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## *Full Length Research Article*

# ANTIBACTERIAL ACTIVITY OF *EXCOECARIA AGALLOCHA* L.LEAF IN CHLOROFORM AND ETHANOL EXTRACTS (GC-MS ANALYSIS)

**[1]Parasuraman, P. and [*,2]Dr. Manikandan, T.**

[1]Govt. Hr. Secodary School, Devikapuram, Tiruvannamalai-606902, Tamilnadu, India
[2]Department of Botany, Arignar Anna Govt. Arts College, Villupuram- 605602, Tamilnadu, India

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GC-MS Analysis.

**ABSTRACT**

The present study was carried out for Antibacterial Activity and GC-MS analysis of *Excoecaria agallocha* L. leaf extracts of Chloroform and Ethanol. Antibacterial assay was carried out against five bacteria viz. *Staphylococcus aureus* MTCC3381, *Bacillus cereus* MTCC430, *Escherichia coli* MTCC739, *Pseudomonas aeruginosa* MTCC 424, *Klebsiella pneumoniae* MTCC432 using agar well diffusion method. Chloroform extract of *E.agallocha* was highly effective on Bacillus cereus strain. There was no effect on *E.coli* and *K.pneumoniae*. It was moderately effective in 2000µg concentration only. *P.aeruginosa* was affected moderately in 1500 and 2000µg concentrations. Ethanol extract was highly effective than Chloroform extract. Ethanol extract was ineffective in 500µg on *E.coli*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. In 1500µg Ethanol extract was ineffective on *P.aeruginosa*. Ethanol extract was ineffective in 1000µg concentration on *K.pneumoniae*. It was highly effective on *S.aureus* at 500,1000,1500,2000µgs. GC-MS analysis was tested in chloroform and ethanol extracts of *E.agallocha* L. In Chloroform extract 34 bio-active compounds were identied. From that Hentriacontane 5.23%, Tricyclo, undec-tetramethyl 16.38%, Tetramethyl, trihydro-napthalene 20.72%, β - amyrin 7.52%, α – amyrin 6.92%, Heptadecanol 8.24% were in high proportions. Ethanol extract showed 17 bio-active compounds, in that Myoinosital 4-c-methyl 37.09%, *Tricyclo undec* (isocaryophyllene) 13.21%, Trimethyl 5,6-dimethylene-deca hydro naphthalene 17.25%, β- amyrin 5.78%, α- amyrin 5.78% were observed as the major constituents.



## INTRODUCTION

Indian medicinal plants having therapeutic values and can also be used in drugs. Most of the people in developing countries depend on traditional medicines from plants for the primary health care needs as estimated by WHO. Plant medicines have minimal toxicity, low cost, pharmacologically active and provide easy remedy for human beings. The effectiveness of plant extracts on micro organisms has been studied worldwide. *Excoecaria agallocha* L. belongs to Euphobiaceae family. It is a small mangrove tree with 15m height. The bark oil is effective against rheumatism, leprosy and paralysis. However it cause temporary blindness if it enters the eyes. The plant is potent as anti- HIV, anti- cancer, anti-bacterial and anti-viral agent (Peter *et al*., 1999). The plant is used as fire wood, timber and also gives tannin, fish poison and medicines for epilepsy, ulcers, hand and feet swellings, toothache (Bandara Nayake, 2002). People in coastal areas destroying the tree for fodder, fuel, wood pulp vegetable tannins, poles for building constructions and medicines (UNEP 1988). Medicines from mangrove plants are used for curing elephantiasis and abdominal troubles. The main aim of the present study is the antibacterial activity and bio-active compounds of *E.agallocha* L. leaf extract in different solvents i.e., Chloroform and Ethanol.

***Corresponding author:*** **Dr. Manikandan, T.**
Department of Botany, Arignar Anna Govt. Arts College, Villupuram- 605602, Tamilnadu, India.

## MATERIALS AND METHODS

Mature leaves of *Excoecaria agallocha* L. were collected during monsoon period in the mangrove belt of Pichavaram, Tami Nadu, India. The present investigation was carried out in the Botanical Garden of Arignar Anna Govt. Arts College, Villupuram, Tamil Nadu, India. The plant material was identified with the help of "FLORA OF THE PRESIDENCY OF MADRAS" (Gamble, 1954). Five different bacteria were used for this study. They were *Staphylococcus aureus*

MTCC3381, Bacillus cereus MTCC430, Escherichia coli MTCC739, *Pseudomonas aeruginosa* MTCC 424, *Klebsiella pneumoniae* MTCC432 from that *S.aureus* and *B.cereus* are gram positive strains. Rest of the three are gram negative strains (Christian Gram, 1884).

**Preparation of plant extracts**

For the preparation of leaf extracts Soxhlet extractor (Franz von Soxhlet in 1879) was used. The leaves were washed in tap water, shade dried and made into a fine powder. The powder was extracted in the thimble of Soxhlet extractor with the solvents Chloroform and Ethanol successively. This extracts were concentrated to dryness using rotary vacuum evaporator.

**Test Organisms**

The extract was tested on the following two gram positive bacteria namely *S.aureus* MTCC3381, *B.cereus* MTCC430. Three gram negative bacteria were also tested**.** They are *E.coli* MTCC739, *P.aeruginosa* MTCC424, *K.pneumoniae* MTCC432. All the strains were collected from Manian Institute of Science and Technology, Coimbatore, Tamil Nadu, India. Test was conducted with the help of MISAT Lab Coimbatore, Tamil Nadu.

**Preparation of Inocula**

Inoculation was done with the help of MISAT lab Coimbatore. The test organisms were sub cultured by streaking them on nutrient agar followed by incubation for 24h at 37°C. Several colonies of each bacteria species were transferred to sterile nutrient broth. The suspensions were mixed for 15sec and incubate for 24h at 37°C on an orbital incubated shaker. Working concentrations of microbial suspension was prepared in 3ml of sterile saline to turbidity equivalent to 0.5mc land scale (10 adjusting the potical density to 0.1 at 600nm yielding a cell density of 1-2x10^5 CFU/ml.).

**GC-MS Analysis**

Chloroform and Ethanol extracts were prepared with the help of Bureau Veritas Consumer product Services (I) Pvt. Ltd. Chennai-32 by GC-MS 5975C Agilent instrument. 100gms of powder was weighed and extracted using 100ml of solvents successively with Chloroform and Ethanol with the help of Soxhlet apparatus. The extracts were evaporated to dryness using rotary evaporator. The dried extract was then subjected to GC-MS Analysis. Gas chromatography technique separates chemicals based on their volatility or ease with which they evaporate into gas. The MS is used to identify the chemicals based on their structure. One micro liter (1µl or 0.000001L) of solvents containing the mixture of molecules were injected into the GC and the sample was carried by Helium through the instrument. Chemicals with high volatility travel through the column more quickly than chemicals with low volatility. The ions travelled through an electromagnetic field then filtrate the ion based on their mass. The spectrum of unknown components was compared with spectrum of the known components stored in NIST library. The name, molecular weight and structure of the components of the test materials were determined.

## RESULTS AND DISCUSSION

Chloroform extract of *E.agallocha* showed mild effect on the micro organisms. The extract showed no inhibition on *E.coli* and K. pneumonia (Gram negative strains). *B.cereus* was inhibited in the range of 10.0± 0.0, 11.5± 0.7, 13.0±0.0, 15.0±0.0 depends on the concentrations.(500,1000,1500 and 2000µgs). *Staphylococcus aureus* was affected only in the 2000µg conc.(10.0±0.0). *P.aereus* was affected in 1500µg (10.0±0.0) and in 2000µg (10.5±0.7) (Table 1 and Plate 1). The inhibitory effect of Chloroform and Ethanol extracts of *Excoecaria agallocha* L. increases with increase in concentration. Chloroform extracts of *Solanum trilobatum* showsinhibition on *S.aureus*, *E.coli*, *K.pneumoniae*, *P.aeruginosa*. This is a negative result with *E.agallocha* L.

**Table 1. Antibacterial activity of *Excoecaria agallocha* L. leaf in chloroform extract**

| Sample | Conc.(µg) | Zone of Inhibition(mm) | | | | |
|---|---|---|---|---|---|---|
| | | S.a | E.c | B.c | P.a | K.p |
| Chloroform | 500 | - | - | 10.0±0.0 | - | - |
| | 1000 | - | - | 11.5±0.7 | - | - |
| | 1500 | - | - | 13.0±0.0 | 10.0±0.0 | - |
| | 2000 | 10.0±0.0 | - | 15.0±0.0 | 10.5±0.7 | - |
| Chloromphenicol | 10 | 20.5±0.7 | 14.0±0.0 | 20.5±0.7 | 14.0±0.0 | 19.5±0.7 |

Values are means of three independent analysis ± Standard Deviation (n=3)

**Antibacterial Activity**

Nutrient agar (NA) plates (12cm diameter) were seeded with 8h broth culture of different bacteria. In each of this plates well were (6mm diameter) cut out using sterile cork borer. Using sterilized dropping pipettes different concentration (500,1000, 1500 and 2000µg /ml) of plant extract was carefully added in the wells and allowed to diffuse at room temperature for 2h (well diffusion Method Perez *et al.,*1990). The plates were then incubated at 37°C for 18-24h. Chloromphenicol (10µg) was used as positive control and DMSO (Dimethyl sulphoxide) as negative control. The antimicrobial activity was evaluated by measuring the diameter of inhibition zone (Agwa *et al.,* 2000). Antibacterial activity was assigned by measuring the inhibition zone formed around the discs.

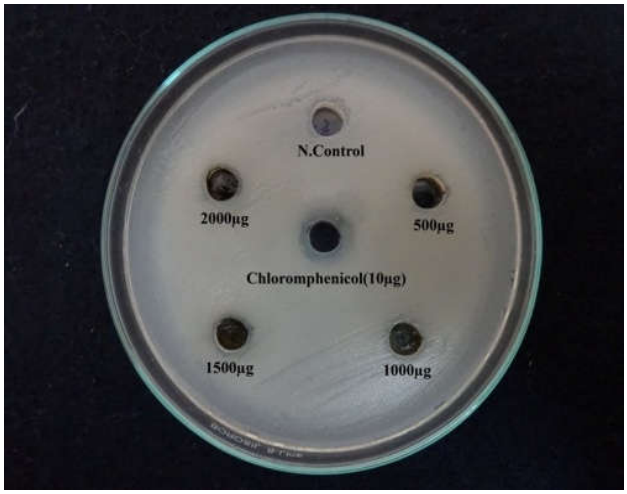
leaf extract (Asirvatham doss and Rangaswamy Dhanabalan, 2008). The Chloroform extract of *E.agallocha* inhibits (50mg/ml) on *P.aeruginosa*, *S.aureus* and *E.coli* (Jayanta Kumar Patra *et al*., 2009). But this is not similar for this present study. *Calotropis gigantia* Linn. Leaf extract of chloroform shows no inhibition on S.aureus and E.coli. It is similar to *E.agallocha* L. (Bharathi *et al*., 2011). In 100mg/ml Chloroform extract of *Solanum nigrum* Linn. Leaf shows no effect on E.coli and S.aureus but effective on *K.pneumoniae* (10mm diameter, Sridhar *et al.,* 2011). It is similar with the present study. The Chloroform extract of *Casuarina equisettifolia* shows inhibition in 100mg/ml on *S.aureus*, *E.coli*, *K.pneumoniae* and *P.aeruginosa* (Nehad Gumgumjee *et al*., 2012). Chloroform extract of *Parthenium hysterophorus* leaf shows 63% inhibition on *B.cereus* (Malarkodi and Manoharan, 2013). It is similar with the present study.

**Table 2. Antibacterial activity of *Excoecaria agallocha* L. leaf in ethanol extract**

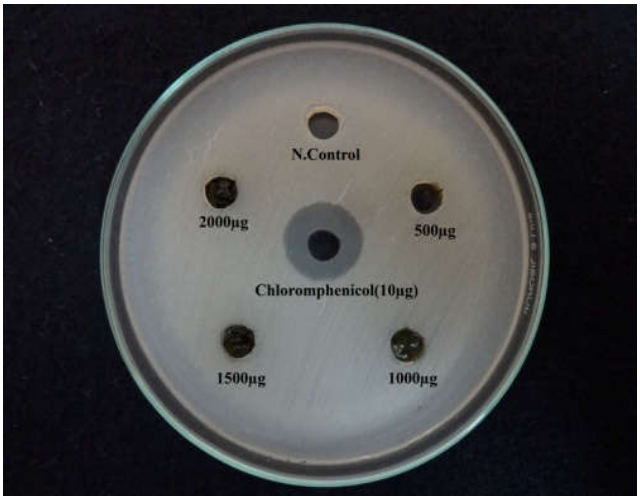
| Sample | Conc (µg) | Zone of Inhibition (mm) | | | | |
|---|---|---|---|---|---|---|
| | | S.a | E.c | B.c | P.a | K.p |
| Ethanol | 500 | 18.0±0.0 | - | - | - | - |
| | 1000 | 20.5±0.7 | 10.0±0.7 | 11.0±0.0 | - | - |
| | 1500 | 22.0±0.0 | 11.0±0.0 | 13.5±0.7 | - | 11.0±0.0 |
| | 2000 | 24.5±0.7 | 13.0±0.0 | 15.0±0.0 | 13.0±0.0 | 10.0±0.0 |
| Chloromphenicol | 10 | 20.5±0.7 | 14.0±0.0 | 20.5±0.7 | 14.0±0.0 | 19.5±0.7 |

Values are means of three independent analysis ± Standard Deviation(n=3)

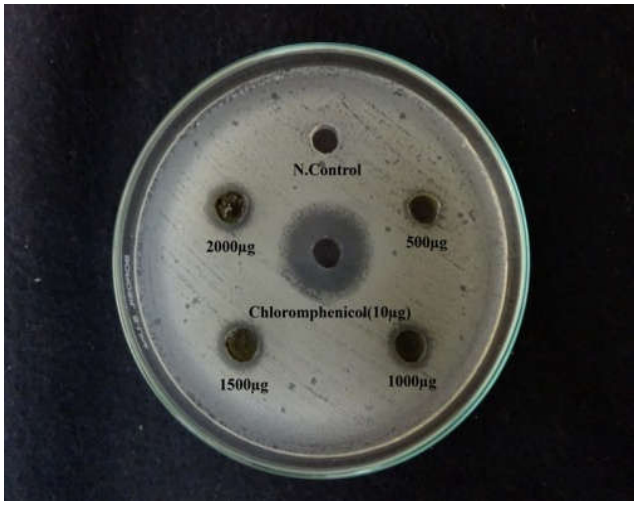
**Plate 1. Antibacterial activity of *Excoecaria agallocha* L. leaf in Chloroform extract**



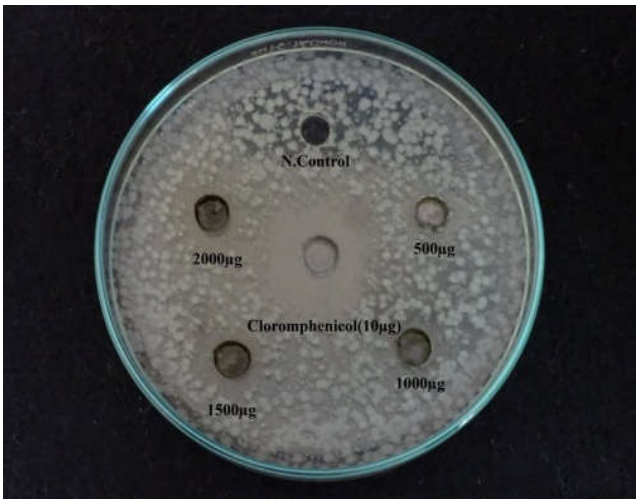

***Excoecaria Agallocha* Chloroform (S.a)**



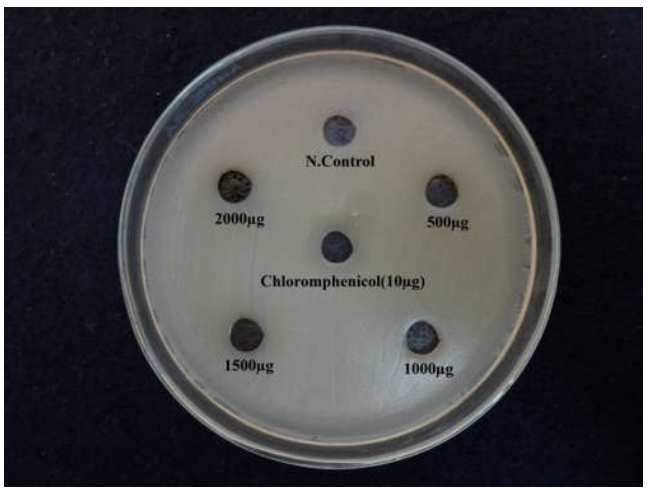

***Excoecaria Agallocha* Chloroform (E.c)**




***Excoecaria Agallocha* Chloroform (B.c)**




***Excoecaria Agallocha* Chloroform (P.a)**




***Excoecaria Agallocha* Chloroform (K.p)**

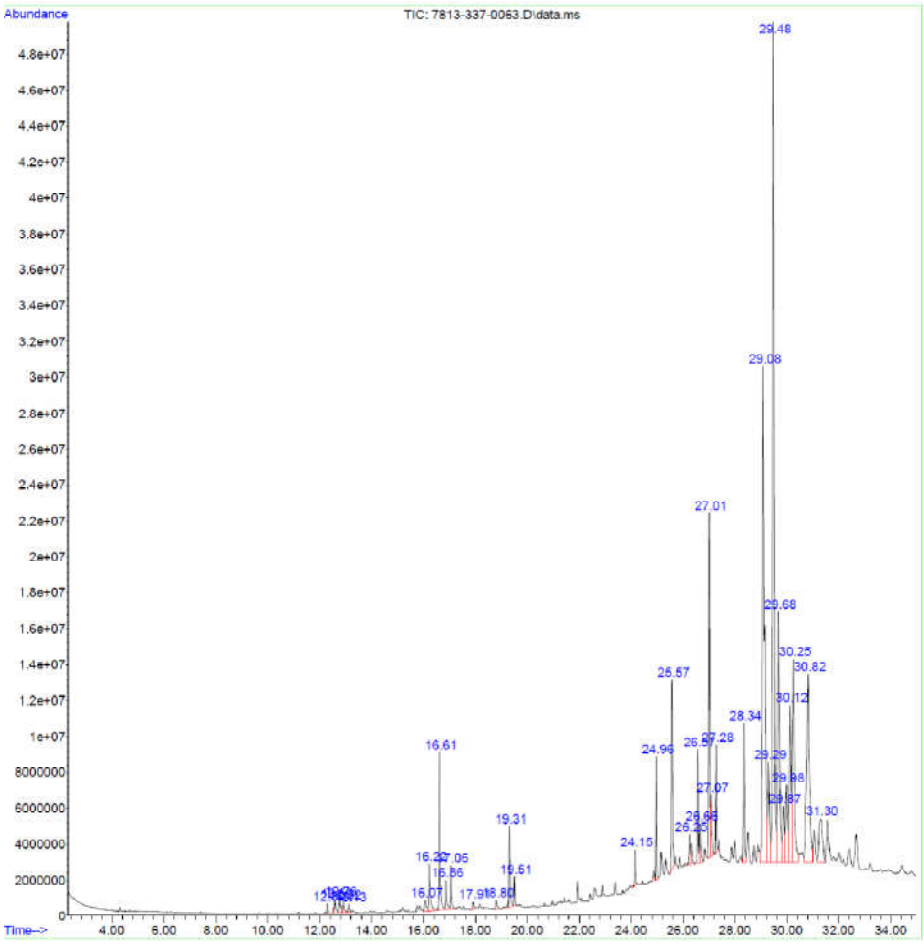
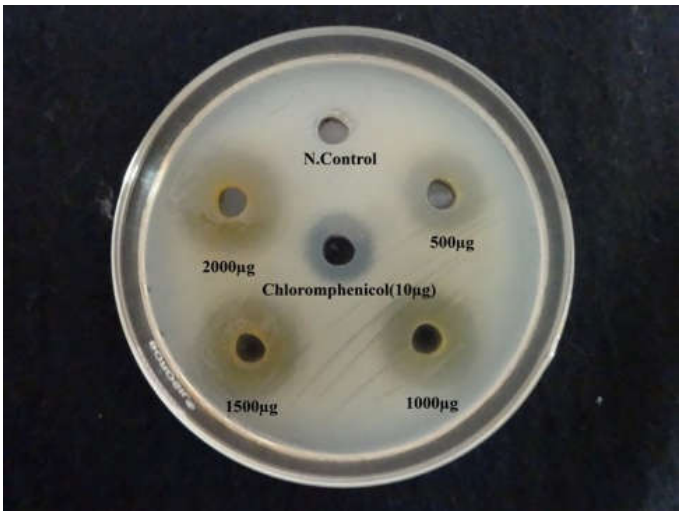



**Fig.1. Peak area of Bio-active Chemicals in Chloroform Extract of Excoecaria agallocha L. leaf (GC-MS)**

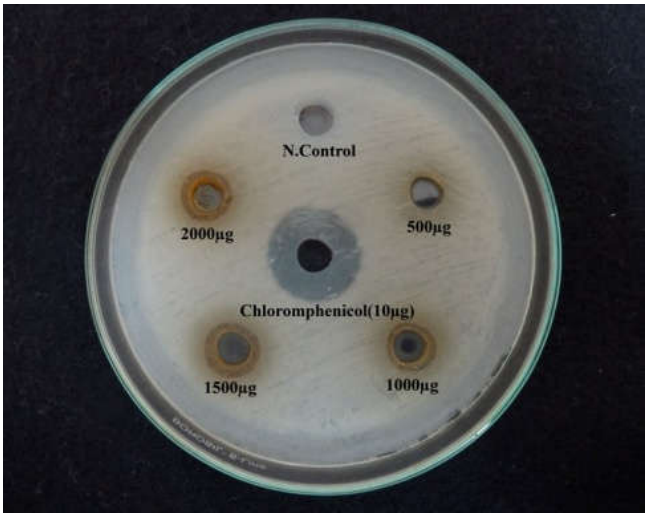
**Table 3. Bio-active chemicals identified in Excoecariaagallocha L. (Chloroform extract) (GC-MS Report TLC)**

| Peak | R.Time(Mins) | Name of the Chemical Compound | M.F. | M.W. | Peak Area | Area% |
|---|---|---|---|---|---|---|
| 1 | 12.293 | B-Curcumene | $C_{15}H_{24}$ | 204 | 12735905 | 0.13 |
| 2 | 12.598 | Benzene ,1-(1,5 dimethyl-4-Hexenyl-4-Methyl- | $C_{15}H_{22}$ | 202 | 26819666 | 0.28 |
| 3 | 12.758 | 1,3-Cyclohexadiene,5(1,5 dimethyl) | $C_{15}H_{24}$ | 204 | 26126978 | 0.28 |
| 4 | 12.918 | β-Bisabolene | $C_6H_8$ | 080 | 22936539 | 0.24 |
| 5 | 13.135 | Cyclohexane,3(1,5 Dimethyl-4-Hexenyl -6-Methylene-s-(RS) | $C_{15}H_{24}$ | 204 | 14225398 | 0.15 |
| 6 | 16.069 | 5-Ethylcyclopent-1-ene-1-Carboxylic acid | $C_8H_{12}O_2$ | 140 | 32786260 | 0.35 |
| 7 | 16.229 | 2-Cyclohexane-1-one 4-Hydroxy- 3,5,6-Trimethyl-4(3-oxo-1Butenyl) | $C_{13}H_{18}O_3$ | 222 | 107623763 | 1.14 |
| 8 | 16.606 | Bicyclo(3,1.1)Heptane,2,6,6 Trimethyl- | $(C_{10}H_{16})n$ | 000 | 230080098 | 2.43 |
| 9 | 16.868 | Phytol, Acitate | $C_{22}H_{42}O_2$ | 338 | 59574193 | 0.54 |
| 10 | 17.057 | 1.Hexadecyne | $C_{16}H_{32}$ | 224 | 67813854 | 0.72 |
| 11 | 17.914 | n-Hexadecanoic acid(palmitic acid) | $C_{16}H_{32}O_2$ | 256 | 11201392 | 0.12 |
| 12 | 18.799 | 1H-Indazole,5,7 Dimethyl | $C_9H_{10}N_2$ | 146 | 18080641 | 0.19 |
| 13 | 19.308 | Phytol | $C_{20}H_{40}O$ | 296 | 124447096 | 1.31 |
| 14 | 19.511 | 5-Methoxy-2-Methylindole-3-acetic acid -Tertbutyl ester | $C_{12}H_{13}NO_3$ | 219 | 31102439 | 0.33 |
| 15 | 24.144 | Eicosane | $C_{20}H_{38}$ | 278 | 46352703 | 0.49 |
| 16 | 24.957 | Squalene | $C_{30}H_{50}$ | 410 | 128286858 | 1.35 |
| 17 | 25.576 | Heptadecane,9-octyl | $C_{25}H_{52}$ | 352 | 277558171 | 2.93 |
| 18 | 26.250 | Heptadecane | $C_{17}H_{36}$ | 240 | 54645644 | 0.58 |
| 19 | 26.569 | 1,19-Eicosadiene | $C_{20}H_{38}$ | 278 | 146660431 | 1.55 |
| 20 | 26.656 | Gamma-Tocopherol | $C_{28}H_{48}O_2$ | 416 | 71959973 | 0.76 |
| 21 | 27.005 | Hentriacontane | $C_{31}H_{64}$ | 436 | 495524359 | 5.23 |
| 22 | 27.063 | 1-Heptacosanol | $C_{27}H_{54}O$ | 394 | 105516830 | 1.11 |
| 23 | 27.281 | Vitamin E | $C_{29}H_{50}O_2$ | 430 | 153143961 | 1.62 |
| 24 | 28.341 | Oxirane,Hexadecyl | $C_{15}H_{32}O$ | 240 | 251390498 | 2.66 |
| 25 | 29.082 | Tricyclo(6.2.1.0(4,11)Undecene 1,5,9,9- Tetramethyl | $C_{15}H_{24}$ | 204 | 1551277372 | 16.38 |
| 26 | 29.285 | Androstan-17-one 16,16-dimethyl-(5-α) | $C_{22}H_{36}O_2$ | 332 | 262470321 | 2.77 |
| 27 | 29.474 | 2,5,5,8a-Tetramethyl-6,7-8,8a- Tetrahydro-5H-Naphthalene-1-one | $C_{10}H_{18}$ | 138 | 1961317086 | 20.72 |
| 28 | 29.677 | β-Amyrin | $C_{30}H_{50}O$ | 426 | 712193762 | 7.52 |
| 29 | 29.866 | Caparratriene | $C_{15}H_{26}$ | 206 | 157222150 | 1.66 |
| 30 | 29.982 | 1,2,5-Oxadiazol-3-amine,4-(3- Methoxy Phenoxy)- | $C_{17}H_{13}F_3N_6O$ | 000 | 218568259 | 2.31 |
| 31 | 30.127 | 9,19-Cyclo lanost-24-en-3-ol,(3β) | $C_{30}H_{50}$ | 426 | 422863456 | 4.47 |
| 32 | 30.244 | α-Amyrin | $C_{30}H_{50}O$ | 426 | 655423785 | 6.92 |
| 33 | 30.825 | Heptadecanal | $C_{17}H_{34}O$ | 254 | 780119808 | 8.24 |
| 34 | 31.289 | 2(Acetoxy methyl)3-Methoxy Carbonyl) bi phenylene | $C_{17}H_{14}O_4$ | 282 | 237767401 | 2.51 |
| | | | | Total | 7697818050 | 99.99 |

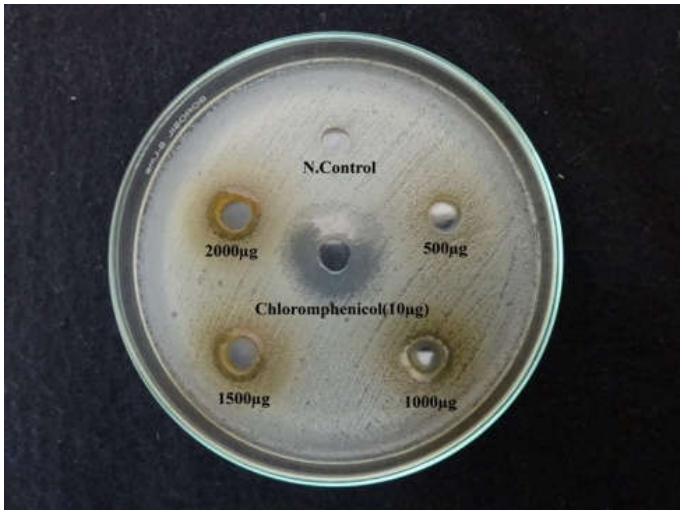
**Plate 2. Antibacterial activity of *Excoecaria agallocha* L. leaf in Ethanol Extract**




***Excoecaria Agallocha*** **Ethanol (S.a)**

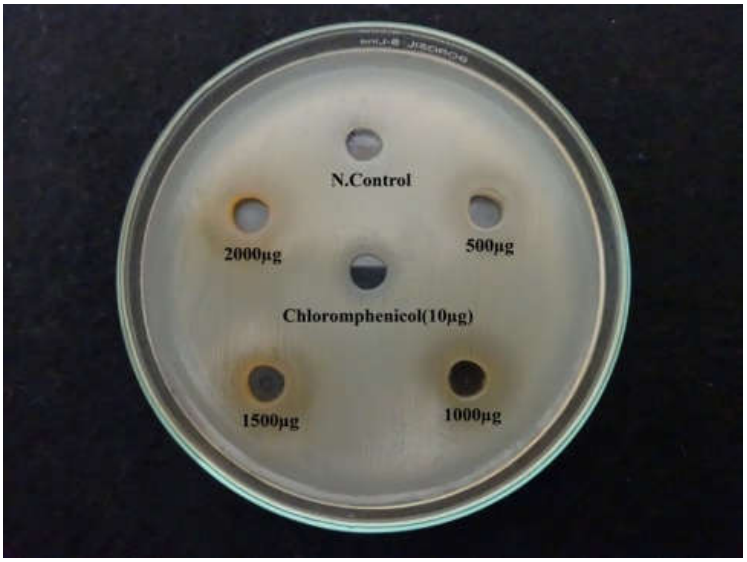



***Excoecaria Agallocha*** **Ethanol (E.c)**




***Excoecaria Agallocha*** **Ethanol (B.c)**


***Excoecaria Agallocha*** **Ethanol (P.a)**




***Excoecaria Agallocha*** **Ethanol (K.p)**

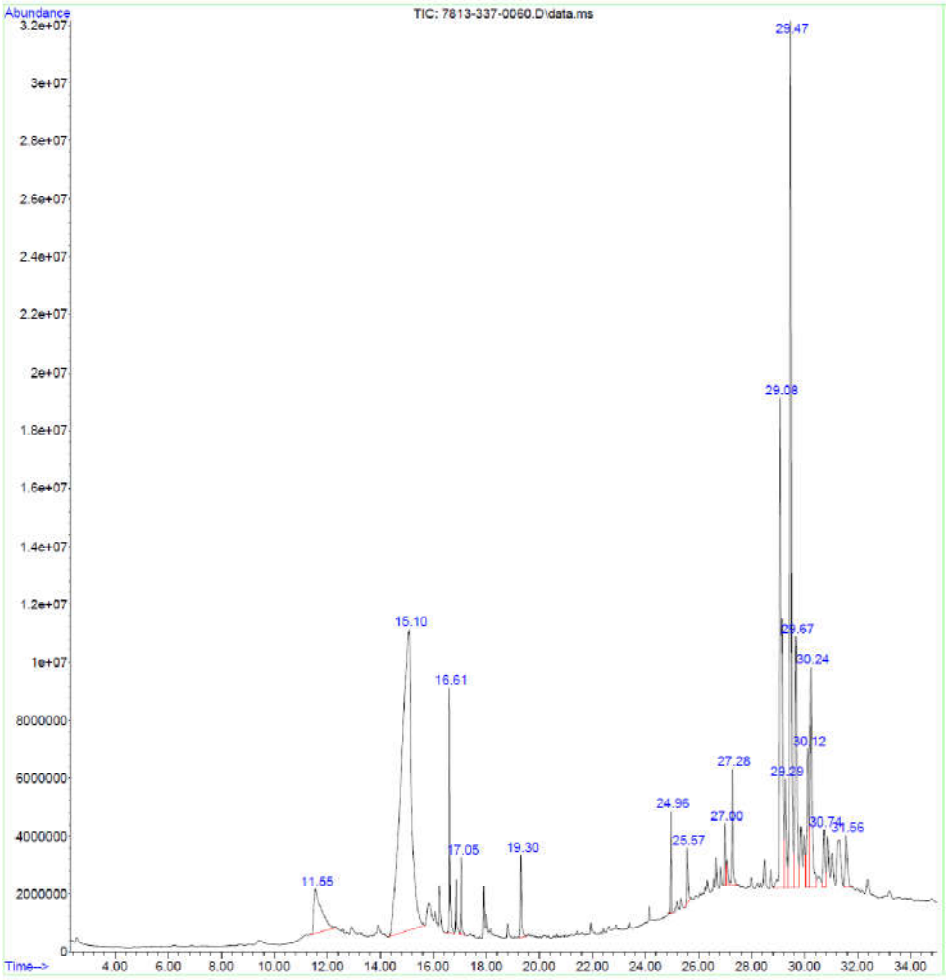



**Fig. 2. Peak area of Bio-active Chemicals in Ethanol Extract of *Excoecaria agallocha* L. (GC-MS)**

**Table 4. Bio-active chemicals identified in *Excoecaria agallocha* L. (Ethanol extract) (GC-MS Report TLC)**

| Peak | R.Time(Mins) | Name of the Chemical compound | M.F. | M.W. | Peak Area | Area% |
|---|---|---|---|---|---|---|
| 1 | 16.607 | Bicyclo(3,1.1)Heptane,2,6,6 Trimethyl- | $(C_{10}H_{16})n$ | 000 | 66904320 | 1.13 |
| 2 | 19.308 | Phytol | $C_{20}H_{40}O$ | 296 | 43891216 | 0.74 |
| 3 | 24.144 | Eicosane | $C_{20}H_{38}$ | 278 | 17190394 | 0.29 |
| 4 | 24.957 | Squalene | $C_{3}0H_{50}$ | 410 | 100848316 | 1.70 |
| 5 | 25.161 | Cyclohexanone-2-2-dimethyl 5(3 methyl) | $C_{8}H_{14}O$ | 126 | 52040461 | 0.88 |
| 6 | 25.567 | Hexadecane 1-iodo- | $C_{16}H_{33}I$ | 352 | 219245211 | 3.70 |
| 7 | 26.569 | Oxirane,Hexadecyl | $C_{15}H_{32}O$ | 240 | 106771039 | 1.80 |
| 8 | 26.657 | Gamma-Tocopherol | $C_{28}H_{48}O_{2}$ | 416 | 50760212 | 0.86 |
| 9 | 27.005 | Hentriacontane | $C_{31}H_{64}$ | 436 | 488711916 | 8.25 |
| 10 | 27.281 | Vitamin E | $C_{29}H_{50}O_{2}$ | 430 | 106482204 | 1.80 |
| 11 | 28.341 | 16-Heptadecanal | $C_{17}H_{34}O$ | 254 | 151004464 | 2.55 |
| 12 | 29.082 | Tricyclo(6.2.1.0(4,11)Undecene 1,5,9,9-Tetramethyl | $C_{15}H_{24}$ | 204 | 1182079977 | 19.95 |
| 13 | 29.285 | Naphthalene | $C_{10}H_{10}O$ | 146 | 184169474 | 3.11 |
| 14 | 29.474 | Tetramethyl-6,7-8,8a-Tetrahydro-5H- naphthalene-1-one | $C_{10}H_{18}$ | 138 | 1547490440 | 26.12 |
| 15 | 29.677 | β-Amyrin | $C_{30}H_{50}O$ | 426 | 410800848 | 6.93 |
| 16 | 29.866 | Caparratriene | $C_{15}H_{26}$ | 206 | 63312855 | 1.07 |
| 17 | 30.128 | Lanosterol | $C_{30}H_{50}O$ | 426 | 164515208 | 2.78 |
| 18 | 30.244 | α-Amyrin | $C_{30}H_{50}O$ | 426 | 399600429 | 6.74 |
| 19 | 30.825 | 1,19-Eicosadiene | $C_{20}H_{38}$ | 278 | 487226123 | 8.22 |
| 20 | 31.057 | Anthracene,9,10 dihydro 9,9,10 trimethyl | $C_{17}H_{18}$ | 222 | 82299986 | 1.39 |
| | | | | Total | 6832980093 | 99.73 |

In Wrightia tinctoria (Roxb) R.br. Chloroform leaf extract shows inhibition on *E.coli*, *S.aureus* but not on *P.aeruginosa* (Vedhanarayanan *et al*., 2013). It is not similar to E.coli with the present study. Ethanol extract of *E.agallocha* L. showed 66.7% inhibition on micro organisms used *S.aureus* was highly inhibited. *E.coli* and *B.cereus* were affected in 1000, 1500, 2000µg concentrations. *P.aeruginosa* was affected only in 2000µg/ml. *K.pneumoniae* was affected only in the 1500 and 2000µg concentrations (Table 2 and Plate 2). Antimicrobial activity of *Eclipta alba* and *Morinda citrifolia* L. extracts of Ethanol is effective on *B.cereus*, *S.aureus*, *E.coli*, *P.aeruginosa*, *K.pneumoniae* (Mukesh *et al.,* 2010). This is not same with the results of E.agallocha L. Ethanol extract of *Aloe vera* showed inhibition on *K.pneumoniae*,

*P. aeruginosa*, *S.aureus*, but no inhibition on *E.coli* (Thiruppathi *et al*., 2010). It is similar with *E.agallocha* L. Ethanol extract of Citrus sinensis shows inhibition on *E.coli*, *P.aeruginosa*, *K.pneumoniae*, *S.aureus* (Uchechi *et al*., 2010). It is not similar with *E.agallocha* L. Ethanol extract of *Calotropis gigantia* Linn. Shows no effect on *S.aureus* and *E.coli*. It is similar with E.agallocha L. but active on *K.pneumoniae* (Bharathi *et al*., 2011). The *Nelumbo nucifera* leaf extract of ethanol shows inhibition on *E.coli* and *K.pneumoniae* in 1500µg/ml (Muthu Mohammad Jamal Moideen *et al*., 2011). It is similar with *E.agallocha* L. *Solanum nigrum* leaf extract was effective on E.coli, K.pneumoniae and S. aureus (Sridhar *et al*., 2011). But this is not similar for E.coli and K.pneumoniae in *Excoecaria agallocha* L. Leaf extract of *Catharanthus roseus* (Linn) inhibits *E.coli*, *S.aureus* and *P.aeruginosa* (Chinna venkatraman *et al*., 2012). This result is not same with *E.agallocha* L. *Casuarina equisettifolia* leaf extract inhibits very high on *S.aureus*, *E.coli*, *P.aeruginosa* and *K.pneumoniae* (Nehad and Gumgumjee *et al*., 2012). But there was moderate effect in *E.agallocha* L. 87% of inhibition was noticed by Malarkodi and Manoharan 2013 in *Parthinium hysterophorus* extract on *B.cereus*. This is high when compared with *E.agallocha* L. *E.coli* was affected low in Ethanol extract of Wrightia tinctoria (Vedhanarayanan *et al*., 2013). It is similar with *E.agallocha* Ethanol extract. In GC-MS analysis of Chloroform extract of *E.agallocha* L. 34 chemical compounds were identified (Table 3). The structural formula, molecular weight, retention time, peak area and area% were given in Figure 1. Among the 34 compounds 7 major components were high in proportion. They are Hentriacontane 5.23%, Tricyclo, undec-tetramethyl 16.38%, Tetramethyl, trihydro-napthalene 20.72%, β - amyrin 7.52%, 9,19-cyclolanost :24-en-3-ol 4.47%, α – amyrin 6.92%, Heptadecanol 8.24%. All the constituents were characterized and identified by comparison of the mass spectra of the constituents of the known components stored in NIST library. The presence of various bio-active compounds in *E.agallocha* justifies the use of leaves for various ailments traditionally. Hentriacontane is in bees wax also (8.9%). In Ethanol extract of *E.agallocha* L. seventeen compounds were identified (Table 4 and Fig.2). From these Myoinositol 4-c-methyl 37.09%, Tricyclo undec(isocaryophyllene) 13.21%, Trimethyl 5,6-dimethylene-deca hydro naphthalene 17.25%, β- amyrin 5.78%, α- amyrin 5.78% were major constituents.

**Conclusion**

This investigation shows that *E.agallocha* L. has lot of antibacterial activity against many bacteria responsible for most of the microbial diseases. GC-MS analysis of Chloroform extract of *E.agallocha* shows 34 bio-active compounds. From that seven compounds are high in proportion. They are very useful for various ailments and diseases. Ethanol extract shows 17 compounds in that 5 compounds, are high in proportion. They have broad spectrum of use. It is used in perfume, food stuffs and beverages. Cyclolanost is an important component in Dandalion coffee. It is very good tonic for liver. Jaundice affect the liver severely. For the jaundice patients, the tonic is very useful. α -amyrin and β – amyrin are used as laxative and used in cancer treatment. Some compounds are used in cleaning agents and used as dying solubilizing agent in textiles. Further studies are needed for the isolation and identification of bio-active compounds using some other solvents and micro organisms gives better understanding of bio- active compounds.

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