IJMPR 2021, 5(4), 209-221

International Journal of Modern Pharmaceutical Research
www.ijmpronline.com

ISSN: 2319-5878
IJMPR
Research Article

SJIF Impact Factor: 5.273

ANTIMICROBIAL PROPERTIES OF *COCOS NUCIFERA* EXTRACTS ON MICROBIAL PATHOGENS

Dr. P. N. Rajarajan*

Assistant Professor, Department of Microbiology, The Madura College, Madurai- 625011, Tamilnadu, India.

Received on: 13/06/2021
Revised on: 03/07/2021
Accepted on: 23/07/2021

***Corresponding Author**
Dr. P. N. Rajarajan
Assistant Professor,
Department of Microbiology,
The Madura College,
Madurai- 625011, Tamilnadu,
India.
r.raja56@gmail.com,

ABSTRACT

The antibacterial activity of extracts of Cocos nucifera, family Arecaceae, had studied against pathogens.The coconut parts were collected from in and around Madurai. In this study selected the different parts of coconut tree they are coconut shell, leave, husk, oil, milk and selected different types of microbial strain *Staphylococcus aureus, Streptococcus species, E. coli, Klebsiella.* In this study selected two different solvents, they are polar solvents and non polar solvents. The two methods follow against antibacterial activity of coconut plant extract. They are agar disc diffusion method & agar well diffusion method. Among the coconut part extracts shell, leaf, husk, milk, has higher antibacterial activity in lowest concentration than the compared to the other. The phytochemical screening of crude extracts shows the presence of tannins, lignin, flavonoid, as secondary metabolites, which may contribute to the antibacterial activity.

KEYWORDS: Phytochemical Screening, Antibacterial, Antibiotics, Secondary Metabolites.

INTRODUCTION

There are so many types of plants existing in land, water sources and rock and all over the world, which may be the higher or lower plants, among them, some are having therapeutic activities, which are coconut plants. The coconut plant parts are effective, in the treatment of various infectious diseases. The coconut plant parts such as husk, leaf, shell, oil, milk extracts shown to possess antimicrobial properties.[1] The antimicrobial property of coconut is, because of its high lauric acid content that has been used as medications for certain oral infections. Examble: Mouth sores. Tender coconut water is readily available economically reasonable and traditionally tolerable, besides having effects. The antimicrobial efficacy of tender coconut water is natural state on *Streptococcus mutans* is not available, tender coconut water against *Streptococcus mutans.*[2]

Coconut is widely used in food products of South East Asian countries including Indonesia, Malasia, Philippines and Thailand. Coconut tree is found in the most regions of the country is called Kalpavriksha. *Cocos nucifera* is an important member of the family Arecaceae (Palm family) popularly known as coconut, coco, or coconut of the beach.[3] The plant is originally from South East Asia (Malasia, Indonesia and Philippines) and the Islands between the Indian and Pacific Oceans. The fruit of the coconut palm is believed to have been brought to India and then to East Africa. The plant is an Arbarescent monocotyledonous tree of around 25 m in height (Giant Coconut) with a dense canopy. The root of the coconut system is fasciculated. The stem is an un-branched type and at its apex, a tuft of leaves protects a single apical bud. The pinnate leaves are feather shaped, having a petiole, rachis and leaflets, under favourable environmental conditions the giant adult coconut emits inflorescence spikes per year. The adult dwarf coconut can emit 18 spikes in the same period. The auxillary inflorescence has globular clusters of female flowers .The plant is monocotyledones male and female reproductive organs on the same plant. Coco and an inner endocarp. The epicarp which is the outer skin of the fruit and the mesocarp which is heavy fibrous and tanned, when dry have many industrial uses Traditional uses of *Cocus nucifera* phytochemical compounds isolated from different parts of the plant and biological activity and toxicological studies to-date. Coconut is a super food with its health benefits, medicinal uses of nutritional value.[4] Coconut trees usually grow in the tropical region 60 species of cocos palm at one time scientist identified with characteristics such as dwarf and tall. Coconut plays a important role in the national economy of India. Indonesia was the largest exporters of coconut in the world. Coconut become invented by 6th century in West travels in India. The coconut life span is 70-100years. Coconut first appear in the Western world in the 15th century growing on tropically Islands throughout the Indian Ocean. 85% of world coconut production, originates Philippines and Indonesia.All parts of the coconut tree can be used. Both the green coconut water and solid albumen ripe fruits are used industrially and in home cooking in many ways, several parts of the fruit and plant have been used by different countries, for the treatment of various pathological conditions.[5]

MATERIALS AND METHODS

Collection of Plant Materials

The various parts of coconut plants are belonging to the species of *Calappa nucifera, Cocos indica, Cocos nana, Palma cocos* were collected from in and around Madurai. The species of plants were identified by the Department of Botany in The Madura College, Madurai.

S.NO.	THE PLANTS	NAME OF FAMILY	USED PART
1.	*Cocos nucifera*	Arecaceae	LEAF
2.	*Cocos nucifera*	Arecaceae	SHELL
3.	*Cocos nucifera*	Arecaceae	HUSK
4.	*Cocos nucifera*	Arecaceae	OIL
5.	*Cocos nucifera*	Arecaceae	MILK

Selection of Solvents

The Non-Polar Solvents and Polar Solvents were choosed for plant parts extraction.

Non-Polar Solvents are Hexane, Benzene, Chloroform. The polar solvents are of 2 types namely Polar-Aprotic and Polar-Protic Solvents are Methanol, Ethanol, Acetic acid and Water.[6]

Tested Organisms

The bacterial strains were belonging to the gram positive *(Staphylococcus aureus Streptococcus)* and gram negative *(Escherichia coli, Klebsiella*) were taken for tests. All the above bacterial strains were prepared in Nutrient Broth were maintained autoclave at 121^0 C for 15 minutes.

Antibiotic sensitivity method

In antibiotic sensitive method, there are two methods. They are as follows Agar disc diffusion method and Agar well diffusion method.

Agar Disc Diffusion (Kirby - Bauer) Method

The agar disc diffusion method was found by Kirby and Bauer, in which the swab culture of the organism is done by Muller - Hinton Agar. On placing the whatman filter paper discs (mixed with extracts) upon the agar, the extracts start diffusing in to the agar and based upon the sensitivity pattern, the zones are observed and measured.[6]

Agar Well Diffusion Method

Muller Hinton Agar and Nutrient Agar plates were prepared.Five wells were preepared in both types of agar plates by 5 mm diameter sterile cork borers. Over night culture of tested organisms (*E.coli, Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus aureus and Bacilius subtillis*) were swabbed on the seperate agar plates.One kind of plant extracts mixed with various solvents were added in thr seperate wells of the each tested organisms agar plates. same procedure was followed for the remaining plant extracts and the test organisms After that all plates were incubated at 37ºC for 24 hrs. After the incubation the plates were observed for the zone of inhibition.[7,8,9]

Agar Disc Diffusion Method

The swab culture of the organism is done by SDA. On placing the whatman filter paper discs (mixed with extracts) upon the agar, the extracts start diffusing in to the agar and based up on the senstivity pattern , the zones were observed and measured.[10,11]

RESULTS

Table 1: Inhibition of bacterial growth by coconut shell extract.

Coconut parts extracts	Test organism	10 g	15 g	20 g	1 g
Shell	*S. aureus*	10	11	12	13
	Streptococcus	12	12	15	17
	E.coli	10	11	12	14
	Klebsiella	5	10	12	13

Among these extracts of coconut shell extracts such as methanol in different concentrations the maximum inhibition was observed by *Streptococcus* in methanol extracts of 17 mm.

Table 2: Inhibition of bacterial growth by leaf extracts.

Coconut parts extracts	Test organism	10 g	15 g	20 g	1 g
Leaf	*S.aureus*	2	3	10	11
	Streptococcus	5	7	9	12
	E. coli	4	5	7	10
	Klebsiella	8	11	13	15

Among these extracts of coconut leaves such as solvent prepared in different concentration. The maximum inhibition was observed by *Klebsiella* in methanol extracts of 15 mm.

Table 3: Inhibition of bacterial growth by husk extracts.

Coconut parts extracts	Test organism	10 g	15 g	20 g	1 g
Husk	*S.aureus*	2	5	7	9
	Streptococcus	2	3	5	7
	E.coli	5	7	9	11
	Klebsiella	1	3	5	7

Among these extracts of coconut husk extracts such as solvent prepared in different concentration. The maximum inhibition was observed by *E.coli* in methanol extracts of 11 mm.

Table 4: Inhibition of bacterial growth by milk.

Coconut parts extracts	Test organism	10 g	15 g	20 g	1 g
Milk	*S.aureus*	2	7	9	11
	Streptococcus	3	8	10	12
	E.coli	2	5	10	12
	Klebsiella	7	9	12	13

Among these extracts of cocconut milk such as solvent prepared in different concentration. The maximum inhibition was observed by *Klebsiella* in methanol extracts of 13 mm.

Table 5: Inhibition of bacterial growth by oil.

Coconut parts extracts	Test organism	10 g	15 g	20 g	1 g
Oil	*S. aureus*	2	9	10	11
	Streprococcus	5	8	9	7
	E.coli	7	5	7	9
	Klebsiella	3	4	5	7

Among these extracts of coconut oil such as solvent prepared in different concentration. The maximum inhibition was observed by *Staphylococcus aureus* in methanol extract of 11 mm.

Table 6: Minimum inhibitory concentration.

Bacterial Culture	Different Concentration of Coconut Extract	Parts Used	OD Value
Staphylococcus aureus	0.1g in 10 ml	Leaf	0.36
	0.15g in 15 ml	Leaf	0.53
	0.2g in 20 ml	Leaf	0.56
	1g in 1 ml	Leaf	0.58
	0.1g in 10 ml	Husk	0.32
	0.15g in 15 ml	Husk	0.36
	0.2g in 20 ml	Husk	0.40
	1g in 1 ml	Husk	0.47
	0.1g in 10 ml	Shell	0.35

	0.15g in 15 ml	Shell	0.37
	0.2g in 20 ml	Shell	0.40
	1g in 1ml	Shell	0.45
	1g in 1ml	Oil	0.50
	1g in 1 ml	Milk	0.54
Streptococcus sps	0.1g in 10 ml	Leaf	0.37
	0.15g in 15 ml	Leaf	0.39
	0.2 g in 20 ml	Leaf	0.41
	1g in 1 ml	Leaf	0.42
	0.1gin 10 ml	Husk	0.13
	0.15g in 15 ml	Husk	0.17
	0.2 g in 20 ml	Husk	0.20
	1g in 1 ml	Husk	0.27
	0.1 g in 10 ml	Shell	0.30
	0.15 g in 15 ml	Shell	0.35
	0.2 g in 20 ml	Shell	0.39
	1g in 1 ml	Shell	0.41
	1 ml	Milk	0.49
	1ml	Oil	0.47
E.coli	0.1g in 10 ml	Leaf	0.32
	0.15 g in 15 ml	Leaf	0.37
	0.2 g in 20 ml	Leaf	0.40
	1g in 1 ml	Leaf	0.45
	0.1 g in 10 ml	Husk	0.36
	0.15 g in 15 ml	Husk	0.39
	0.2 g in 20 ml	Husk	0.43
	1g in 1ml	Husk	0.46
	1ml	Milk	0.50
	1ml	Oil	0.53
	0.1g in 10 ml	Shell	0.32
	0.15g in 15 ml	Shell	0.39
	0.2g in 20 ml	Shell	0.42
	1g in 1 ml	Shell	0.45
Klebsiella	0.1g in 10 ml	Leaf	0.31
	0.15g in 15 ml	Leaf	0.35
	0.2 g in 20 ml	Leaf	0.37
	1 g in 1ml	Leaf	0.39
	0.1g in 10 ml	Husk	0.35
	0.15 g in 15 ml	Husk	0.37
	0.2 g in 20 ml	Husk	0.39
	1g in 1ml	Husk	0.42
	0.1g in 10 ml	Shell	0.39
	0.15 g in 15 ml	Shell	0.42
	0.2 g in 20 ml	Shell	0.50
	1g in 1ml	Shell	0.53
	1ml	Milk	0.25
	1ml	Oil	0.31

The mean values for zone of inhibition were higher with positive control. The coconut leaf extracts higher sensitive to the organisms on *Staphylococcus aureus* species in the Standard Deviation value in 9.237. The coconut leaf extracts lowest sensitive to the organism on *E.coli* Species in the Standard deviation value on 4.618. The coconut husk extracts highest sensitive to the organism on *Staphylococcus aureus* in the Standard Deviation value on 5.196. The coconut husk extracts lowest sensitive to the organism on *Streptococcus* species in the Standard Deviation value on 4.416. The coconut oil extracts highest sensitive to the organism on *Staphylococcus aureus* in the Standard Deviation value on 6.928. The coconut oil extracts lowest sensitive to the organism on *E.coli* in the Standard Deviation value on 2.308. The coconut milk extracts highest sensitive to the organism on *E.coli* in the Standard Deviation value on 8.660. The coconut milk extracts lowest sensitive to the organism on *Klebsiella* species in the Standard Deviation value on 5.196.

Table 7: The result of Minimum Inhibitory Concentration (MIC) is sensitivity on the isolates at various dilution concentrations.

S.No.	Particulars	N	Mean ± SD	P
1.	Fresh tender coconut milk	15	3.08 ± 0.2	0.0001
2.	Pasteurized milk	15	14.23 ± 0.2	0.0001
3.	Fresh tender oil	15	12.13 ± 0.2	0.0001
4.	Pasteurized oil	15	15.43 ± 0.2	0.0001
5.	Positive control	15	60.27 ± 0.2	0.0001
6.	Negative control	15	15.28 ± 0.1	0.0001
7.	Fresh coconut shell	15	5.06 ± 0.2	0.0001
8.	Pasteurized coconut shell	15	16.32 ± 0.2	0.0001
9.	Fresh coconut husk	15	7.09 ± 0.2	0.0001
10.	Pasteurized coconut husk	15	19.23 ± 0.2	0.0001
11.	Fresh coconut leaf	15	9.08 ± 0.1	0.0001
12.	Pasteurized coconut leaf	15	20.12 ± 0.2	0.0001
13.	Positive control	15	62.57 ± 0.2	0.0001
14.	Negative control	15	17.45 ± 0.1	0.0001
15.	Positive control	15	65.71 ± 0.2	0.0001
16.	Negative control	15	13.45 ± 0.2	0.0001
17.	Positive control	15	15.56 ± 0.2	0.0001
18.	Negative control	15	13.78 ± 0.2	0.0001
19.	Positive control	15	15.61 ± 0.2	0.0001

Table 8: Different concentrations of coconut extract.

Organism	Different concentration of coconut extract	Components used	Zone of Inhibition (in mm) Mean values
Staphylococcus aureus	5 ml in 5 g 10 ml in 10 g 15 ml in 15 g 1 ml in 1 g	Leaf extract	10
	5 ml in 5 g 10 ml in 10 g 15 ml in 15 g 1 ml in 1 g	Shell extract	11
	5 ml in 5 g 10 ml in10 g 15 ml in15 g 1 ml in 1 g	Husk extract	9
	1000 µl	Coconut milk	10
	1500 µl	Coconut oil	5
Streptococcus	5 ml in 5 mg 10 ml in 10 g 15 ml in 15 g 1 ml in 1 g	Leaf extract	10
E.coli	0.05 g in 5 ml 0.15 g in 15 ml 10 ml in 0.1g 1 g in 1 ml	Shell extract	10

Table: 9: Antibacterial screening.

Antibiotics	Inhibition Zone in(cm)			
	Staphylococcus aureus	***Streptococcus sp***	***E.Coli***	***Klebsiella***
Ceprofloxacin (5µg)	2.80	3.00	2.50	2.30
Crentamycin (10µg)	3.00	2.50	2.40	2.20

Amilacin (10µg)	2.00	2.50	2.00	1.80
Rifampicin (15µg)	1.50	1.25	1.50	2.20
Kanamycin (80µg)	1.50	1.60	1.60	1.30
Erythomycin (15µg)	3.80	1.65	0.80	1.00
Carbenicillin (100µg)	0.80	3.30	1.50	1.40
Streptomycin (10µg)	2.80	-	-	-
Methicillin (10µg)	2.80	2.30	0.80	0.70
Methicillin (10µg)	-	-	-	-
Amaeycillin (10µg)	0.70	-	-	1.00
Chloramohenicol (10µg)	-	-	-	-

Table 10: Antimicrobinal efficiency of methanol Extracts of coconut parts extracts against Selected Cremative and Cream Negative microbial pathogons.

Extracts / Bacterium	CLE Inhibiton Zone(cm)	CSE Inhibiton Zone(cm)	CHE Inhibiton Zone(cm)	CME Inhibiton Zone(cm)	COE Inhibiton Zone(cm)
Staphylococcus aureus	10.0 ± 0.20	11.2 ± 0.20	9.1 ± 0.90	10.1 ± 0.10	5.2 ± 0.5
Streptococcus	15.1 ± 0.15	12.05 ± 0.20	11.3 ± 0.11	12.2 ± 0.12	13.5 ± 0.13
E.Coli	10.0 ± 0.20	6.5 ± 0.6	15.5 ± 0.15	12.3 ± 0.12	18.3 ± 0.18
Klebsiella	10.0 ± 0.20	2.5 ± 0.25	13.2 ± 0.13	16.2 ± 0.16	13.3 ± 0.13

Table 11: Biochemical Test.

Characteristics and tests	*Staphylococcus aureus*	*Streptococcus sps*	*E.Coli*	*Klebsiella*
Gram starning	+	+	-	-
Shape	Cocci	Rod	Rod	Rod
Motility	Non motile	Motile	Motile	Non motile
Capsule	Non Capsulated	Capsulated	Non Capsulated	Capsulated
Spore	Non Sporing	Sporing	Non Sporing	Non Sporing
Flagella	-	Swimming or foeming	Single flagella	-

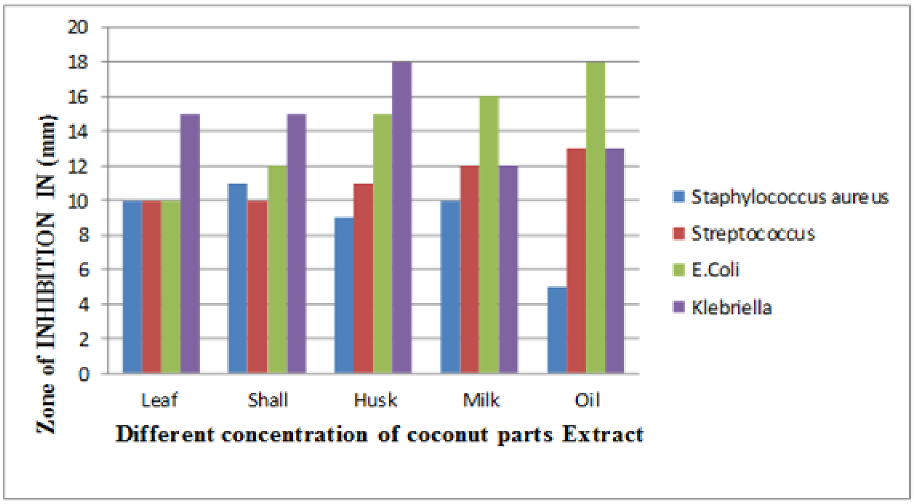

Figure 1: Antimicrobial activity of coconut parts extract using microbial pathogens.

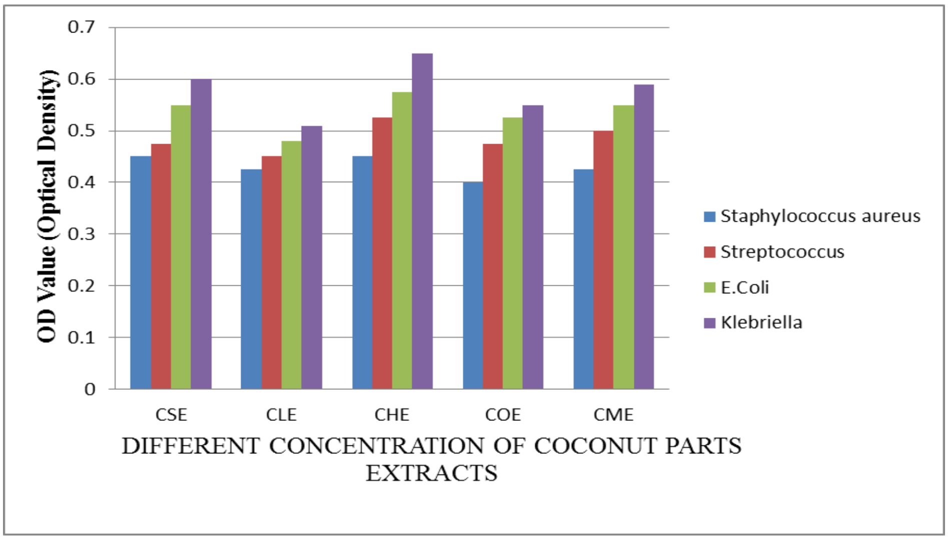

Figure 2: Different concentration of coconut part extracts

Figure 3: Antimicrobial activity of coconut milk by muller hinten agar using *staphylococcus aureus.*

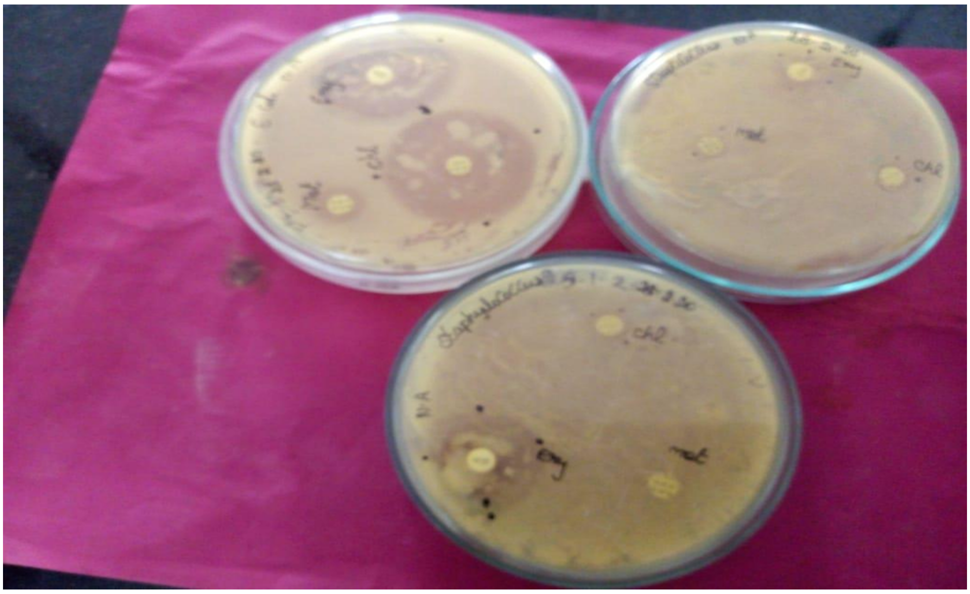

Figure 4: Antimicrobial activity of coconut shell extract against a muller hinton agar *streptococcus species.*

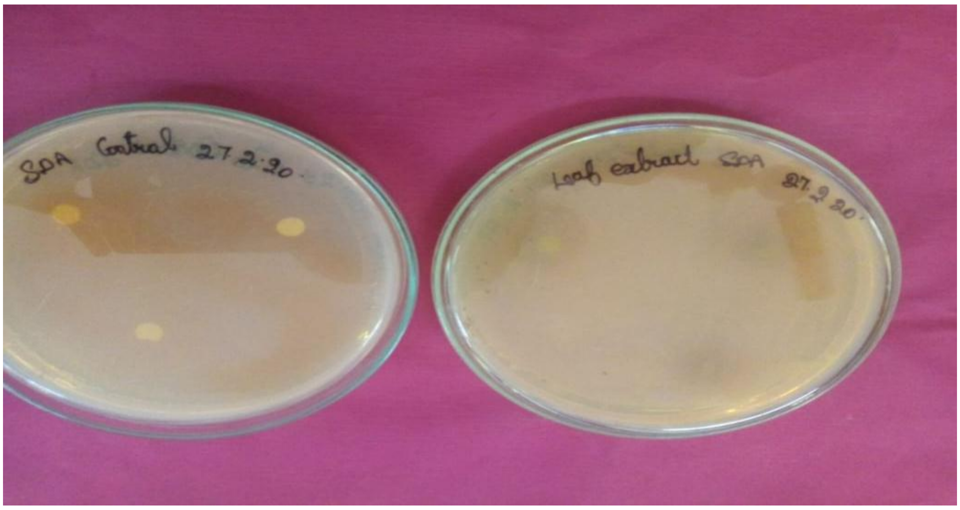

Figure 5: Antifungal activity of coconut leaf extract against a sabouraud dextrose agar using *Aspergillus niger* .

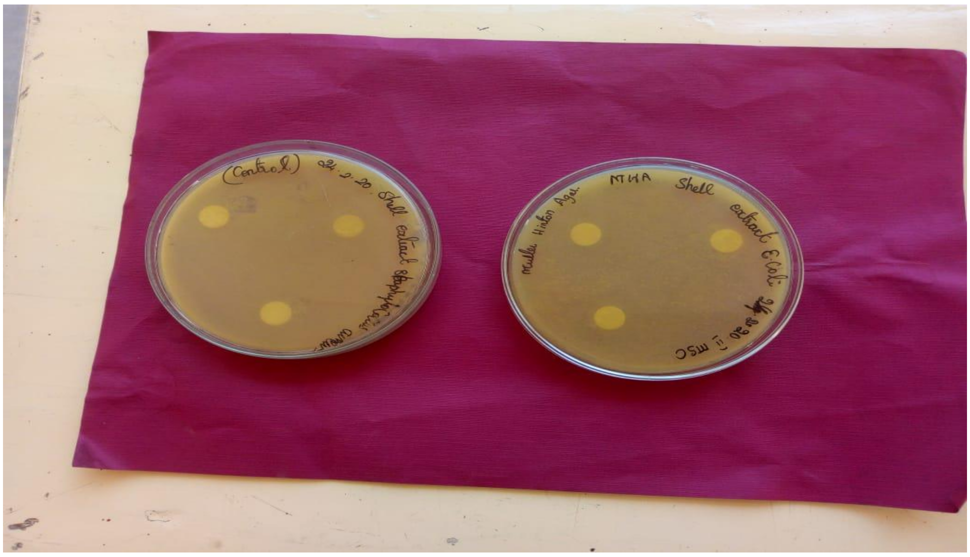

Figure 6: Anti microbial activity of coconut shell extract against a mullerhinten agar using *E.coli* .

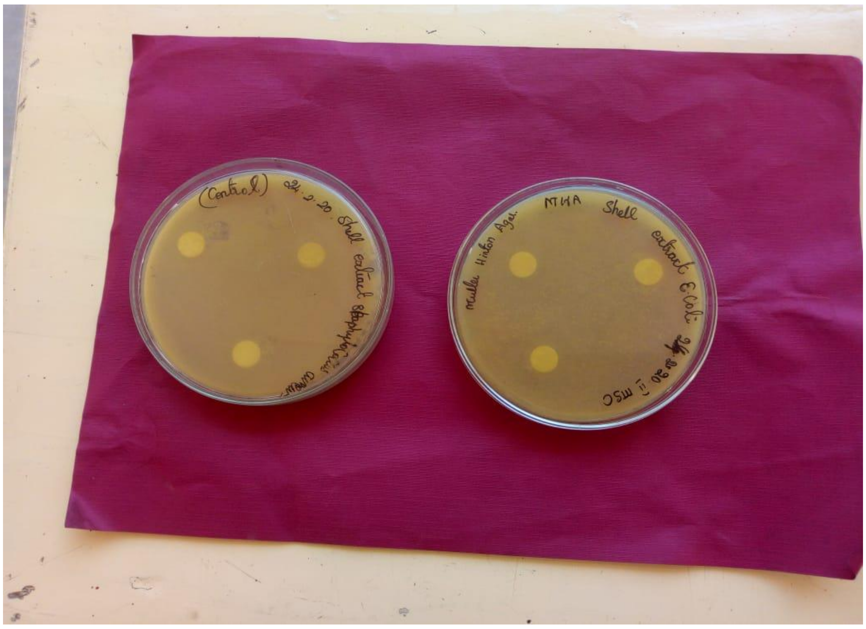

Figure 7: Antimicrobial activity of coconut milk extracts against *E.coli* species

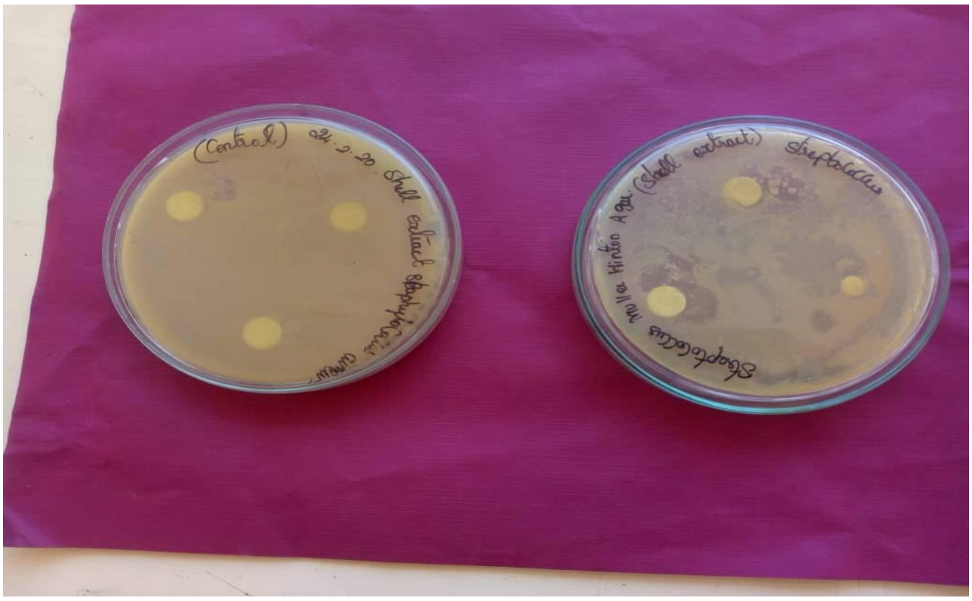

Figure 8: Antimicrobial activity of coconut leaf extracts against a muller hinton agar using *streptococcus species*

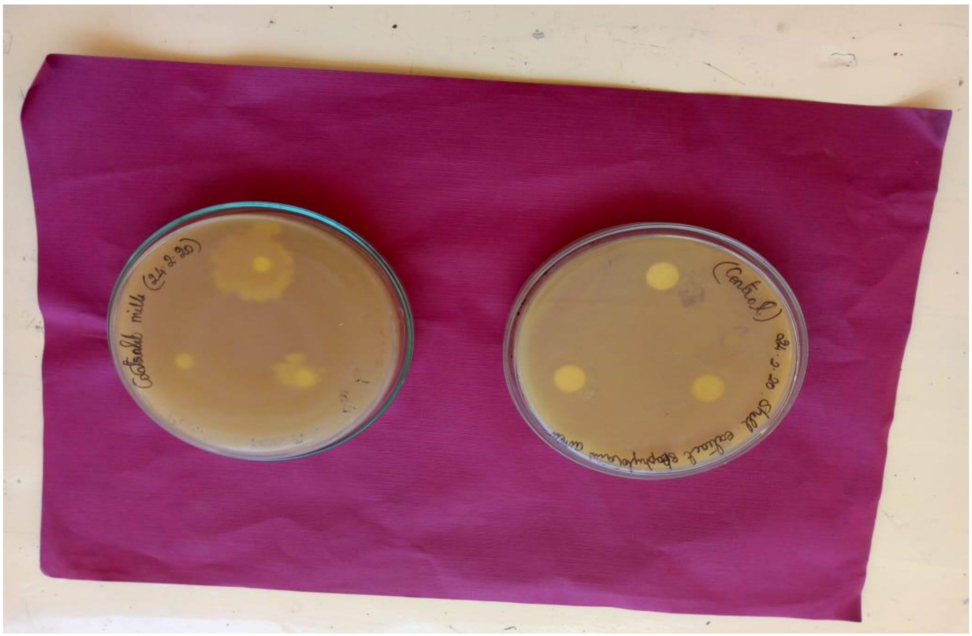

Figure 9: Antimicrobial activity of coconut milk against muller hinton agar using *Staphylococcus aureus.*

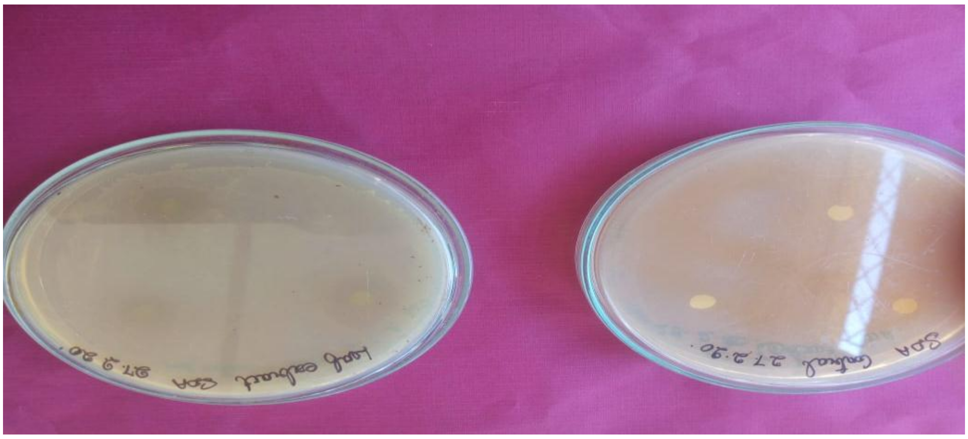

Figure 10: Antifungal activity of coconut leaf extracts against sabouroud Dextrose agar using *Aspergillus niger.*

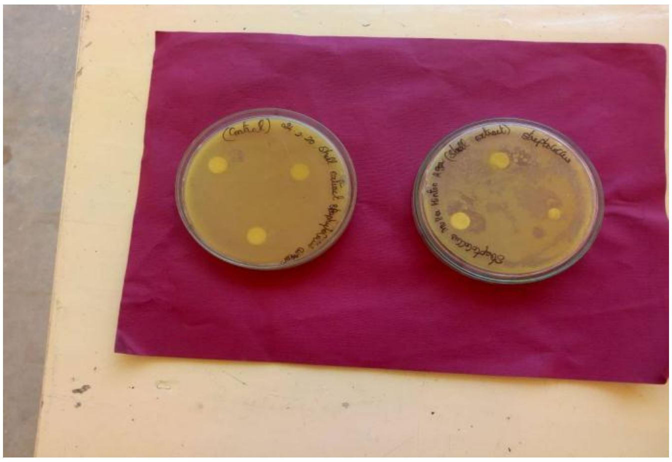

Figure 11: Antimicrobial activity of coconut oil against by muller hinton agar using *Streptococcus species.*

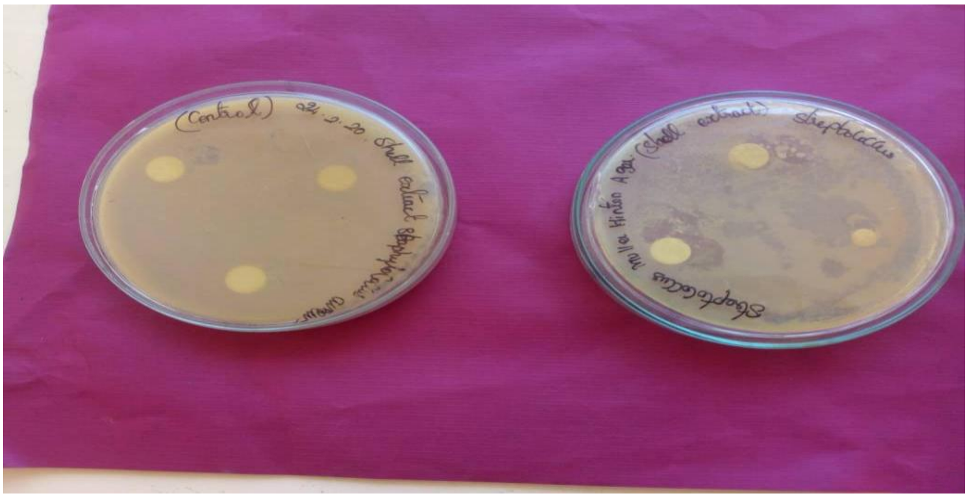

Figure 12: Antimicrobial activity of coconut shell extract against muller hinton agar using *streptococcus species.*

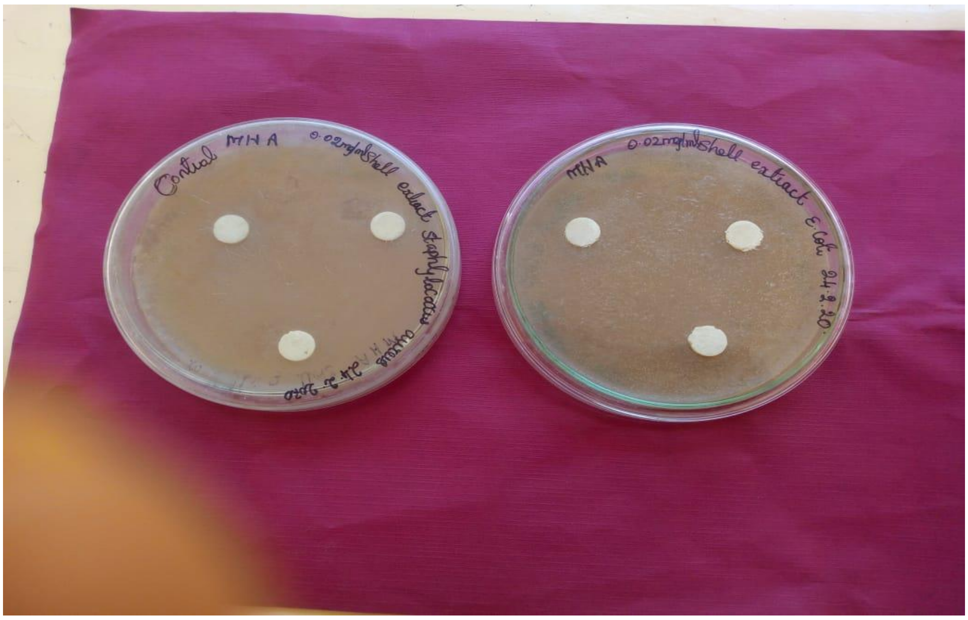

Figure 13: Antimicrobial activity of coconut husk extract against muller hinton agar using *E.coli* species

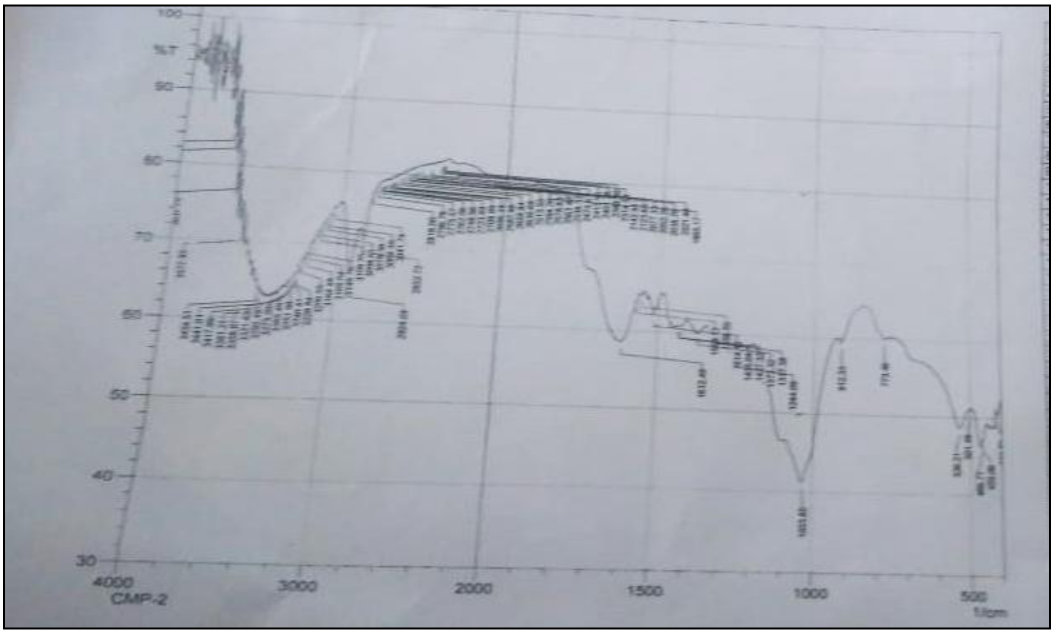

Figure 14: FTIR analysis of Husk

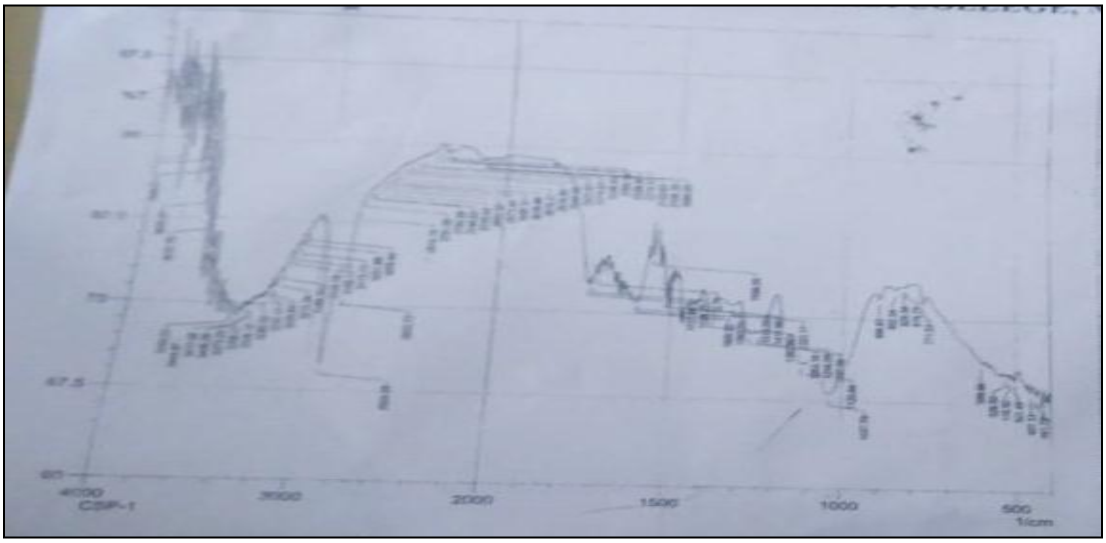

Figure 15: FTIR analysis of Leaf.

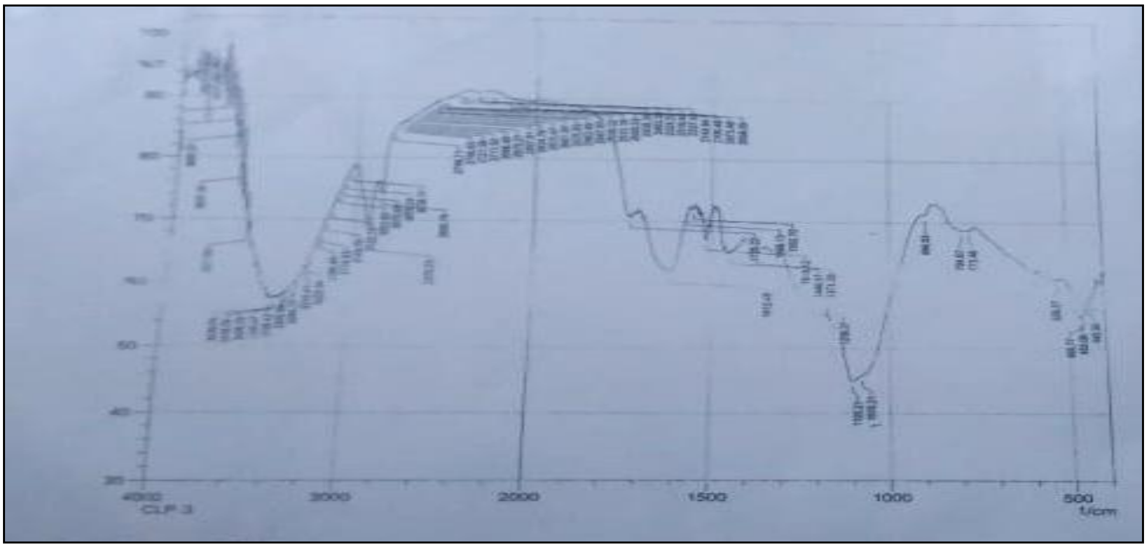

Figure 16: FTIR analysis of shell.

DISCUSSION

The antibacterial activity of the extracts and their potency was quantitatively, assesed by the presence or absence of inhibition zone and zone diameter, only, methanolic extracts was found to better solvent for extractions of anti-microbically active substance compared to water. The coconut shell extract with methanol solvents shows the maximum clear zero than compared to the other concentration, so it has the higher bactericidal activity against the other organism.

CONCLUSION AND FUTURE SCOPE

The current work, extracts isolated from Cocos were found to possess secondary metabolites like tannin, lignin and flavonoid. These secondary metabolite might contribute to the antibacterial and antifungal efficacy extract. The extract is used traditionally as antiseptic which foresight the screening of extract towards its coagulant and antiseptic property.

ACKNOWLEDGEMENT

I would like to thankful to the Head, Department of Microbiology supporting to do the research work and also thank Department of Chemistry, The Madura College, Madurai for Tehnical studies.

REFERENCES

1. Dahanukar, S.A., Kulkarni, R.A., And Rege, N.N., pharmacology of medicinal plants and natural product, Indian Journal of Pharmacology, 2000; 32,S81, S118.
2. Eisbaey, M., AbdulBar, F, M., Cytotoxic and antioxidant activity of Coconut shells (endocarp), the extract used are methanolic extract and cytotoxic activity performed towards prostate cancer in cell line (pc3) compared to normal cells International journal of pharmacognosy and phytochemical research, 2017; 9(10): 12881292.
3. Rafael, Vincent., Maries, Silvestre., Anti Alzheimers activity of ethanolic leaf extract of cocos nucifera, Biomedicines, 2017; 15-20.
4. Verma, V., Bharway, A., Development of a new generation Antibiotic by six different solvent extract of cocus nucifera, ICSA Journal of biological science, 2012; 1(2): 48-54.
5. R,Cristiane, D.O., Nascimento., Tavares, figeira., Antimicrobial and cytotoxic activities of crude extract of coconut, Journal of chemical and pharmaceutical research, 2016; 8(8): 276-282.
6. Mounyr, Balouiri., Moulay, Sadiki., Saad Koraichi Ibnsouda., Methods for in vitro evaluating antimicrobial activity: A review Journal of Pharmaceutical Analysis, 2016; 6(2): 71-79.
7. Srinivass, k., Vijaya srinivas., Antibacterial activities of cocos nucifera linn, Indain journal of pharmaceutical sciences, 2016; 4(2): 252-232.
8. Rajeev, K., Antioxidant and Antimicrobial activities of Cocos nucifera Linn (Arecaceae) endocarp, Indo global Journal of pharmaceutical sciences, 2011; 1(4): 354-361.
9. Maji, jose., Antimicrobial properties of Cocos nucifera, an extrapolation to oral health by alcoholic extract of coconut husk, Journal of national science, biology and medicines, 2014; 5(2): 359-364.
10. Rukmini, J, N., Sunkari, Manara., Umashankar, G, K., Antibacterial efficacy of cocos nucifera linn on Streptococcus mutans, Journal of international society of preventive and Community dentistry.
11. Ernest basseyassikong., Akeh, Martins., Antimicrobial activity of coconut oil and its derivatives, International Journal of medical science and clinical Inventions, 2017; 4(8): 3173-3177.