid smoke (CS-LS) . The common wound pathogens includes bacteria, fungi, protozoa and viruses among which the most common are *Streptococcus pyogenes*, *Staphylococcus aureus, Pseudomonas aeruginosa*, Proteus, *Escherichia coli*, Enterococcus, Acinetobacter, Klebsiella, and other Coliforms. Although wounds may heal through the body's natural process of regenerating dermal and epidermal tissues, chronic forms cause significant impact on health and economic growth. Current methods used to treat chronic wounds include debridement, irrigation, antibiotics, tissue grafts and proteolytic enzymes, which have major drawbacks and unwanted side effects. Topical antimicrobials may be indicated when the clinical signs and symptoms of an active infection are present. Complications of deep tissue infections such as bacteremia can be treated with systemic antibiotic. However, the increase in antibiotic resistant strains together with the lack of and high cost of new generation antibiotics increased wound-related morbidity and mortality. A wider range of plants are being used in the treatment of wounds and other diseases in the traditional health care system. Crude extracts of plants and others used elsewhere revealed strong antibacterial activities indicating that these plants can serve as sources of effective drugs against wound-causing bacteria.

Herbal medicines are being used by about 80% of the world population mostly in the developing countries for primary health care. These medicines have stood the test of time for their safety, efficacy, cultural acceptability and lesser side effects. Indian medicinal drugs and their derivatives have been an invaluable source of therapy due to their antibacterial, antihelmintic, anti-ulcer, anti-inflammatory and even anticancer and antioxidant activity. In recent years, among the world population, there is an increasing trend towards the usage of herbal medicines Coconut Shell Liquid Smoke (CS-LS) known to be good preservative agent substituting formalin, due to its antibacterial activity thus has inhibitory effect of pathogens. CS-LS has Antimicrobial, antifungal, anti-inflammatory and antioxidant activity [1,2] which is mainly due to the presence of aldehyde, carboxylic acid and phenols. It is also used for burns, corns, warts, eczema, athelet' s foot, ring worm and other bacterial and fungal organisms such as *Candida, Aspergillus*, *Staphylococcus aureus* and Alopecia areata a type of hair loss etc, It is also used as activated carbon and charcoal.

According to World Health Organization(WHO), the prevalence rate of superficial fungal infection worldwide has been found to be 20-25%. [1] In developing countries

superficial fungal skin diseases are very common if not treated properly will become more complicated and life threatening rarely. These are more prevalent in tropical and subtropical countries like India where the heat and humidity is high for most part of the year.

Material and Method

A cross sectional study was conducted at Clinical Microbiology Laboratory, Saveetha Medical College and Hospital, Department of Microbiology during the period January 2022 to June 2022. Institutional Review Board approval was obtained and the approval no. is SCAHS/IRB/2022/May/326. Continuous sampling was done.
Test Organisms
Specimen yielding *Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa* were included in this study . Totaly 90 isolates of bacteria were isolated and they were processed by Standard Conventional procedure like agar plating and Biochemical identification test

Indentification of Bacteria:

Microscopy-Gram staining ,Culture ,Identification of bacteria by Bio-chemical test.
Microscopy: Smears were prepared by placing a loopful of sample on a clear glass slide and gram staining was done for microscopic examination.

Gram stain procedure

The smear was flooded with crystal violet and was allowed to stand for 1 minute.The stain was poured off and rinsed with tap water and excess water was shaken off.Then Grams iodine solution was allowed to stand for 1 minute.Smear was rinsed in tap water.Slide was held in slanting position and decolorized with acetone and it washed quickly in tap water.Finally, safranin was added and left for 30 seconds and then washed off.Smear was blot dried and examined under oil immersion objective.

Culture: All samples were inoculated in Nutrient agar, blood agar and Mac conkey agar and the plates were incubated overnight at 37^{o}C.

Staphylococcus aureus

Microscopy examination: Gram-positive cocci in clusters, Culture characteristics: It produces golden yellow pigmentation on nutrient agar and beta hemolytic colonies on blood agar. Biochemical test: catalase positive, coagulase positive.

Escherichia coli

Microscopy examination: Gram-negative bacilli, Preliminary test: Catalase positive and oxidase negative, Culture characteristics Blood agar: Circular, grey, moist colonies, hemolysis variable MacConkey agar: flat pink, lactose fermenting colonies

Biochemical test: Indole Positive, Mannitol motility Fermented and Motile

Pseudomonas aeruginosa
Microscopy examination: Gram negative bacilli, Preliminary test: Catalase positive and oxidase positive Culture characteristics Nutrient agar: opaque irregular colonies with blue green diffusible pigment Blood agar: beta hemolytic grey moist colonies. MacConkey: non lactose fermenting colonies Biochemical test: based on indole production

Aspergillus niger

Colonies - black, cottony type, reverse is white LPCB (Lactophenol cotton blue staining): Vesicles globular – shaped, phialides in two rows, conidia from entire vesicle Conidia are black in color
Aspergillus fumigatus

Colonies – smoky green,velvety to powdery, reverse is white LPCB: Vesicle is conical – shaped, phialides arranged in single row, conidia arise from upper third of vesicle, conidia are hyaline.

Rhizopus

Colonies on SDA show white cottony wooly colonies with black spores (salt and pepper appearance) LPCB: colonies sporangium with rhizoid present

Candida albicans

Colonies appear creamy white, smooth and pasty with typical yeasty odor Gram staining of the colonies show gram positiveOnce the pot is sealed, the sides and top of the pot are covered by cow dung cakes, which are then burnt. The resultant heat produces medicinal oil from the coconut shell pieces inside the pot [3]

Mueller Hinton Agar:

In vitro antibiotic susceptibility tests were done for all isolates to antimicrobial agents by the Kirby-Bauer disc diffusion method. Mueller-Hinton agar is recommended for the disc diffusion method of antibiotic susceptibility testing. Antimicrobial susceptibility test discs impregnated with known amount of antibiotics were placed on the agar surface. The plates were incubated at 37°c for 24 hrs, next day the plates were examined for the presence and size of inhibitory zones. The diameter of the inhibitory zone includes the diameter of the disc. Inhibitory zones for each antimicrobial agent were measured and the zone of inhibition was interpreted as sensitive, intermediate, or resistant with the standard chart.[7]

Procedure :Lawn culture of the organism was made on a sterilized Mueller Hinton agar from standard inoculums Readymade antibiotic discs were placed on the surface of inoculated plates. The plates were incubated at 37°C for 24 hrs. After incubation, the

plates were observed for the zone of inhibition. An inhibitory zone around the disc indicated that the growth of the organism was inhibited by the antibiotics. The results were compared with the standard charts and were tabulated.

Extraction of CS-LS

·Extraction of coconut shell liquid smoke was done as per the procedure given in the 'Kuzhi Thalika' method. Under this method, thin wires are inserted into a clay pot through small circular holes. These thin wires are pulled out of holes on the bottom of the pot and tied into a knot so that it holds firm. After that, the upper ends of the pot are filled with pure, well dried coconut shell pieces. Once the pot is sealed, the sides and top of the pot are covered by cow dung cakes, which are then burnt. The resultant heat produces medicinal oil from the coconut shell pieces inside the pot [3]

Agar well diffusion for antibacterial activity

Agar well diffusion technique were carried out in this study to evaluate the antibacterial and antifungal property,Agar using lawn culture technique the plate where inolicilation bacterial suspension of standard inoculums 0.5 McFarland standard. Three hole were made in the agar surface at equal distance and CS-LS is incouclation the hole at different concentration 5µL,10µL,20µL. Then plate is incubated in 37° C for 24 hours. Observing the zone of inhibition and diameter is measured in millimeter [4]

Disk diffusion method for antifungal activity

Antifungal activity of CS-LS was tested by using Disc diffusion method by Mueller-Hinton agar. *Candida albicans,Aspergillus niger*, *Aspergillus fumigates* and *rhizopus* are impregnated on disc and incubated for 25° C for 48 hours. Concentration of CS-LS 5µL, 10µL ,20µL and Fluconazole (10mcg) was used as standard control drug, zone of inhibition is measured in millimeter.[5]

Components identification:

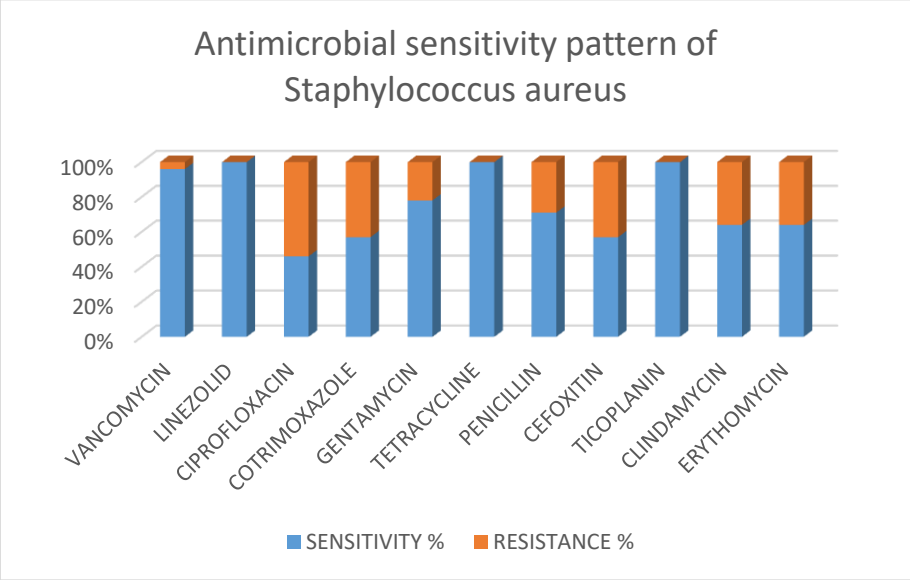
The CS-LSextract is collected in the sterile air tight cointainer and transported to performed Gas Chromatography Mass spectrometry (SRM, SCIF) to identify the components

Result

The study was done with total of 90 isolates of *Escherichia coli* (34), *Pseudomonas aeruginosa* (28) and *Staphylococcus aureus* (28). The antibiotic susceptibility pattern of Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli were explained in Figure 1,2 and 3 respectively. Methicillin Resistant Staphylococcus aureus (MRSA) prevalence was noted to be 43%. All strains were sensitive to Tetracycline and

Linezolid. Vancomycin Resistant Staphylococcus aureus (VRSA) prevalence was identified as 4%.

Figure 1: Antibiotic Susceptibility Result Staphylococcus aureus (28 isolates)



In this study Multidrug resistant (MDR) *Pseudomonas aeruginosa* was identified as 54%

Figure 2: Antibiotic Susceptibility Result Pseudomonas aeruginosa (28 isolates)

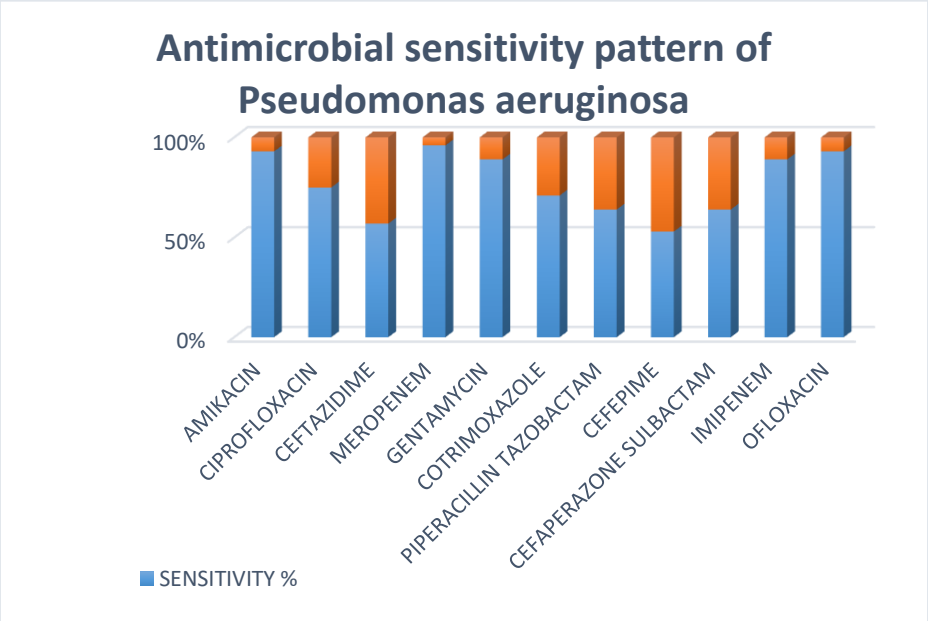
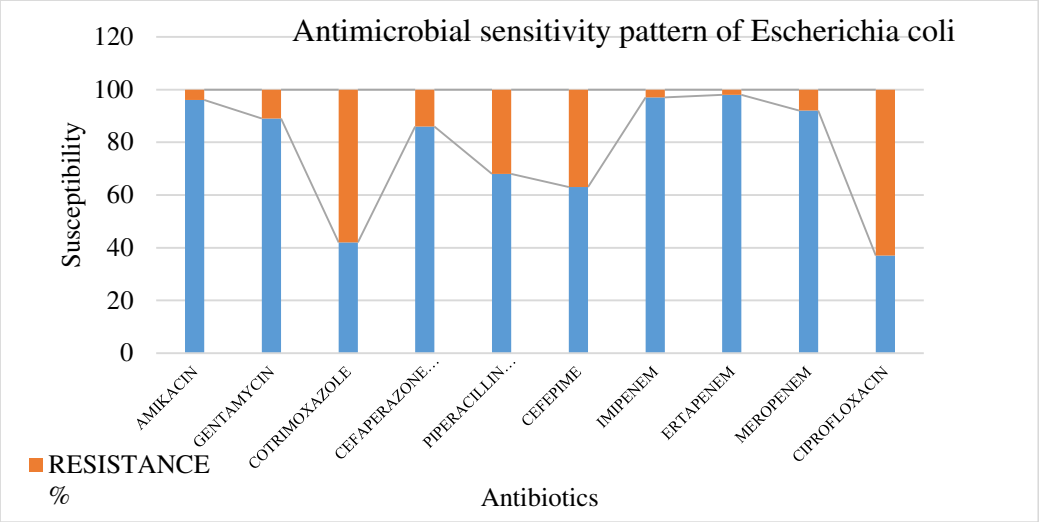


Figure 3: Antibiotic Susceptibility Result *Escherichia coli* (34 isolate)



Coconut Shell liquid smoke was equally effective as that of routine antibacterial agents. It has shown sensitivity to all bacterial strains with 20 microlitre concentration with reducing effects as the concentration reduced. So this can have concentration dependent susceptibility to bacterial agents. Table 1 represents the antibacterial susceptibility of CS-LS compared with betadine.

Table 1: Antibacterial susceptibility of CS-LS in comparison with Betadine

Volume		Staphylococcus aureus No. Of Isolates Sensitivity %	Escherichia coli No. Of Isolate Sensitvity %	Pseudomonas aeruginosa No. Of Isolate Sensitivity %
CS-LS	5µL	7	7	23
	10µL	80	100	100
	15µL	100	100	100
POVIDONE IODINE	10µL	-	-	3

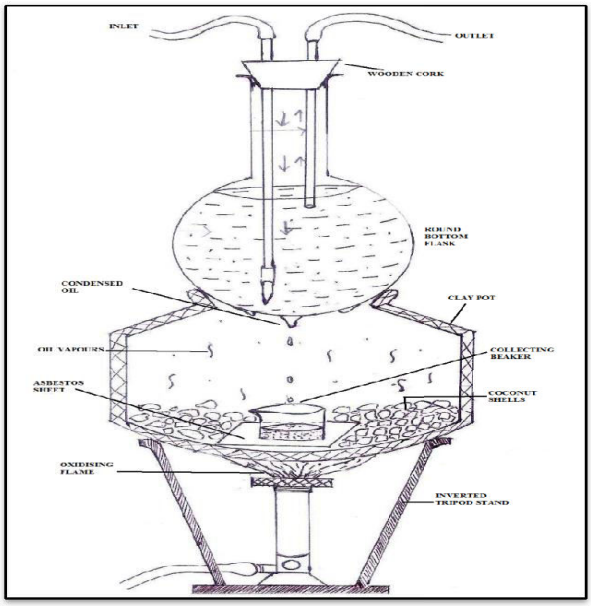
Table 2**:** reflects the antifungal activity of Coconut Shell Liquid smoke (CS-LS). In this study CS-LS does not have any effect with Rhizopus (the mold form of fungi). Apart from that Aspergillus species like fumigatus and niger were susceptible at 20 microlitre of CS-LS. There may be a drawback like it gives profound growth in culture plates and thus microtiter plate method or Minimum inhibitory concentration methods would have been adapted. The yeast form of fungi (Candida albicans) was susceptible to CS-

LS. The study would have been carried out for multiple clinical strains of fungus as there exists a resistance among the yeast also.

Table 2: Antifungal susceptibility of CS-LS in comparison with Fluconazole

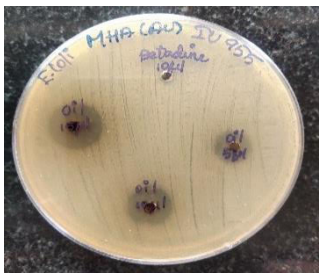
Volume		Rhizopus	Aspergillus Fumigatus	Aspergillus Niger	Candida Albicans
		(zone of inhibition in mm)			
CS-LS	5 µL	0	12	8	15
	10 µL	0	13	14	17
	20 µL	8	21	21	22
Fluconazole		0	0	0	22

Figure 4 A,B shows the preparation of Coconut Shell Liquid Smoke (CS-LS),.



(A)

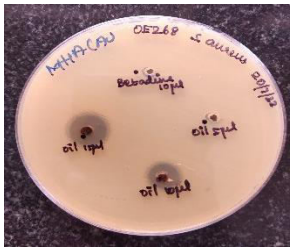
(B)



(C)

(D)

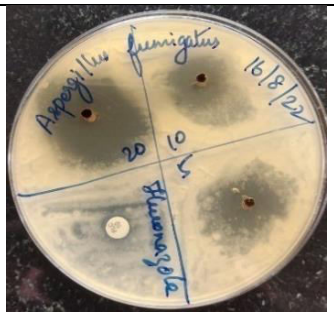
(E)



(F)

(G)

(H)



(I)

Figure 5): A, B and C Represent test zone of inhibition for Escherichia coli; D, E and F Represent test zone of inhibition for Staphylococcus aureus;G and H Represent test zone of inhibition for Pseudomonas aeruginosa with different concentration of CSLS extract compared with Povidone iodine. I Represent test zone of inhibition forAspergillus fumigatus against CSLS extract compared with Fluconazole

Figure 7

Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Name
1	3.903	3.860	4.010	38522	0.12	12033	0.39	3.20	2-Ethoxytetrahydrofuran
2	4.074	4.010	4.170	42831	0.14	8322	0.27	5.15	BUTANOIC ACID, 2-PROPENYL ESTER
3	5.362	5.230	5.555	174533	0.56	17092	0.55	10.21	2(3H)-FURANONE, DIHYDRO-
4	5.660	5.625	5.720	6094	0.02	2556	0.08	2.38	1-(1-METHOXYCYCLOPROPYL)ETHANC
5	5.820	5.750	5.865	17721	0.06	4759	0.15	3.72	SILICIC ACID (H4SIO4), TETRAETHYL ES
6	5.950	5.865	5.970	18017	0.06	4421	0.14	4.08	BENZENE, 1,1'-[3-(2,4-CYCLOPENTADIEN
7	6.037	5.970	8.090	17064518	55.07	1513239	48.67	11.28	Phenol
8	6.694	6.625	6.925	133019	0.43	14092	0.45	9.44	2-Cyclopenten-1-one, 2-hydroxy-3-methyl-

Figure 8

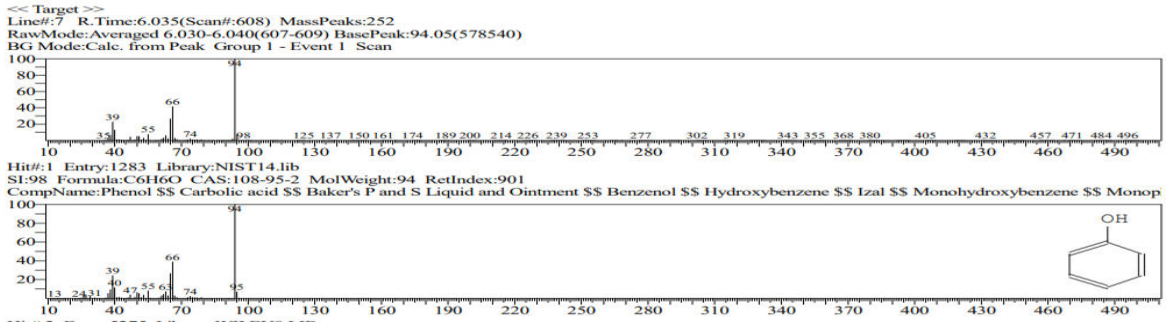


Figure 7 and 8 represents the Gas chromatography mass spectrometry that showed 55% presence of phenol content in CSLS

Discussion

Nosocomial infections have been recognized for more than a century as a critical problem affecting the quality of the health care which is provided in the hospital. All over the world, nosocomial infections are Results also showed that higher concentration (15 and 20 microlitres) of liquid smoke gave better inhibitory effect to the growth of bacteria.

The result of the study impact of herbal medicine on bacterial and fungal organism in the Agar well diffusion method are summarized in the figures respectively. Coconut shell oil showed activity against all the tested organisms. It was more active against bacterial agents at concentration of 15 microlitre than the fungal agents.

A significant proportion of the infections result from cross-contamination, and transmission of microorganisms by the hands of the health care workers is the main route of spread . There were several goals during management of skin and soft tissue infections that too with Healthcare associated infection. This study compared the use

of CS-LS, a traditional medicine as an alternative agent to those commonly used, i.e., Povidone iodine and with Fluconazole for comparison with bacterial and Fungal agents respectively.
Our results are comparable with the study conducted by Nithyalakshmi et al where 61% of the samples were from exudates.[6]

In our study the resistance percentage of Imipenem for Escherichia coli and Pseudomonas aeruginosa were 3% and 11% respectively. In the study by Nithyalakshmi et al, the susceptibility for Imipenem was 96.6%. In the study by Abbas et al in Egypt,the susceptibility to Imipenem was 90%. Both the studies have higher imipenem susceptibility compared to our study.

In our study the Pseudomonas aeruginosa resistance to ceftazidime was 43%. The study by Abbas et al had a susceptibility percentage of 98% which was high compared to our study. Ceftazidime had susceptibility percentage of 57.3%. In the study by Nithyalakshmi et al the susceptibility to Ceftazidime was 24.2% which was less compared to our study. In the study by Abbas et al, all the isolates were resistant to Ceftazidime.

High prevalence of ESBL producing Pseudomonas was reported by Vijay Mane et al,Varun Goel et al and Silpi Bask et al who observed 57%,42,3% and 40% respectively.In contrast the study by Abbas et al,none of the isolates were ESBL producers.

In this study, CS-LS sensitivity was 100% effective against bacterial agents when compared to fungal agents. All strains except 3 strains of Pseudomonas aeruginosa were resistant to Povidone Iodine. All four strains of Fungus were resistant to fluconazole. The study done by Tarawan et al also stated that CS-LS were more effective in comparison with 10% Povidone Iodine.
The study done by Sodha R et al reported that petroleum ether extract was more effective and equally shown sensitivity against Staphylococcus aureus and Escherichia coli in comparison with Chloroform extract. The antifungal property was also analyzed in this study which have shown positive results against Aspergillus niger and Rhizopuss stolonifera which is contrary to our study.
The study done by Kailaku et al reported that CS-LS has antibacterial activity only against Staphylococcus aureus and Escherichia coli and no antifungal activity for the growth of Candida species. This result is also contrary to our study.The study done by Jayashree et al proved that CS-LS had antifungal activity similar to that of Amphotericin B at different concentrations even.[8]
Study done by CP Prince et al showed that Chirattai thailam (CS-LS) activity against all the organisms they have tested for. Least activity was recorded against *Pseudomonas aeruginosa*.

Conclusions

Drug resistance pose a major problem. The coconut shell liquid smoke (CS-LS) was tested for its antibacterial and antifungal activity. It gave satisfactory result against bacterial and fungal agents except Rhizopus. Majority samples (49%) were from exudate specimens. MRSA and VRSA prevalence was 43% and 4% respectively. MDR prevalence of *Pseudomonas aeruginosa* was 54% All bacterial strains were 100% sensitivity to CS-LS at 15 microlitre concentration. Out of 4 fungal agents that were tested Candida albicans, Aspergillus fumigatus and Aspergillus niger were sensitive to CS-LS with 20 microlitre concentration and Rhizopus doesn't have any effect in presence of CS-LS It is already proved that for CSLS the LD50 value is more than 15.000 mg/kg body weight of mice and it also has wound healing activity. This helps us to support the clinicians in treating the multidrug resistant isolates causing skin infections especially.

Reference

1. Zakaria Z.A., Reezal I., Mat Jais A.M., Somchit M.N., Sulaiman M.R., Marmin A.H.I., Sidek H., Husin S.H., Rahim M.H.A. and Abdul Rahman L., The anti inflammatory, anti-pyretic and wound healing activities of Cocos nucifera L. (MATAG types) fresh juice and kernel extract in experimental animals, Toxicol., 1, 516–526 (2006)
2. Jean W.H. Yong, Liya Ge, Yan Fei Ng and Swee Ngin Tan, The Chemical Composition and Biological Properties of Coconut (Cocos nucifera Molecules, 14, 5144-5164 (2009)
3. C.P. Prince and V. Gopal. (2016) Department of Microbiology, antimicrobial evaluation of chirattai thailam, Vol. 6 (1), 01-06 ISSN 2277 – 1506
4. Magaldi S, Mata-Essayag S and Hartung de Capriles C 2004 Well diffusion for antifungal susceptibility testing Int J Infectious Dis 8 39–45
5. Balouiri M, Sadiki M and Ibnsouda SK 2016 Methods for in vitro evaluating antimicrobial activity: A review. J Phar Anal 6 71-9
6. Nithyalakshmi .J, Mohana Krishnan. ESBL producing Pseudomonas aeruginosa in clinical specimens: Is it a scary nightmare or paper tiger? Indian J Microbiol Res 2016;3(3):287-291
7. Bhuvaneshwari G,et al.,(2017) Multiple Antibiotic resistance indexing of Non fermenting gram negative bacilli.Asian J Pharm Clin Res 2017;10(6):78-80.
8. Kailaku, M Syakir ,I Mulyawanti , ANA Syah.,(2016) Antimicrobial activity of coconut shell liquid smoke206 012050
9. Jayasree D, Vasundara devi, Arthi.,(2019) Antifungal activity of Chirattai thailam (coconut shell oil) Vol 18, Issue 7 Ser. 1 , PP 54-57

10. Milly PJ, Toledo RT and Chen J., (2008) Evaluation of liquid smoke treated ready-to eat (RTE) meat products for control of listeria innocua J Food Sci 73 179-183
11. Rajeev K Singla, Nitesh Jaiswal2, Varadaraj Bhat Hitesh Jagani.,(2011) Antioxidant and Antimicrobial Activities of Cocos Nucifera Linn. (Arecaceae) Endocarp Extracts, Indoglobal Journal of Pharmaceutical Science. 1(4) 35-361
12. O'Neill J. Tackling drug-resistant infections globally: final report and recommendations. Review on Antimicrobial Resistance; 2016.
13. Van Boeckel TP, Brower C, Gilbert M, Grenfell BT, Levin SA, Robinson TP, et al. Global trends in antimicrobial use in food animals. Proc Natl Acad Sci U S A 2015; 112 : 5649-54.
14. Akiba M, Senba H, Otagiri H, Prabhasankar VP, Taniyasu S, Yamashita N, et al. Impact of wastewater from different sources on the prevalence of antimicrobial-resistant Escherichia coli in sewage treatment plants in South India. Ecotoxicol Environ Saf 2015; 115 : 203-8.
15. Fukuda T, Boeckh M, Carter RA, Sandmaier BM, Maris MB, Maloney DG, Martin PJ, Storb R, Marr KA. Risk and outcomes of invasive fungal infections in recipients of allogeneic hematopoietic stem cell transplants after nonmyeloablative conditioning. Blood 2003; 102:827-833.
16. Husain S, Alexander BD, Munoz P, Avery RK, Houston S, Pruett T, Jacobs R, Dominguez EA, Tollemar JG, Baumgarten K, Yu CM, Wagener MM, Linden P, Kusne S, Singh N. Opportunistic mycelial fungal infections in organ transplant recipients: emerging importance of nonAspergillus mycelial fingi. Clin Infect Dis. 2003; 37:221- 229.
17. Lin S-J, Schranz J, Teutsch SM. Aspergillosis case-fatality rate: systematic review of the literature. Clin Infect Dis 2001; 32:358-366.
18. Thyagarajan,R (2004), "Siddha Materia Medica-Thaathu Vaguppu", Department of Indian Medicine and Homeopathy Chennai,4th edition, 555
19. Vita Murniati Tarawan, MD, PhD1 Journal of Evidence-Based Complementary & Alternative Medicine 2017, Vol. 22(3) 436-440 ª The Author(s) 2016 Reprints and permission: sagepub.com
20. Nitiema LW, Aly S, Jacques S, Dayeri D and Alfred ST 2012 In vitro antimicrobial activity of some phenolic compounds (coumarin and quercetin) against gastroenteritis bacterial strains Int J Microbiol Res 3(3) 183-7
21. Zuraida I, Sukarno and Budijanto S 2011 Antibacterial activity of coconut shell liquid smoke (CS-LS) and its application on fish ball preservation Int Food Res J 18 405-10
22. Verma V, Bhardwah A, Rathi S and Raja R.B Potential Antimicrobial Agent from Cocos nucifera mesocarp extract; Development of a New Generation Antibiotic, Journal of Biological Sciences 3202, 1(2), 48-54 (2012)

23. Kumar K, Kindo AJ, Kalyani J, Anandan S. Clinicomycologicalprofi le of dermatophytic skin infection in a tertiary care centre. Sri Ramachandra Journal of Medicine 2007;1:12-5.
24. Elangovan, S; Jeeva, gladys R; Kalai, arasi R; Mubarak, H and Velpandian,V (2013), "Antimicrobial Screening of Vanga Vennai and Mathan Thailam for Diabetic Foot Ulcer", IOSR Journal of Pharmacy and Biological Sciences", Volume 6, Issue 5,01-05
25. Toledo RT 2007 Wood Smoke Components and Functional Properties Proc. Int. Conf. on Smoked Seafood (Alaska: Sea Grant College Program)
26. Valgas C, DeSouza SM and Smânia EFA 2007 Screening methods to determine antibacterial activity of natural products Braz J Microbiol 38 369–80
27. Nitiema LW, Aly S, Jacques S, Dayeri D and Alfred ST 2012 In vitro antimicrobial activity of some phenolic compounds (coumarin and quercetin) against gastroenteritis bacterial strains Int J Microbiol Res 3(3) 183-7