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FATTY ACID COMPOSITION OF BHALLATAKA OIL AND THEIR BIOLOGICAL PROPERTIES

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ABSTRACT

Semecrpus anacardium L.f is a plant well known for its therapeutic value in ayurvedic and siddha system of medicine, in our survey we found that, the Anacardiaceae family has 77 genera and 850 species surviving as trees, shrubs and vines. Out of these, the commonly known Cashew family (or) Sumac family has great viable importance. Due to the presence of several anticancer drugs isolated from Anacardiaceae family, we have selected *Semecarpus anacardium* L.f, for its high medicinal value in Ayurveda and Siddha systems and isolated numerous active constituents. In our research, *Bhallataka oil (*cape 32) found to have a free fatty acid (0.50) hence; alkaline *Tran's*-esterification was done by using anhydrous methanol at a molar ratio of 6:1 and 3g/ Litre of sodium hydroxide as catalyst, and the results showed that Fatty acids with highest weight percentage 18:1 - 51.25403 %, 16: 0 -13.41810 %, 18:3 - 6.042404 % and also it consists of the antimicrobial activity against both gram positive and gram negative bacteria.

KEW WORDS: ***Anacardiaceae family, Semecarpus anacardium L.f, Fatty acid methyl esters, Antimicrobial activity.***

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INTRODUCTION

Herbal drugs are contributing a lot to human health in our tradition especially in 21st century. Natural medicine progresses the inner immune system of the human body and there will be no side effects. Hence, the herbal drug acts more effectively than the modern medicine. The word, Semecarpus[1] is derived from Simeion in Greek means marking/tracing and carpus in Greek means nut. Anacardium means like cardium, i.e. heart shaped marking nut. Bhallatak literary means sharp like spear. Bhallatak or marking nut is known to the world since ancient times. The stem, sap, fruit and seeds are used by mankind for diverse purposes such as timber, paint, waterproofing, food and medicine. In view of its several potent medicinal properties, it is acclaimed as Ardhavaidya in Ayurveda and as a Golden acorn at the time of Galen in the western world. Severe skin manifestations, precludes its use for medical purposes. In India, the plant is used by Ayurvedic practitioners, traditional healers across the country albeit with caution. However, manufacturing units are overtly scared in view of its apparent toxic nature, and hence there are a few takers. The plant belonging to Anacardiaceae family has potential to produce allergic manifestations through contact dermatitis. Phytoconstituents, viz. alkyl catechols, phenols, quinols and resorcinols are believed to be responsible for skin reactions. The plant growing naturally in tropical and dry climate is a deciduous tree having a height of about 10 m. Flowering time is May to October, whereas fruiting time is December to March. The fruit is eaten when ripe; whereas the kernel of the seed is eaten after removing the pericarp. Marking nut seed, which sinks in water, is used for medicinal purposes, Ayurvedic properties of Bhallatak are madhur, kashay ras, ushna virya, madhur vipak and laghu, snigdha, tikshna, and ushna gunas[2]. It has several karmas like Kaphavatashamak(alleviates kapha & Vata dosha), Bhootanashan (anti-devil) Pittasanshodhak (expels out pitta dosha), Medhya (beneficial to brain), Vanhikar (improves digestive fire), Vrishya (aphrodisiac), Chedana (excisional functions), Bhedan (incisional function), Bruhan (anabolic in effect), and hence indicated for many diseases like Arsha (haemorrhoids), Udar (ascites), Grahani (inflammatory bowel diseases), Shotha (inflammation), Krumi (helminthiasis), Kushtha (skin disorders, like psoriasis), Vran (wounds), Shwitra (vitiligo), Gulma (abdominal mass), Jwar (fever), Adhman (flatulence), etc.

PHARMACOLOGICAL ACTIVITIES

Recent studies suggested that single and compound formulations of *Semecarpus anacardium* L.f. has multiple pharmacological activities such as anti-inflammatory activity[3], anti-arthritic activity[4], hypoglycaemic activity [5] Anti-cancer activity [6], contraceptive agent [7], lipoxygenase inhibitory activity[8],hypolipidemic activity & hypocholesterolemic activity[9] antimicrobial activity[10],antistress activity[11] and immune modulatory activity[12] in (Fig:1)

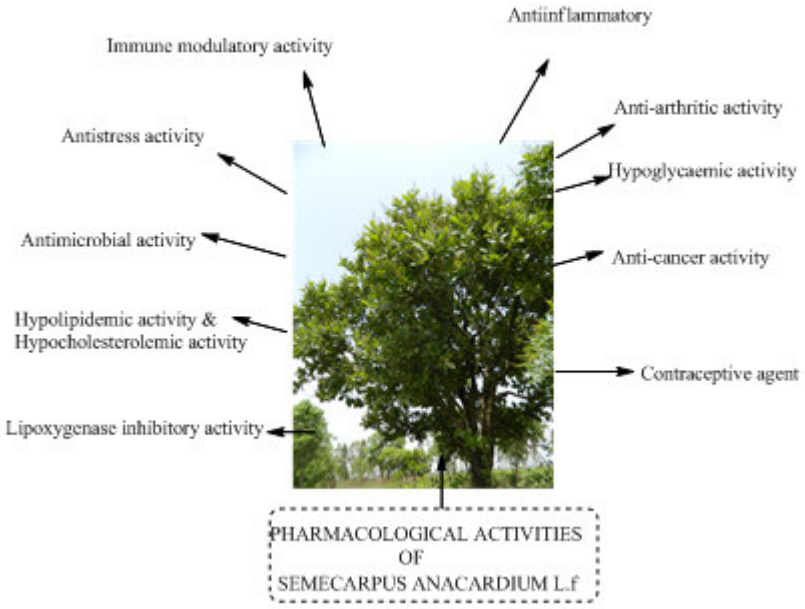


Figure1
Pharmacological activity properties shown by Semecarpus anacardium L.f.

MATERIALS AND METHODS

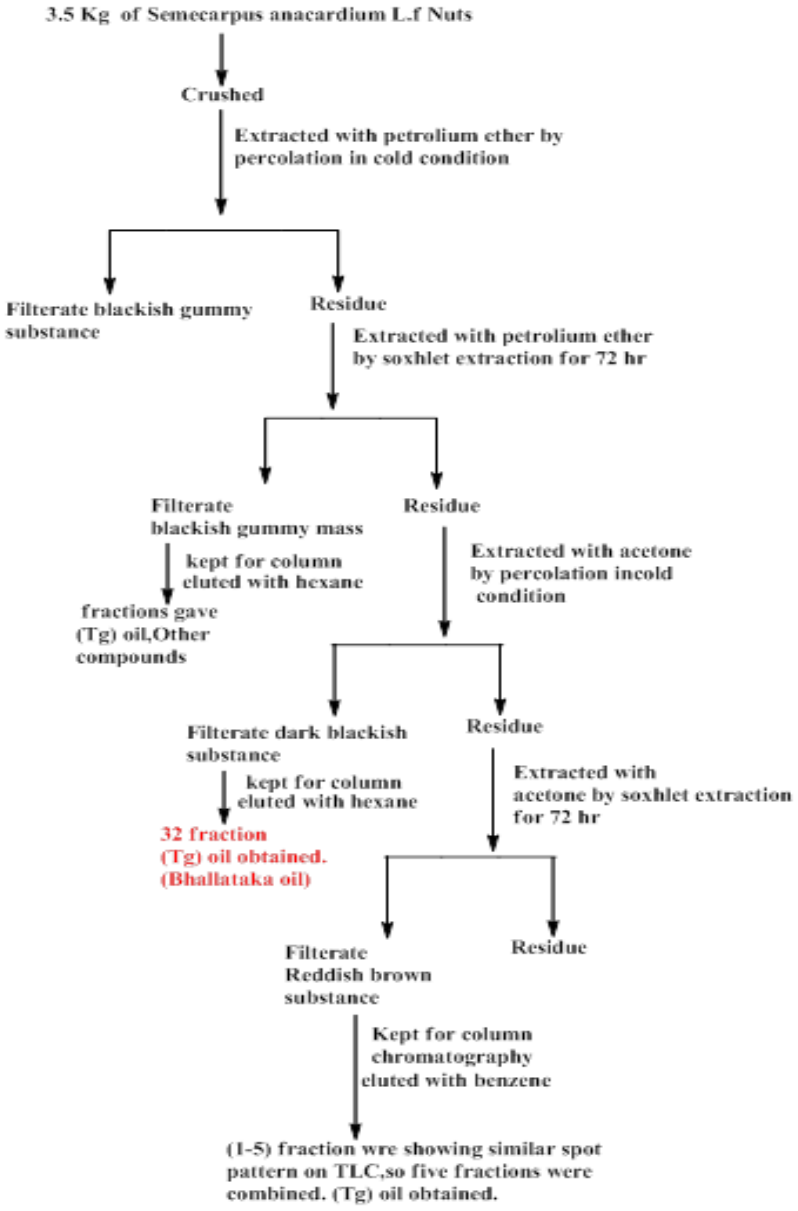
Semecarpus anacardium L.f. nuts were collected from field area very nearer to the village Nandgaon, Kolhapur City, Maharashtra, India. All plant material specimens were identified by Dr Vatsavaya S. Raju, Plant Systematic Laboratory, Department of Botany, Kakatiya University, Warangal (A. P. State) and conformed as *Semecarpus anacardium* L.f. (syn: *Anacardium latifolium* Lam., *A. orientale* Steud.) of Anacardiaceae

and plant specimen deposited at Kakatiya University Herbarium, Warangal (KUW) with accession number1874. It is locally known as 'nalla jeedi' and popularly known as 'marking nut/dhobi nut.

Extraction and purification

The crushed nuts (3.5 kg) were extracted with cold petroleum ether. Solvent from this extract was removed under reduced pressure. 129gms of blackish gummy substance was obtained, this was designated as *Semecarpus anacardium* cold hexane extract (SaCHE).The residue left after cold extraction was Soxhlated with hot petroleum ether (60-80^0) and this was concentrated under reduced pressure which resulted blackish gummy mass of 680gms and was designated as *Semecarpus anacardium* hot hexane Extract (SaHHE). The left residue was extracted with cold acetone; the solvent from this extract was removed under reduced pressure which resulted dark blackish substance of 420gms which designated as *Semecarpus anacardium* cold acetone extracts (SaCAE). Finally the residue was soxhlated with hot acetone, the solvents from this extract was removed under reduced pressure, resulted the yield of dark reddish brown substance of 959gms which is designated as *Semecarpus anacardium* hot acetone extract (SaHAE).All the four filtrates (SaCHE, SaHHE, SaCAE, and SaHAE) were subjected for column chromatography on silica gel and column was eluted with increasing polarity of solvents individually. And the oil obtained from the fraction of CAPE (cold acetone petroleum ether extract) 32 was under reduced pressure the oil (named CAPE 32(Bhallataka Oil-3) was concentrated by vacuum evaporation and given for spectral analysis.

Process of Flow Diagram of Semecarpus anacardium L.f Nut oil(Bhallataka Oil-3).



Fidure 2
Process of Flow Diagram of Semecarpus anacardium L.f Nut oil(Bhallataka Oil-3).

RESULTS AND DISCUSSION

In our investigation, the seed oil of *Semecarpus anacardium L.f* (Bhallataka oil) has number of bioactive compounds and the Oil was characterized by FT-IR, ^{1}H NMR, ^{13}C NMR,GC methods, it was found that it contains free fatty acids, triglycerides.Bhallataka Oil-3 was obtained from the cold acetone extract of 31-32 fractions which was eluted with Hexane, the fractions were examined by TLC, individually, Basing on TLC, fractions from 31-32 were showing similar spot pattern and designated as CAPE-32, it was obtained as Brown oil with B.P 74-78.5^{0}C.The FT-IR Spectrum, displayed bands at 3474.70 cm^{-1} (O-H, hydrogen bonded alcohol, phenols), 3006.14 cm^{1} (hydrogen bonded alcohol, phenols), 2925.96 cm^{-1}, 2855.77cm^{1}(CH,alkanes),1746.76cm^{1}(C=O,aldehydes, ketones, carboxylic acids, esters), 1461.47 cm^{-1}, 1376.63 cm^{-1} (C-H, alkanes), 1237.81 cm^{-1}, 1118.90 cm^{-1},1163.85cm^{-1} (C-O, alcohol, acids, ethers, carboxylic acids, esters.), 722.78 cm^{-1} (C-H, alkanes).The proton NMR Spectrum of Bhallataka Oil-3(CAPE 32) :5.360-5.323 δppm is due to the unsaturated acids -CH=CH- Olefinic protons, The Sn-2 proton (attached to the carbon CHO) of the Glycerol backbone of the triacyl glycerols causes the small clusters of the peaks at 5.220 ppm,4.322 – 4.113 ppm due to Sn-1 and Sn-3 protons(attached to the two terminal carbons; CH_2O) of the Glycerol backbone of the triacylglycerides, 2.789-2.751ppm[bisallylic protons, i.e., protons attached to carbons situated directly next to two C=C double bonds –CH=CH-CH_2-CH=CH-],2.334-2.284ppm[Protons on the second carbon in the fatty acid chains –O-C(=O)-CH_2], 2.019-2.000ppm due to allylic protons i.e., protons attached to carbons next to C=C double bonds,1.606 ppm due to Methylene protons due to all acyl chain –CH_2-CH_2-COOH,1.301-1.255 ppm.–$(CH_2)_n$- due to all acyl chains,0.109-0.857 due to Terminal methyl protons –$(CH_2)_n$-CH_3

^{13}CNMR ($CDCl_3$)

173.228, 172.804 (C-1,Sn-2 due to triacylglyceride moiety), 130.175 (C-10 due to oleyl), 129.965, 129.657 (C-9 oleyl),128.031, 127.851 (Linoleyl), 68.856 (Triacylglycerides), 62.070 (CH_2-Sn-1,3 Triacylglycerides), 35.810, 34.164, 34.003 (C-2,Sn-1,3 ;acyl chain), 31.884 31.750 (W_3 ,sat., n-9 andn-6 acids), 31.497, 31.262, 30.878, 29.679, 29.500, 29.295, 29.092,((CH_2)$_n$ acyl chains, 27.174 (due to oleyl and linoleyl), 25.599 (Diallylic,linoleyl,linolenyl), 22.655, 22.544, 14.0073and 13.768 (due to acyl chain).

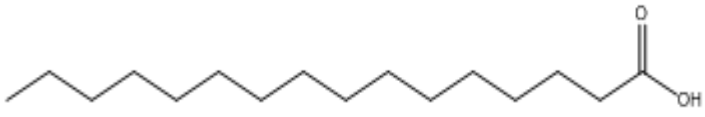
Fatty acid composition of Bhallataka Oil-3

Table 1
Fattt acid composition of Bhallataka Oil-3

S.No	LIPID VALUE	FATTY ACID	WT%
1	12:0	Lauric acid	0.18
2	14:0	Myristic acid	0.25
3	16:0	Palmitic acid	13.41
4	16:1	Palmitoleic acid	0.1474
5	18:1	Oleic acid	51.254
6	18:2	Linoleic acid	5.639
7	18:3	α- Linoleic acid	5.320
8	20:0	Arachidic acid	3.819
9	20:1	Paullinic acid	1.09
10	20:2	Eicosadienoic acid	1.189
11	22:0	Behinic acid	5.076
12	23:0	Dihomo gammaLinolenic acid	1.02
13	24:0	Tetracosanoic acid	1.18

The structural elucidation of the Bhallataka Oil-3 is finalized by the linkage of connecting the major composition of the fatty acids by the increasing order of Molecular weight of the fatty acids. According to this fatty acids present in Bhallataka Oil-3 are

(1) Palmitic acid: lipid value (16:0) with weight percentage 13.41



Palmitic acid

Figure 3
Palmitic acid

(2) Oleic acid: Lipid value (18:1) with weight percentage 51.254

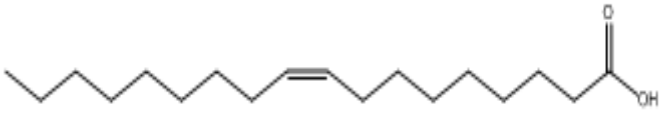


Figure 4
Oleic acid

(3) α- Linoleic acid : Lipid value (18:3) with weight percentage 5.320

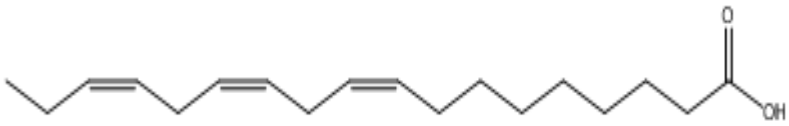


Figure 5
α- Linoleic acid

The tentative structure of Bhallataka Oil-3 is

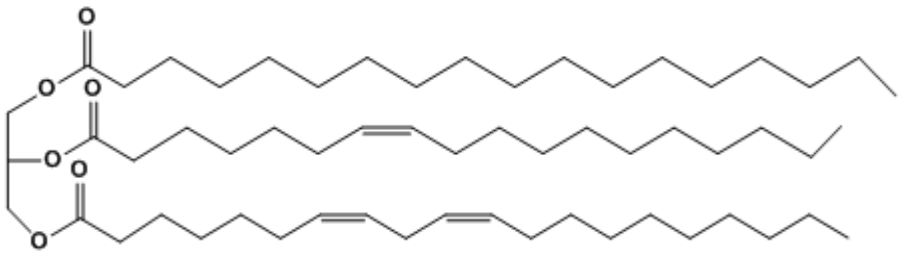


Figure 6
Structure of [(7Z, 10Z)-2-((Z)-octadec-7-enoyloxy)-3-(stearoyloxy) propylicosa-7,10-Dienoate

The proposed structure of Bhallataka Oil -3 (CAPE 32) is confirmed by the Spectral and chemical Trans-esterification.

Tran's -esterification process [13, 14]

In our investigation, *Semecarpus anacardium* nut oil cape 32 had found to have a free fatty acid (0.50) hence; alkaline *Tran's*-esterification was done by using anhydrous methanol at a molar ratio of 6:1 and 3g/Liter of sodium hydroxide as catalyst. The processor was stirred at 600 rpm and at a temperature of 60^0 C for 2 hours, after which the mixture was poured into a decanter and allowed to settle for 3 hours so that the reaction can be driven to completion. By following mechanism as in Figure 6 and found that the mixture has been separated in to its corresponding methyl ester. The glycerol at the bottom was drained off by gravity. The excess methanol in the ester was removed by using a flash rotary evaporator. The impurities were removed from the methyl ester by washing with distilled water of volume ratio 3 to 1 three times. Finally, the washed methyl ester was dried by passing it through anhydrous sodium sulphate (Na_2SO_4).

Figure 7
General Mechanism of Trans-esterification process of Oil.

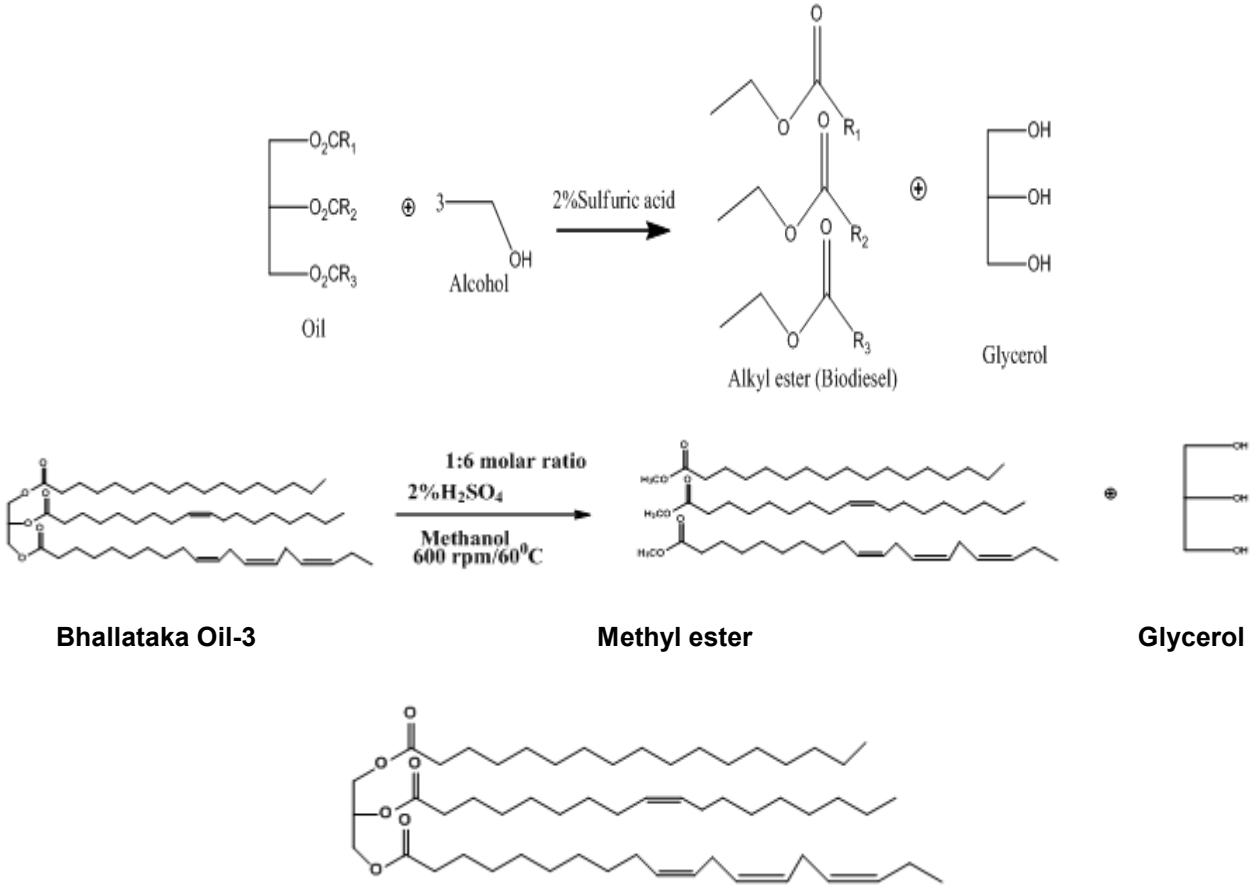


Figure 8
Structure of [(7Z, 10Z)-2-((Z)-octadec-7-enoyloxy)-3-(stearoyloxy) propyl icosa-7, 10-dienoate, Bhallataka oil-3(Kabala triacylglyceride) of major fatty acids.

The Identification of Cold acetone pet.ether 32 fraction of Nuts was Identified according to IUPAC Name was (7Z, 10Z)-2-((Z)-octadec-7-enoyloxy)-3-(stearoyloxy) propyl icosa-7, 10-dienoate] and found to be as new triacylglyceride "isolated from Semecarpus anacardium L.f was characterized and named as Kabala triacylglyceride" Basing on UV, FT-IR, ^{1}H NMR, ^{13}C NMR, MASS, HPLC, LCMS, GCMS, GC methods, it was found that it contains free fatty acids, triglycerides and others. Basing on spectral data and their physical properties *Semecarpus anacardium* L.f. nut oil was selected for biodiesel production.

The spectral data of CAPE 32 was given below as:

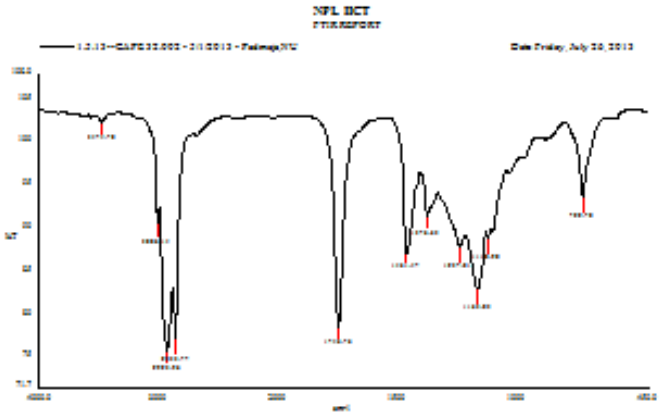


Figure 9
FT-IR of CAPE 32

Table 2
FT-IR Spectral data of Bhallataka Oil (CAPE 32)

CAPE -32	Functional group assignment	Group frequency cm-1
3474.70	Hydrogen bonded Alcohol, Phenols.	3200-3600
3006.14	Aromatic H-X group	3000-3100
2925.96	Alkanes	2850-3000
2855.77	Alkanes	2850-3000
1746.76	Saturated aldehyde	1720-1740
1461.47	Alkenes	1610-1680
1376.63	Alkanes	1340-1470
1237.81	Alcohol, Ether, Carboxylic acid, Ester	1050-1300
1163.85	Alcohol, Ether, Carboxylic acid, Ester.	1050-1300
1118.90	Alcohol, Ethers, Carboxylic acids, Esters.	1050-1300
722.78	Alkanes	679-995
	Alkanes	679-995
	Alkanes	679-995

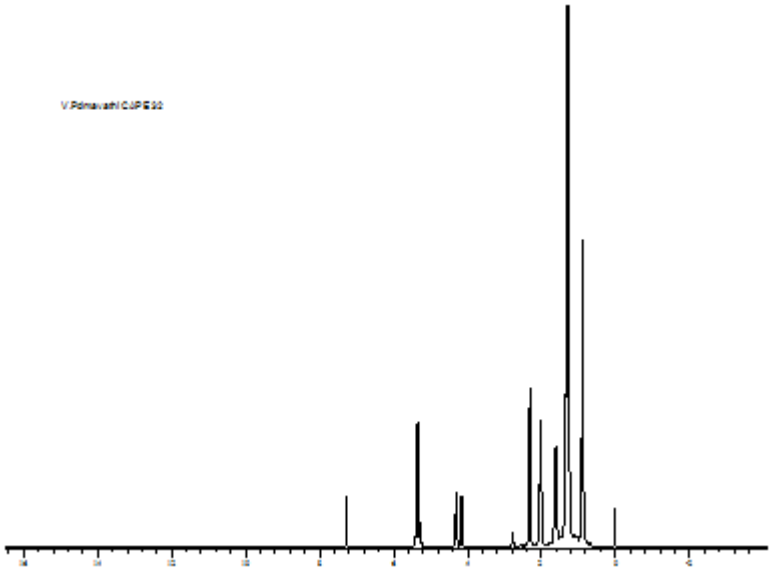
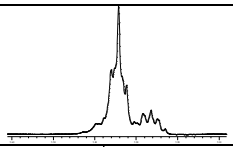
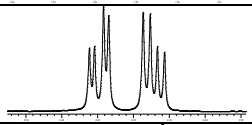
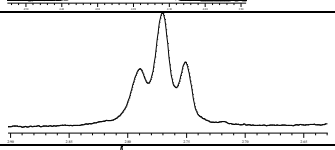
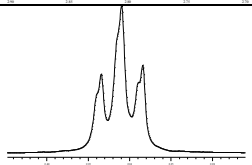


Figure 10
^{1}HNMR of CAPE 32

Table 3
FT-IR Spectral data for Bhallataka Oil

Expanded form of CAPE 32 ^{1}HNMR

S.NO	PEAK	δ(ppm) Values	Assignment of compound
1		(5.44-5.22) δ(ppm)	Unsaturated acids -CH=CH- olefinic protons, the SN-2 protons (attached to the centre carbon; CHO) of the glycerol backbone of the triacylglycerols causes the small cluster of peak at 5.25 ppm.
2		(4.22-4.10, 4.40-4.22) δ(ppm)	SN-1 OR SN-3 unsaturated fatty acids -CH-OCOR, (attached to the two terminal carbons; CH_2O) of the glycerol back bone of the triacylglycerols.
3		(2.85-2.70) δ(ppm)	*bis*-allylic protons, *i.e.* protons attached to carbons situated directly next to two C=C double bonds, -CH=CH-CH_2-CH=CH-.
4		(2.40-2.25) δ (ppm)	Protons on the second carbon in the fatty acid chains-O-C (=O)-CH_2-.

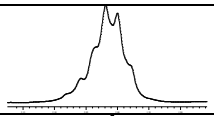
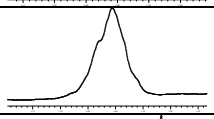
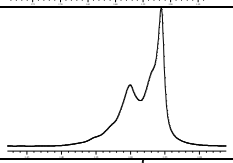
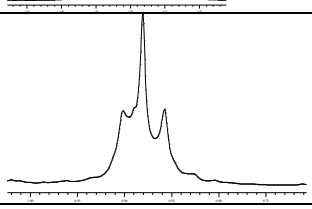
5		(2.10-1.90) δ (ppm)	allylic protons, *i.e.* protons attached to H=CH-. Carbons next to C=C double bonds, -CH_2-CH_2-CH=CH-.
6		(1.75-1.50) δ(ppm)	methylene protons –(CH_2)n-.
7		(1.40-1.20) δ(ppm)	fatty acids –$(CH_2)_n$-
8		(0.95-0.75) δ(ppm)	terminal methyl protons -$(CH_2)_n$-CH_3; the small peaks

Table 3
Expanded form of CAPE 32 ^{1}HNMR

^{13}CNMR Spectral data for CAPE 32:

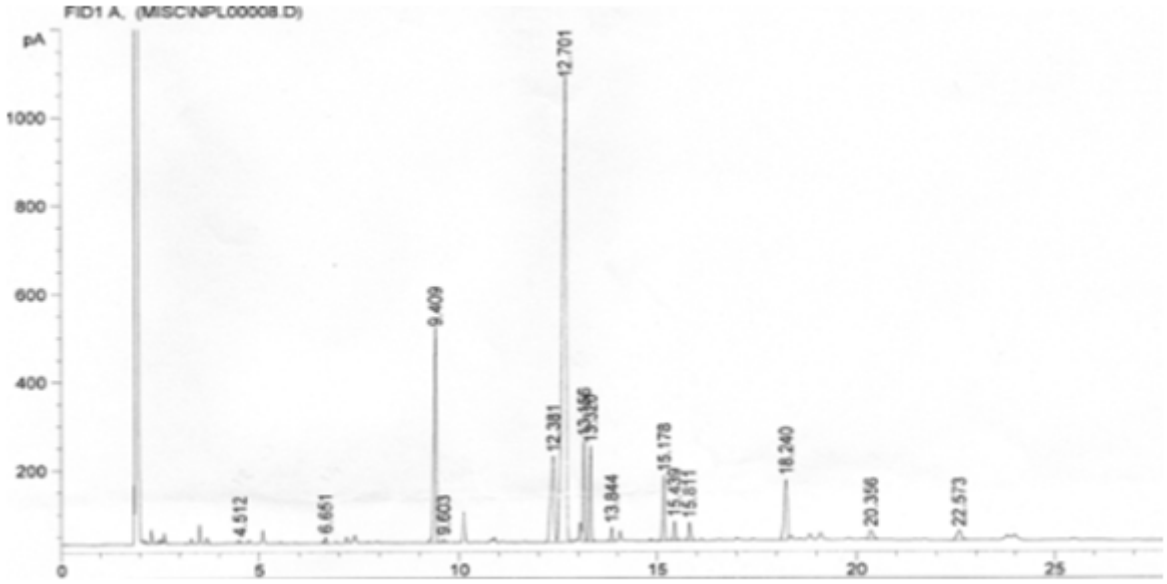
CAPE 32	peaks
173.228	C_1,Sn-1,3
172.804	C_1,Sn-2
130.175	C_{10}
	C_{10}
129.965 129.657	C_9
128.031	C_{10}
127.851	C_{12}
68.656	-CHO- Sn-2
62.070	CH_2O Sn-1,3
	C-2 Sn-2
35.810	c-2 Sn-2
34.164 34.003	C-2 Sn-1,3
	C-2 Sn-1,3
	W3 saturated
31.884 31.750	
31.497 31.262 30.878	saturated
29.679 29.500 29.295 29.092	(CH_2)_ allyl chain
27.174	Acyl chain c8-c11;c-8-c-14
25.599	Acyl chain
	Acyl chain
	Diallylic c-11,c-14
24.819	Acyl chain
22.655	Acyl chain
22.544	Acyl chain
14.073 13.768	Terminal methylene.

Table 4
^{13}CNMR Spectral data for CAPE 32 GC analysis

The fatty acid composition was determined by GC and the results are given below, the acid value and it was found that it is varied from 0.52-0.31.For the analysis the method followed for Gas chromatography (GC) is as follows:GC Apparatus:Agilent Technologies, USA., Model no →6890 N ,Column → DB-225 [(50% cyanopropylphenyl)-dimethylpolysiloxane, 30M×0.250mm1D×0.25µm film thickness Inlet temp →

230^{0}c,Oven prog → 160^{0}c(2min)-5^{0}c/min-230^{0}c(20min) , Det. temp → 270^{0}c ,HY → 30ml/min ,Air → 300ml/min ,Inlet type → Split/Split less , Det. type →FID ,Soft ware →GC Chem. station , A.10.02(1757) The fatty acid composition was determined by GC and the results are given below; it was found that the acid value varied from 0.52-0.31.Fatty acid composition of the Bhallataka oil (CAPE 32).

Figure 11
GC analysis of Semecarpus anacardium L.f Oil

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rted By             :      Retention Time
ltiplier            :      1.0000
lution              :      1.0000
mple Amount         :      1.00000  [ng/ul]   (not used in calc.)
e Multiplier & Dilution Factor with ISTDs

gnal 1: FID1 A,

ak RetTime Sig Type    Area      Height     Area
#   [min]           [pA*s]      [pA]        %
--|-------|---|----|----------|----------|--------|
 1   4.512  1  BB      25.64367    6.22549  0.18675
 2   6.651  1  BP      35.49586   13.30136  0.25849
 3   9.409  1  BB    1842.54785  480.79678 13.41810
 4   9.603  1  PB      20.24084    7.05006  0.14740
 5  12.381  1  BV    1326.60620  192.38126  9.66082
 6  12.701  1  VB    7038.10791 1063.49280 51.25403
 7  13.156  1  VV     774.37714  228.03784  5.63929
 8  13.320  1  VB     730.65979  212.54608  5.32093
 9  13.844  1  BP      99.02870   30.61722  0.72116
10  15.178  1  BP     524.48413  145.53618  3.81948
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:a File C:\...\DATA\MISC\NPL00008.D                    Sample Name

Peak RetTime Sig Type    Area      Height     Area
  #   [min]           [pA*s]      [pA]        %
----|-------|---|----|----------|----------|--------|
  11  15.439  1  BB     150.90208   43.02037  1.09892
  12  15.811  1  BP     163.37068   40.40532  1.18972
  13  18.240  1  BV     697.12018  135.39716  5.07668
  14  20.356  1  PB     141.16777   18.89869  1.02803
  15  22.573  1  PB     162.06049   21.10181  1.18018

Totals :              1.37318e4  2638.80843

 Results obtained with enhanced integrator!
=====================================================================
                    *** End of Report ***
```

Table 5
Fatty acid composition of CAPE-32

Fatty acid	12:0	14:0	16:0	16:1	18:1	18:2	18:3	20:0	20:1	20:2	22:0	23:0	24:0
Wt%	0.18675	0.25849	13.41810	0.14740	51.25403	5.63929	6.04209	3.81948	1.09892	1.18972	5.07668	1.02803	1.18018

Antimicrobial activity

Determination of Antimicrobial activity

The antimicrobial activity of the isolated compounds and their derivatives was determined by using well diffusion method (Amsterdam, 1996) against different pathogenic reference strains procured from the Microbial Type Culture Collection and Gene Bank (MTCC), CSIR-Institute of Microbial Technology, Chandigarh, India. The pathogenic bacteria and *Candida* reference strains were seeded on the surface of the media Petri plates, containing Muller-Hinton agar with 0.1 ml of previously prepared microbial suspensions individually containing 1.5×10^8 cfu ml^{-1} (equal to 0.5 McFarland). Wells of 6.0 mm diameter were prepared in the media Petri plates using a cork borer and the isolated compound and their derivatives at a dose range of 300 - 1.4 µg $well^{-1}$ was added in each well under sterile conditions in a laminar air flow chamber. Standard antibiotic solutions of Neomycin and Miconazole at a dose range of 300 -1.4 µg $well^{-1}$ and the well containing methanol served as positive and negative controls, respectively. The plates were incubated for 24 h at 30°C and the well containing the least concentration showing the inhibition zone was considered as the minimum inhibitory concentration. All experiments were carried out in duplicates and mean values are represented.

Table 6
Antimicrobial activity of Bhallataka oil

COMPOUNDS	*Staphylococcus aureus MTCC 96*	*Klebsiella planticola MTCC 530*	*Bacillus subtilis MTCC 121*	*S.aureus MLS16 MTCC 2940*	*Micrococcus luteus MTCC 2470*	*Escherechia coli MTCC 739*	*Pseudomonas aeruginosa MTCC 2453*	*Candida albicans* MTCC 3017
BO	---	150	75	75	----	---	150	150
FAME BO	9.37	9.37	18.75	9.37	9.37	9.37	9.37	9.37
Neomycin	18.75	18.75	18.75	18.75	18.75	18.75	18.75	--
Miconazole	--	--	--	--	--	--	--	9.37

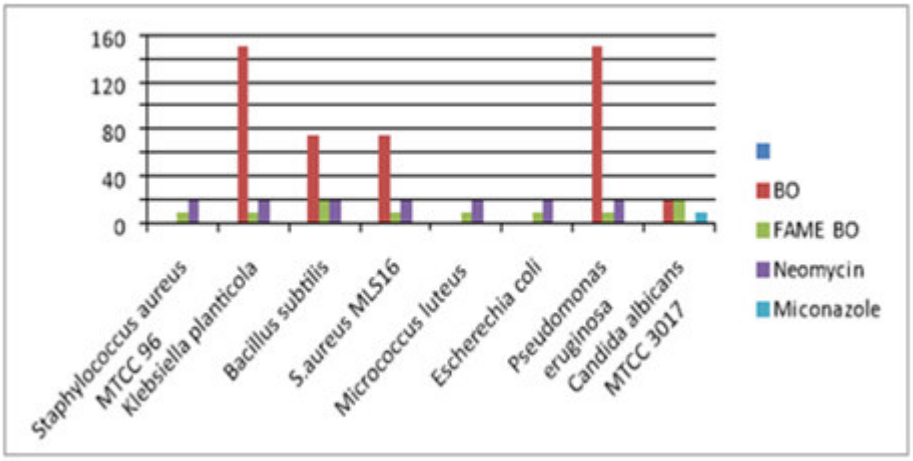

Figure 12
Graphical representation Antimicrobial activity of Bhallataka oil

The seed oil of *Semecarpus anacardium* (Bhallataka oil) has number of bioactive compounds. In our investigation, Oil was characterized by FT-IR, ^1H NMR, ^{13}C NMR, GC methods; it was found that it contains free fatty acids, triglycerides and others.*Semecarpus anacardium* nut oil cape 32 had found to have a free fatty acid (0.50) hence; alkaline *Tran's*-esterification was done by using anhydrous methanol at a molar ratio of 6:1 and 3g/Liter of sodium hydroxide as catalyst. SA Nut oil to yield Fatty Acid Methyl ester, and glycerine as a by-product and the Derivative (FAME) determined by Trans-esterification process (Fig.7) compared with the Various Oil Spectral data & Various properties, which were not reported earlier [15-24], Bhallataka oil derivative is most active against *both gram +ve and gram –ve bacteria when compared to Bhallataka oil* (Table.6). It was found that it contains free fatty acids, triglycerides and others. Basing on spectral data and their physical properties *Semecarpus anacardium* L.f. nut oil was selected for biodiesel production.

CONCLUSION

Bhallataka oil has a specific free fatty acid value of 0.50. Hence, alkaline Tran's esterification process was chosen. These results were studied by Gas Chromatography and found the methyl esters of the following acids as : Oleic acid(18:1)-51.25403%;

Palmitic acid(16:0) -13.41810%; α-Linoleic acid(18:3)-6.04209%;Linoleic acid(18:2) -5.63929%;Docosanoic acid(22:0) -5.07668; Eicosanoic acid(20:0) -3.81948;Eicosadienoic acid(20:2)-1.18972;Lignoceric acid(24:0)-1.18018;Eicosenoic acid(20:1)-1.09892; Mead acid(23:0)-1.02803;Myristic acid(14:0)-0.25849;Lauric acid(12:0)-0.18675;Palmitoleic acid(16:1)-0.14740%.These results showed, that all the above fatty acids were totally converted to their corresponding methyl esters. Further we found that, the Bhallataka oil derivative is most active against gram +ve and gram –ve bacteria when compared to Bhallataka oil. The applications of these various compositions of Bhallataka Oil as Biodiesel and Biolubricants are under process.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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