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GCMS ANALYSIS AND ANTIBACTERIAL ACTIVITY OF COCOS NUCIFERA AND MUSA ACUMINATA AGAINST MULTI DRUG RESISTANT ESCHERICHIA COLI AND STAPHYLOCOCCUS AURESUS

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ABSTRACT

The present study was undertaken to determine antibacterial activity of *Cocos nucifera* and *Musa acuminata* leaf extracts against multi drug resistant *Staphylococcus aureus* and *Escherichia coli.* The leaf extracts of *C. nucifera* and *M. acuminata* were prepared by Continuous Soxhlet extraction and cold extraction method. Antibacterial activity was done by agar well diffusion method and MIC values were determined by using macro-broth dilution method. Antibacterial activity present in both the extracts; methanol extract of *Musa acuminata* and *Cocos nucifera* showed more antibacterial activity against multi drug resistant *Staphylococcus aureus* followed by the methanol extract of *Cocos nucifera* against *Escherichia coli*. GCMS analysis of both plant extracts showed presence of biologically active compounds 6-Octadecenoic acid, methyl ester, (Z)-, Neophytadiene and Squalene that possess pharmacologically potential activity. The *Cocos nucifera* and *Musa acuminate* leaf extracts may have therapeutic value in control of certain multidrug resistant bacterial pathogens.

KEYWORDS: Antibacterial activity, GCMS analysis, *Cocos nucifera, Musa acuminata, Staphylococcus aureus, Escherichia coli.*

INTRODUCTION

Antimicrobial agents that are commonly used have become inefficient to controlling infectious diseases. Additionally, there are several problems associated with the antimicrobial

agents including serious adverse effects and severe allergic reactions. Bioactive compounds derived from natural resources presents a promising solution; Phytomedicines derived from herbal plants are widely used in many parts of the world due to the presence of diverse bioactive compounds. This dragged the attention of the researchers to identify and develop new antimicrobial agents derived from medicinal plants in order to fulfill the current therapeutic problem. India is a hub of medicinal plants and use traditional medicines like Siddha, Ayurveda and Unani for treating various diseases. Phytoconstituents from plant extracts are considered as secondary metabolites to cure various human diseases (Umamaheswari, 2017). Phytochemical screening of plant extracts reveals the secondary metabolites like alkaloids, tannin, lignin, flavonoid, reducing sugar, saponins, steroid etc. which may have therapeutic property when ingested or applied locally (Anumol joy *et al*., 2019). The reported pharmacological activities of *M. acuminate* include antioxidant, antidiabetic, immunomodulatory, hypolipidemic, anticancer, and antimicrobial especially anti-HIV activity (Simmonds and Shepherd, 1955). Various antibiotics have been developed from solvent extract of *Cocos nucifera*. There are a very few reports about antibacterial activity of *Cocos nucifera* and *Musa acuminata*on antibiotic resistant bacterial pathogens. Therefore, the present work was undertaken to study the effect of *Cocos nucifera* and *Musa acuminata* on antibiotic resistant *Staphylococcus aureus and Escherichia coli.*

MATERIALS AND METHODS

Collection of plant material

Fresh plant leaf materials of *Musa acuminate* and *Cocos nucifera* were collected from Shelgi, Solapur district, Maharashtra, India. Plant material were identified and authenticated in the Department of Botany, D.B.F. Dayanand college of Arts and Science, Solapur, Maharashtra, India. Leaf materials were cleaned with distilled water and dried at room temperature for 4-5 days under shade. It was ground to obtain coarse powder by using an electric grinder.

Preparation of plant extract

Leaf constituents were extracted by cold extraction method. For this, 10 g of powder was added in 100 ml of methanol and water each. The mixture was soaked at room temperature for overnight and then filtered using Whatman filter paper no.1. The filtrate was collected and solvent was allowed to evaporate at room temperature. Percentage yield was noted. Shade dried leaves (20g) coarsely powdered and subjected to successive solvent extraction by

continuous Soxhlet extraction. The extraction was done with methanol (150 ml) at 62°**C** for 4-5 hrs.

Test organisms

The bacterial sp. used for the study was *Escherichia coli* (*E.coli*), *Pseudomonas aeruginosa* (*P.aeruginosa*), and *Staphylococcus aureus* (*S.aureus*) sp. The organisms were periodically sub cultured and maintained on nutrient agar slant at 4°C**.**

Preliminary phytochemical analysis of plant extracts

The preliminary phytochemical analysis of plant extracts was done by the standard methods given by (Raaman N., 2006).

Antibacterial activity assay (Cruickshank *et.al*., 1965)

Antibacterial activity of the extract was determined by agar well diffusion method. Sterile Muller-Hinton agar medium was used as culture medium. The suspension containing 0.1 ml of 24 hrs incubated cultures of the respective bacterial strains (with 10^8number of cells/ml) was spread separately on the agar media. Wells were made using a sterile stainless steel cork borer under aseptic conditions. The concentration of both extract of *Musa acuminata* and *Cocosnucifera*30mg/ml of extract dissolved in methanol were tested for antibacterial activity and loaded into corresponding wells. Chloramphenicol disc with concentration 30mcg was used as positive control and methanol in one well was used as negative control. The plates were incubated for 24 hrs at 37°C and after incubation the diameter of the zone of inhibition in mm was measured.

Resistance of bacterial pathogens

Resistance of test organisms was done by using Hexa universal poly disc of antibiotic. The test organisms 0.1ml of suspension *Staphylococcus aureus* and *E. coli* were matched to 0.5 McFarland standard and spread on nutrient agar plates and poly disc containing different six antibiotics was placed on plate. The plates were incubated at 37°C for 24 hrs. After incubation the zone of inhibition was measured in mm.

Gas Chromatography-Mass Spectroscopy (GC-MS) analysis

GCMS analysis was performed using GCMS unit, model Shimadzu GCMS QP 2020 with AOC 20i auto injector and AOC 20s auto sampler. In GCMS 2ml sample of methanol extract of *Musa acuminate* and *Cocos nucifera* were used. Various components were identified using

graph of plot of intensity against retention time. From graph the compounds are identified comparing the data with existing software libraries NIST17.lib.The name, molecular weight and structure of component were obtained.

Determination of minimum inhibitory concentration [MIC]

Tube dilution method was used to determine MIC of the methanol extracts of *Musa acuminata* and *Cocos nucifera*. A series of two-fold dilutions of each extract ranging from 4mg/ml to 0.125mg/ml were done in Nutrient broth. 0.1 ml of suspension of *Staphylococcus aureus* and *E.coli* matched to 0.5 McFarland standard was seeded into each dilution. Two controls were used for each test batch. These included tubes containing extract and growth medium without inoculums and organism control i.e. tube containing the growth medium and inoculums. The tubes were incubated at 37°**C** for 24 hours and checked for turbidity. Minimum inhibitory concentration was determined as highest dilution of the extract that showed no visible growth.

RESULTS AND DISCUSSION

Percentage of yields of *Musa acuminata and Cocos nucifera* extract

Musa acuminata and *Cocos nucifera* extracts obtained in methanol were weighted and percentage yield was determined. In [Table 1] *Musa acuminate* yielded higher yield as compared to *Cocos nucifera* in methanol.

Table 1: Percentage of yields of *Musa Acuminata* and *Cocos nucifera.*

Sample used	Solvent used (100 ml)	Amount of sample (g)	Amount of extract (mg)	Percentage yield (%)
Musa acuminata	Methanol	10	900	9
Cocos nucifera	Methanol	10	700	7

The percentage extractive yield ranged from 7.0 % methanol extract of *Cocos nucifera*, to 9.0 % methanol extract of *Musa acuminata*.

Preliminary phytochemical screening

The preliminary phytochemical analysis showed that carbohydrates, saponins, tannins, alkaloids and cardiac glycosides were present in the methanol extract of *Musa acuminate & Cocos nucifera* (Table 2). (Mahkota *et. al*., 2011) reported that the phytochemicals analysis of *Musa paradisiaca* flower extracts showed the presence of alkaloids, glycosides, steroids, saponins, tannins, flavonoids and terpenoids.

Table 2: Preliminary phytochemical analysis of methanol extract of *Musa acuminata* and *Cocos nucifera.*

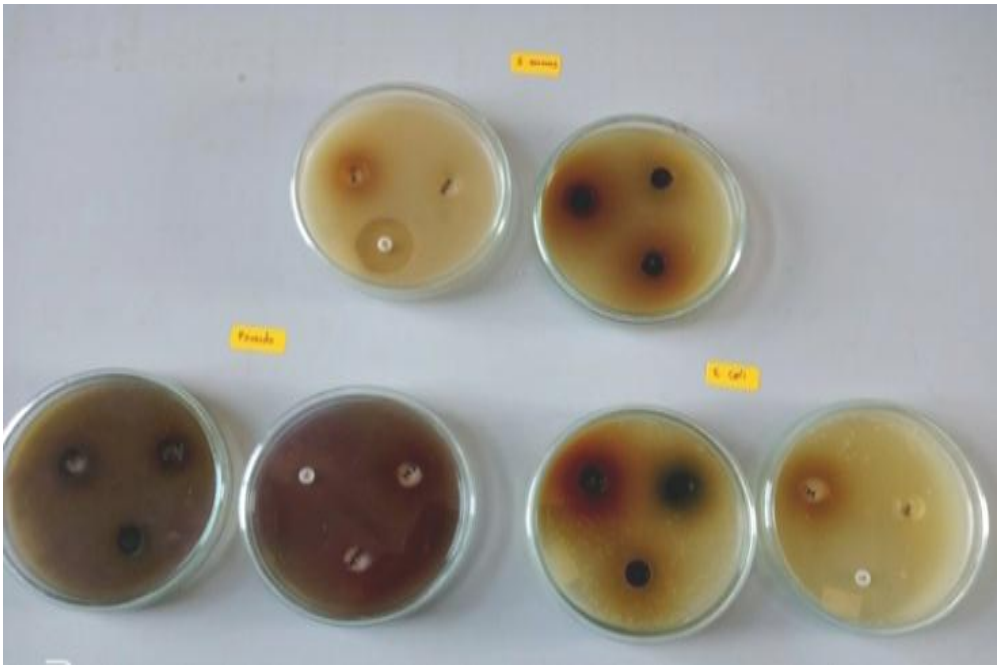
Phytochemical	Test	Methanol extract	
		Musa acuminata	***Cocos nucifera***
Carbohydrates	Benedict's test	+	+
Proteins	Ninhydrin test	-	-
Saponins	Foam test	+	+
Tannins	Ferric chloride test	+	+
Flavonoids	Magnesium and hydrochloric acid reduction test	-	-
Arthraquinones	Ammonia test	-	-
Alkaloids	Mayer's test	+	+
Cardiac glycosidase	Killer kiliani test	+	+

(+) indicates presence of phytochemical, (-) indicates: absence of phytochemical

The glycosides detected in phytochemical screening are used in the treatment of congestive heart failure and cardiac arrhythmias. Tannins are believed to have some general antimicrobial and antioxidant activities where at low concentrations it can inhibit bacterial growth and act as an antifungal agent at higher concentrations (Sumathy *et. al*., 2011).

Table 3: Antibacterial activity of Crude extract of *Musa acuminata* and *Cocos nucifera* against bacterial pathogen.

Name of test organism	Diameter of zone of inhibition (mm)				
	***Musa acuminata* (30mg)**		***Cocos nucifera* (30mg)**		**Chloramphenicol (30 mcg)**
	Methanol extract	**Aqueous extract**	**Methanol extract**	**Aqueous extract**	
Staphylococcus aureus	22	0	24	0	21
E. coli	21	0	19	0	17

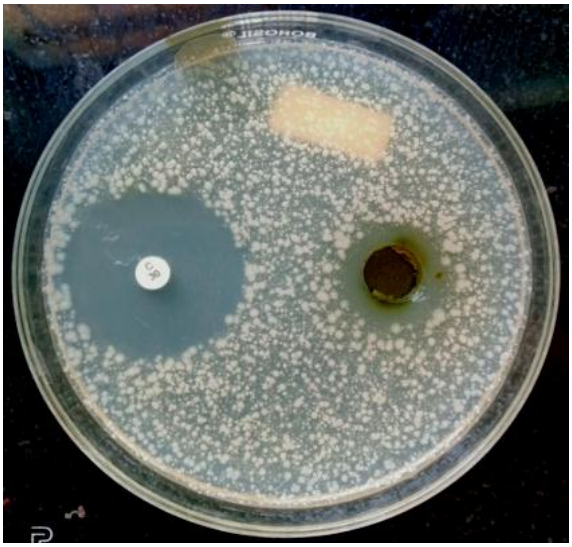


Photograph 1: Antibacterial activity of Crude extract of *Musa acuminata* and *Cocos nucifera* against bacterial pathogen.

[Table 3] shows that the methanol extract of *Musa acuminata* and *Cocos nucifera* showed maximum zone of inhibition against *Staphylococcus aureus* (22mm and 24mm) and *E. coli* (21mm and 19mm) respectively. The aqueous extract of *Musa acuminate* and *Cocos nucifera* showed zero mm of zone of inhibition against all test organisms. Therefore, in further studies the aqueous extracts were discontinued and studies were focused only on methanol extracts of *Musa acuminata* and *Cocos nucifera*. During further studies, the plant extracts obtained after extraction by Soxhlet extraction method were used. The table 3.4 shows the antibacterial activities of *Musa acuminata* and *Cocos nucifera* methanol extract (Soxhlet extraction) against *Staphylococcus aureus* and *E. coli*.

Table 4: Antibacterial activities of *Musa acuminata* and *Cocos nucifera* in methanol extract (Soxhlet extraction) against bacterial pathogen.

Name of test organism	Diameter of zone of inhibition mm		
	***Cocos nucifera* 30mg/ml**	***Musa acuminata* 30mg/ml**	***Chloramphenicol* 30 mcg**
Staphylococcus aureus	21mm	22mm	27mm
E. coli	20mm	17mm	28mm



Photograph 2: Antibacterial activity of *Musa acuminata* against *Staphylococcus aureus.*

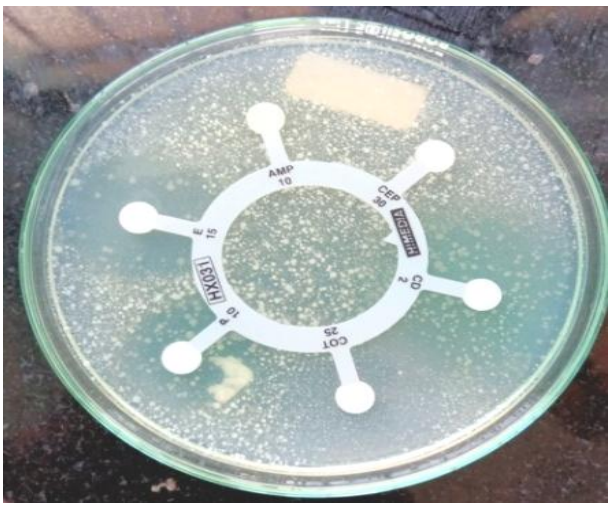
The methanol extract of *Musa acuminata* showed highest zone of inhibition (22mm) against *Staphylococcus aureus* and the *Cocos nucifera* extract showed maximum zone of inhibition (21mm) against *Staphylococcus aureus* and *E. coli* (20mm).Thus, the methanol extract of *Musa acuminata* and *Cocos nucifera* showed significant activity against tested bacterial pathogen. (Okorondu *et. al*., 2012) reported that the higher antibacterial activity of *Musa* leaf extracts was against *S.aureus*. The antimicrobial properties of plant extracts had been attributed to the presence of alkaloids and flavonoids found present in this plant.(Umamaheswari *et.al*.2017) reported that antibacterial activity of the methanol extract of *Musa acuminate* bract was significant against gram-positive (*S.aureus*) and gram-negative (*E.coli*) bacterial species in a dose dependent manner. On comparison, it was observed that the methanol extract of Musa *acuminate bract* showed greater antibacterial activity against gram negative bacteria (*E. coli*) than the gram positive bacteria (*S.aureus*). These reports corroborate the results of our studies. The in vitro antimicrobial susceptibility of *M. acuminata* flower against Gram positive, Gram negative, yeast and molds were studied by (Sumathy *et.al*. 2011).

Drug Resistance of *S. Aureus* and *E.Coli.*

The multidrug resistance pattern of *S.aureus* and *E.coli* was studied by using hexa poly disc of different antibiotics. The [Table 5] shows the zone diameters of inhibition of growth of *S.aureus*.

Table 5: Resistance of multidrug resistant *S.aureus* against various antibiotics.

Name of test organism	Diameter of zone of inhibition mm					
	Amphicillin	Cephalothin	Clindamycin	CO-Trimoxazole	Penicillin G	Erythromycin
Staphylococcus aureus	0	0	0	0	0	10



Photograph 3: Resistance of multidrug resistant *Staphylococcus aureus* against various antibiotics.

The [Table 5] revealed that *Staphylococcus aureus* showed higher resistance to various antibiotics like Amphicillin, Cephalothin, Clindamycin, CO-Trimoxazole and Penicillin G, except erythromycin which showed (10mm) zone of inhibition against *staphylococcus aureus*. The *Staphylococcus aureus* is one of the most common causes of food borne infections in most of the countries of the world, especially in India; rate of infection is still higher because of warm and humid climate. The pathogenicity of *Staphylococcus aureus* is due to the toxins, invasiveness and antibiotic resistance. It is present as a normal flora of human beings and colonizes skin, but may become pathogenic and result in minor skin infections and abscesses, to life threatening diseases such as pneumonia, meningitis, endocarditis, toxic shock syndrome (TSS), septicemia, mastitis, phlebitis, urinary tract infections, osteomyelitis and endocarditis (Bhatia A *et. al.* 2007).

Table 6: Resistance of multidrug resistant *E. coli* against various antibiotics.

Name of test organism	Diameter of zone of inhibition mm					
	Amphicillin	Gentamicin	Tetracycline	Ciprofloxacin	Cefalexin	CO-Trimoxazole
E. coli	0	0	7	0	24	0

Photograph 4: Resistance of multidrug resistant *E. coli* against various antibiotics.

In [Table.6] revealed that *E. coli* showed higher resistance to the various antibiotics like Amphicillin, Gentamicin, Ciprofloxacin and CO-Trimoxazole and showed zone of inhibition Cefalexin (24mm) and tetracycline (7mm). *Escherichia coli* is an important cause of extra-intestinal infections, enteric disease, and systemic infections in humans and animals. The emergence of MDR *E.coli* causing urinary tract infections with high virulence potential is alarming. High frequencies of resistance were observed toward trimethoprim–sulfamethoxazole (72.7%), ampicillin (70.9%), ampicillin–sulbactam (55.5%), piperacillin–tazobactam (55.5%), ciprofoxacin (47.3%), and levofoxacin (43.6%) (Ramírez-Castillo *et al.*, 2018). *E. coli* is the predominant facultative flora in the gastrointestinal tract of humans and animals. Prolonged exposure of *E. coli* to antibiotics contributes to the development of antibiotic resistance (Ponmurugan *et al*., 2013).

Determination of minimum inhibitory concentration of plant extracts

Table 7: MIC of the methanol extract of *Musa Acuminata and Cocos nucifera.*

Sr. No.	Organism	MIC (mg/ml)	
		Musa acuminata	***Cocos nucifera***
1	*Staphylococcus aureus*	1	2
2	*E. coli*	2	2

The [Table 7] shows minimum inhibitory concentration of methanol extracts of *Musa acuminate* against *Staphylococcus aureus* [1mg/ml] and against *E. coli* [2mg/ml]. The minimum inhibitory concentration of methanol extract of *Cocos nucifera* against *Staphylococcus aureus* [1mg/ml] and against *E.coli* [2mg/ml].(Ponmurugan Karuppiah & Muhammed Mustaffa 2013) reported that MIC values of *Musa* against 9 pathogens ranged

between 15.63 mg/ ml to 250 mg/ ml. The minimum inhibitory concentrations (MICs) of the *Cocos nucifera* husk extract against the susceptible bacteria generally ranged between 0.6 and 5.0 mg/ml. The MIC value ranged from 1.562mg/ml to 12.5mg/ml for the cultures tested. The extract had the highest antimicrobial activity against *S. aureus* with the lowest MIC value of 1.562mg/ml. The antimicrobial activity observed from the disc diffusion and broth dilution method could be due to the active compounds that are present in the *M. acuminata* flower extract (Sumathy *et.al*. 2011).

Gas Chromatography -Mass Spectroscopy (GCMS) Analysis

The present studies for identification and quantification of phytochemical compounds present in extracts of *Musa acuminate* and extract of *Cocos nucifera* were determined by GCMS. The results are shown in the Tables 8, 9, 10, 11 and "Fig. 1 and 2."

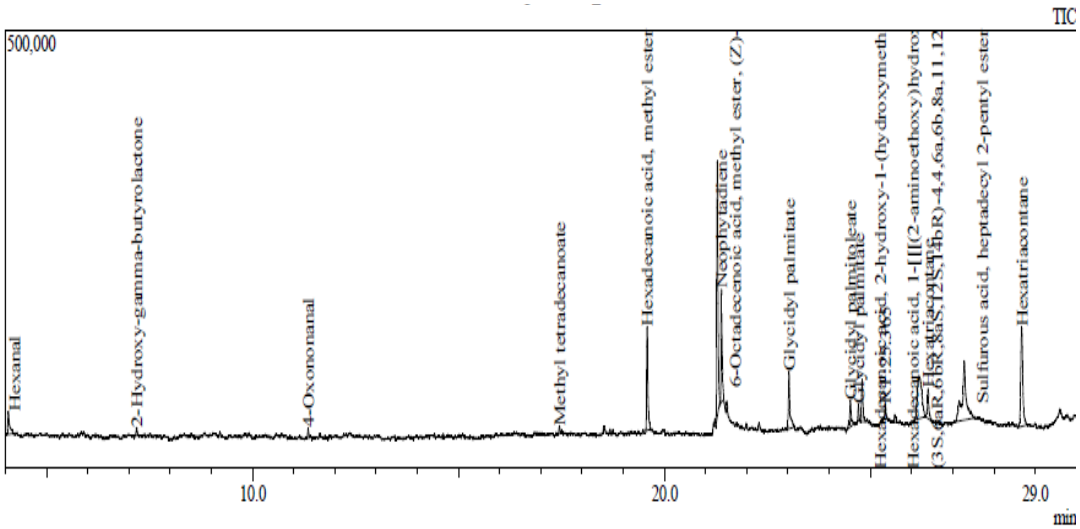


Fig. 1: Showing Chromatograph of extract of *Musa acuminate.*

The chromatograms of the fractions of methanol extract of *Musa acuminata* were obtained from the gas chromatography – mass spectrometry analysis with the peaks are shown in "Fig.1"

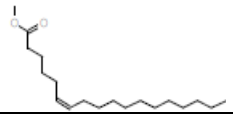
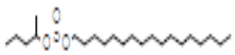
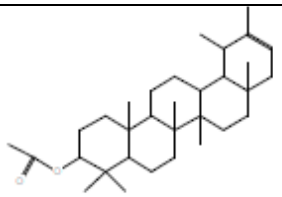
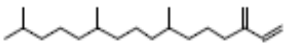
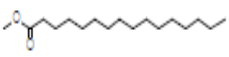
Table 8: Chemical compounds found in methanol extract of *Musa acuminata.*

Peak	R. Time	Area	Area%	Name of compound
1	21.288	590022	19.92	6-Octadecenoic acid, methyl ester, (Z)-
2	27.282	462318	15.61	Sulfurous acid, heptadecyl 2-pentyl ester
3	28.677	391878	13.23	Hexatriacontane
4	26.185	368915	12.46	3S,6aR,6bR,8aS,12S,14bR)-4,4,6a,6b,8a,11,12,14b-octamethyl1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,12,12a,12b,13,14,14a,14b-icosahydropicen-3-

				yl acetate
5	21.387	258586	8.73	Neophytadiene
6	19.583	240502	8.12	Hexadecanoic acid, methyl ester

In [Table 8] exhibits different compounds found in methanol extract of *Musa acuminata*. It showed the presence with maximum area of following compounds 6-Octadecenoic acid, methyl ester, (Z)-(19.92%), Sulfurous acid, heptadecyl 2-pentyl ester(15.61%), Hexatriacontane(13.23%),3S,6aR,6bR,8aS,12S,14bR)-4,4,6a,6b,8a,11,12,14b-octamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,12,12a,12b,13,14,14a,14b-icosahydropicen-3 yl(12.46%), Neophytadiene(8.73%), Hexadecanoic acid, methyl ester(8.12%).

Table 9: Structures of chemical compounds found in methanol extract of *Musa acuminate.*

Sr. No	Name of compound	Molecular formula	Molecular weight	Structure of Compound
1	6-Octadecenoic acid, methyl ester, (Z)-	C19H36O2	296	
2	Sulfurous acid, heptadecyl 2-pentyl ester	C22H46O3S	390	
3	Hexatriacontane	C36H74	506	
4	3S,6aR,6bR,8aS,12S,14bR)-4,4,6a,6b,8a,11,12,14b-octamethyl1,2,3,4,4a,5,6,6a, 6b,7,8,8a,9,12,12a,12b,13,14 ,14a,14b-icosahydropicen-3-yl acetate	C32H52O2	468	
5	Neophytadiene	C20H38	278	
6	Hexadecanoic acid, methyl ester	C17H34O2	270	

In the [Table 9] structures of chemical compounds found in methanol extract of *Musa acuminate* are shown Neophytadiene shows carminative gastrin inhibitor, antiulcerative, histamine release inhibitor antiprotozoal, antiparasitic biological activity. 6-Octadecenoic acid, methyl ester, (Z)-, Hexadecanoic acid, methyl ester anti-inflammatory, intestinal Calcium channel (voltage-sensitive) activator, Antihelmintic (Nematodes) Reductant, Antimutagenic, Antiprotozoal (Leishmania) with this different biological activity (Md.Adnan *et.al*.2019). Sulfurous acid, heptadecyl 2-pentyl ester is ester compound consist antibacterial activity, Hexatriacontane is included in alkane hydrocarbon with antioxidant activity and Hypoglycaemic (Babu *et al*., 2017).

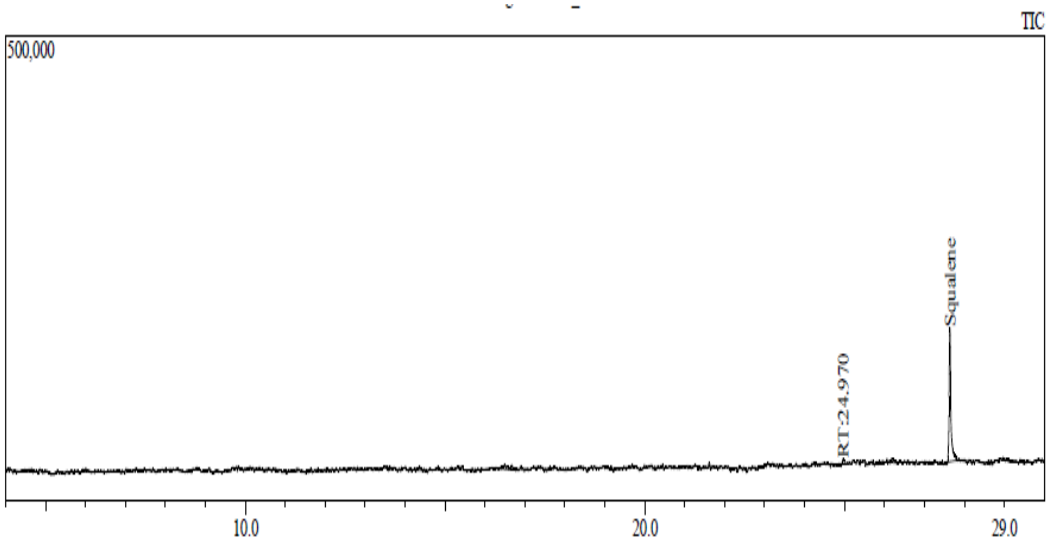


Fig. 2: Showing chromatogram of extract of *cocos nucifera.*

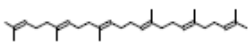
The chromatograms of the fractions of methanol extract of were obtained from the gas *Cocosnucifera* chromatography – mass spectrometry analysis with the peaks are shown in "Fig. 2"

Table 10: Chemical compounds found in methanol extract of *Cocos nucifera.*

Peak	R. Time	Area	Area%	Name
1	27.640	455730	79.14	Squalene

The [Table 10] exhibits single compound found in methanol extract of *Cocos nuciera.* It showed the presence of Squalene (79.14%) with maximum area. It is considered a potent chemo preventive and chemotherapeutic agent, which inhibits the tumor growth in the colon, skin, lung, and breast, and it stimulates the immune system for the application of drugs in the treatment of diseases such as HIV, H1N1, leukemia, papilloma, and herpes, among others. Squalene is also found in the human body, is secreted by the sebaceous glands for skin protection (M. Azalia Lozano-Grande *et al*., 2018).

Table 11: Structures of chemical compounds found in methanol extract of *Cocos nucifera.*

Sr. no	Name of compound	Molecular formula	Molecular weight	Structure of Compound
1	Squalene	C30H50	410	

In the [Table 11] structure of chemical compound found in methanol extract of *Cocos nucifera* are shown. Thus the GCMS analysis of extracts of *Musa acuminate* and *Cocos*

nucifera showed presence of several antibacterial substances that are responsible for their potential antibacterial activity even against the multidrug resistant human pathogenic bacteria.

CONCLUSION

Musa acuminata and *Cocos nucifera* leaf extract showed potential antibacterial activity against the multidrug resistant *Staphylococcus aureus* and *E. coli*. The extract showed various phytochemical and bioactive compounds like Tannin, Saponins and Cardiac glycosides which are responsible for the antibacterial activity. This revealed active pharmaceutical agents in extract which are responsible for effective antibacterial activity. The presence of pharmacological effect in both extracts indicates the potential of these plant extracts for manufacturing of traditional therapeutic drug.

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