Antibacterial Activity from The Cashew Nut Shell Extracts

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Abstract. Cashew nut shells were considered as a waste material of the cashew industries; therefore, we used this material as a source of antibacterial drugs and as a source of chemical constituent. *Anacardium accidentale* Linn (*Anacardiacea* family) popularly known as "Cashew", is grown in the sandy loam soil, and has a spreading root system. Nutshell from cashew nut represents one of the major cheapest sources of non-isoprenoid phenolic lipid. This study investigated of antibacterial activities of cashew nut shell extract (CNSE). Samples were maceration by hexane, dichloromethane, ethyl acetate, methanol, and water solvent were then evaporated at 50 ºC to give n-hexane, dichloromethane, ethyl acetate, methanol, and water extract. The yield were obtained was 125.54 gr (28.91 %), 11.19 gr (2.58 %), 43.15 gr (9.94 %), 2.46 gr (0.08 %), and 46.50 gr (10.71 %), for hexane, dichloromethane, ethyl acetate, methanol, and water solvent espectively. The extract was examined for bacterias, namely *Bacillus subtillis*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus*, using the agar disc diffusion methods in using concentration of Cashew nut shell of 0.125%. 0.25%, 0.5 % and 1% respectively. The ethyl acetate extract and n-hexane extract of CNSE showed an antibacterial effect on *B. subtillis* with an inhibition zone of 10.333 mm and 9.67 mm. An n-hexane extract showed inhibition zone (11.667 mm) for *S. aureus,* 11.33 mm for *P. aeruginosa*. Methanol extract exhibited inhibition zone higher for *S. aureus*, followed by *E. coli*, *P. aeruginosa* and *B. subtillis*. Water extract did not affect the growth of *E. coli*. Four the ethyl acetate, methanol, hexane, and dichloromethane extract showed better antibacterial effects compared to water extract. Chemical constituents of hexane extract of cashew nut shell were investigated by GC-MS.

1 Introduction

Cashew is another name for Anacardium occidentale L [1] is a tree that can grow within the region between 23°N and 23°S of the equator and also adapts to grow in sandy loam soil because the cashew has a spreading root system. Its height is above 12 m, sometimes reaching

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up to 30 m, [2]. The tree is also called (Tahiti), Fetau (Samoa), Damanu (Fiji Island), Tetai, and Mete (Indonesia).

According to Mathias et al., 2017, the distribution map of cashew in the world is quite extensive because cashew was cultivated in various tropical regions in Asia, Africa, India, Mozambique, and Tanzania. Although the cashew tree is a plant originates from Brazil, and cashew tree is found a lot of the Northeast and Midwest of Brazil, and in Rio grande and in Piaui Creare area and comprises 600 species. The cashew tree was widely distributed in the tropical rain forest and the cashew tree was cultivated in tropical regions. e.i Africa, Asia, and, Tanzania, India, and Mozambique [3], [4].

Matias et.al 2017 and Morais et. al, 2017 also state that the extensive cultivation area of the cashew tree is great economic importance in Northeastern Brazil. Cashews have very good economic value for Brazil. About 90% of the world's cashew production is produced in Brazil about 670,000 ha of cashew farms were found in the Northeastern Brazil region [3], [4], [5].

The food and Agriculture Organization (FAO) estimated 2.7 million tons per annum cashew were produced, while Vietnam produced 960,800 tons, Nigeria produced 594,000 tons, Brazil produced 147,6529 tons and Indonesia 122,000 tons). In some countries [6], Cashews belong to the Anacardiacea family [2]. The Anacardiaceae family has 76 genera comprising 600 species [6]. Cashew nut shell from Anacardium occidentale Linn is a waste material from rejection and regarded as worthless, but now known to have high values because the fruit of cashew and cashew nut shell contains a lot of phenolic compounds, which phenolic molecules have antioxidant activities [4], [7]. Cashew nut shell also was useful for analgesia, anti-inflammatory, antitumor, anti-bacterial, anti-fungal, and anti-mollusca [8]. In addition, cashew nut shells produce oil which brown in color. The cashew nut shell oil (CNSO) used for commercial applications such as resin and plastic industry also used as an anti-corrosive for metal and brake linings. Consumption of cashews has evidenced lower risk of cardiovascular and diabetes disease. CNSO is also very usefull for treatment of ringworn, sores, and scurry [1].

The cultivation and reproduction of the cashew tree are very important in Brazil, Tamilnadu (India), and South-Western Nigeria also in Indonesia [9], Cashew fruit a lot in Wonogiri Regency and Kupang Regency in Indonesia. The cashew tree was used for reforestation, to influence productivity. The nut of fruit cashew was of great economic importance as a large large-value exported commodity. The nut has high economic value because it was traded as a large export commodity [10]. The main products from cashew trees traded in the international market are kernel, raw cashew nuts, and cashew nut shell liquid [11]. The cashew nut contains a lot of monounsaturated fatty acid which is conducive to the increasing of good health, and also rich fat in cashew nut in no way poses a risk [12]. The cashew nut shell liquid (CNSL) was also used in the automotive industry as electric insulation, and waterproofing [5], [13].

The study about the extraction method for isolating CNSL by using protic and aprotic solvents and studying their physic-chemical parameters were carried out by Gandhi et al., 2013. [14]. The analyzed the vitamin composition and fiber fraction of cashew nut shells, this research the implication for animal nutrition, and Andayane, W.R and., Ernawati, N. 2019 also investigated the effect of cashew nut shell extract against instar of silver leaf whitefly (Bemisia tabaci Genn)", these result showed that cashew nut shell extract has the effect to nymphal. Cashew nut shells are considered a waste material the the cashew industry and have never been utilized in Indonesia. Therefore in this study, we used this waste material and tried to investigate the chemical constituent in cashew nut shells and tested for its activity as antibacterial agents.

2 Materials and Methods

2.1 Materials

483.7 gr Cashew nut shells were obtained from Wonogiri Regency, Central Java Province. Test bacteria were obtained from Indonesian Culture Collection (InaCC-BRIN), *B. subtillis* code FNCC 8059, *S. aureus* code FNCC 0047, *E. coli* code FNCC 0195, and *P. aeruginosa* code FNCC 0063. Streptomycin 0.1 % =1.000 ppm in DMSO was used as standard. Mueler Hinton Broth (MHB) Himedia M391, and nutrien agar M001 HiMedia Laboratory PVt. Ltd Lot no 00005570736 was used as media.

2.2 Instruments

The instruments used in this study were as follows: GC (Agilent type 19091S-433), MS were recorded with detector MSD (Mass selective Detector, Agilent 7890B) for analysis of chemical constituents in the CNSE, with a column93.92873 DB-5MS UI, 5% Phenyl methyl silox. Silica gel 70-230 mesh and 230-400 mesh were used as absorbents in the chromatography method. Thin layer chromatography (TLC) precoated kiesel gel 60F254 (silica gel plates 60 F_{254} thin 0.25 mm0.25 mm thick of 20x20 cm, Merck) was used for detection of the amount of spot or chemical constituent in extract samples. Spots or constituents were visualized under UV light (254 and 365 nm) irradiation and by spraying with 10% sulphuric acid solution followed by heating at 110^{o}C. The melting point was analyzed by using a Buchi 520 instrument.

2.3 Methods

Samples were crushed with a laboratory milling machine to obtain the powder. Dried powder was stored in darkness before used. The powder was extracted successfully by hexane, dichloromethane, ethyl acetate, methanol, and water at room temperature by using 4 L of each of the solvents (solvents were purchased from the local market and distilled before use. The extracts were concentrated by vacuum evaporator (Buchi) and were then dried in a desiccator. MHB was prepared by dilution of 2.1 gr MHB in 100 ml H_2O. Nutrient Agar (Himedia M002) amount 28 gr was diluted with 1 lt H_2O and sterilized before used, MHB was applied for pre-incubation (culture) overnight at 37^{o}C for all bacterial and for the measurement of Minimum inhibition concentration (MIC), nutrient agar also used as medium for the measurement of MBC.

2.3.1 Antibacterial assay

The antibacterial activities of the CNSE extract were evaluated against the selected strains using the disc diffusion method of Balouri Sadiki (2016). The agar plates are inoculated with a standardized inoculum of the test microorganism. The agar medium (28 g/1L distilled water) was poured into a plate of about 15-20 mL to a uniform depth of 5 mm and then 100 µl of each bacterial was spread in the agar plate and allowed to solidify. Sterile filter paper discs (about 6 mm in diameter) were placed on the surface of inoculated plates containing the test compound at a desired concentration, In this research the extract of cashew nut shell (1%) was dissolved in DMSO to initial concentration then a four serial dilution were made in order to obtain concentration range 0.125; 0.25; and 0.5 and 1% [15]. In addition, 10 µl each concentration of cashew nut shell was pipetted into sterile paper discs. Each extract was tested with the triplicate for each of the strains, the control discs contained 10µl sterile

DMSO Streptomycin (10 µl/disc) in each cased 0.1 % (1mg/1 ml DMSO) was used as standard (positive control). The Petri dishes were incubated under suitable conditions (at 37ºc for 24 hours) for each of the strains and the results were expressed as average values. Generally, an antibacterial agent diffuses into the agar and inhibits germination and growth of the test microorganism, and then the diameters of inhibition growth zones are measured by transparent rule. Analysis was done 3 times for each test, were then calculated the mean of the triplicate test ± their standard error of meant (SEM).

2.3.2 Microorganisms

Microorganisms in this study were obtained from the Indonesian Culture collection-BRIN. (InaCC – BRIN). The **a**ntibacterial activity was evaluated against *E. coli* FNCC 0195, *B. subtillis* FNCC 8059, *S. aureus* FNCC 0047, *P. aeruginosa* FNCC 0063, all bacterial cultures were grown/sub-cultured on muller-Hilton Broth medium before used and 10 µl of suspense bacteria, were then diluted with 1 ml of NaCl 0,85%, the results were diluted again by pipetting 40 µl bacterial to 40 ml NaCl 0,85% to obtained a microbial suspension about 1.00 x 10^6 colony-forming unit (CFUs)/mL for further used.

2.3.3 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Cashew Nut Shell

Gas Chromatography (Agilent 7890B mode) with an Agilent 5977A (Mass selective Detector (MSD) was used for the analysis of chemical constituents in the hexane and CH_2CL_2 extracts of cashew nut shell. Parameter analysis is: injector temperature 270ºC, injector volume 1 µl, initial column temperature was set 40ºC for 1 minute, with increasing at flow 10ºC/min until 300ºC, hold 4 minutes. In this research was used column (DB 5 MS-UI column length 30 m, 0.25 mm ID, particle size 0.25µm). Helium was used as carrier gas at a flow rate of 1 ml/min. The ion source temperature was 200ºC, electron ionization system was operated with an ion energy of 70 eV. The relative peak area was acquired as a percentage of the total volatile composition from the total run ion current (TIC). The identification of compounds in the cashew nut shell extract was done by matching with the load mass spectra software data of the GC/MS system and also by comparing the retention time (RT) sample to RT data of the GC/MS system. The amount of each compound in cashew nut shell extract was calculated as a peak area compared to the total peak area of the chromatogram.

3 Results and Discussion

3.1 Extraction results

Powder of Cashew nut shell 434.20 gr was successively extracted with hexane, dichloromethane, ethyl acetate, methanol and water, the yield of hexane = 125.54 gr (28.91%), CH_2Cl_2 = 11.19 gr (2.58%), Ethyl acetate = 43.15 gr (9.94%), methanol=3.46 gr (0.80%) and H_2O = 46.50 (10.71%) respectively. On the basis of the chemical parameter of the extract of the CNS, we concluded that ethyl acetate solvent was more efficient than other solvents.

3.2 TLC results

Extract of hexane CH_2Cl_2, EtOAc, MeOH, and H_2O were subjected to TLC (Thin Layer Chromatography. Figure 1) on silica gel-coated plates (thickness 0.5mm) eluted with hexane : Ethyl acetate = 7:3. TLC profile of hexane, CH_2Cl_2, ethyl acetate, methanol and water extract showed the appearance of characteristic spots confirming the amount of compound by using eluent hexane: ethyl acetate 3:7, the result were showed in the Figure 1. eluent was used for TLC was hexane : ethyl acetate = 7 : 3.

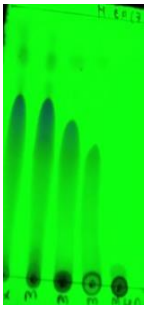


Fig. 1. Cromatoplate of CNSE extract

Cromatoplate corresponds to the extract of hexane, CH_2Cl_2, EtOAc, MeOH, and H_2O of CNS. The TLC study identified secondary metabolites present in hexane, CH_2Cl_2, EtOAc, MeOH, and H_2O extracts of CNS. Cromaplate from the CNS has shown the presence of several compounds in all extracts, but in water extracts, there are few compounds. To determine the antibacterial activity of each extract, tests were carried out by using *B. subtilis, P. aeruginosa, E. coli,* and *S. aureus* bacteria shown in Table 1.

Table 1. Antibacterial activity of CNS on zone inhibition (mm) in hexane, dichlorometane, ethyl acetate, methanol, and water extracts

Extract (%)	*B. subtilis*	*P. aeruginosa*	*E. coli*	*S. aureus*
Hexane (Hx)				
1	13.67 ± 0.47	14.00 ± 0.81	12.33 ± 0.47	13.67 ± 0.94
0.5	11.33 ± 0.47	12.33 ± 0.47	11.33 ± 0.47	12.33 ± 1.24
0.25	10.00 ± 0.81	11.00 ± 0.81	10.33 ± 0.47	13.00 ± 1.14
0.125	9.67 ± 0.47	11.33 ± 0.47	8.67 ± 0.47	10.00 ± 2.83
Dichlorometane (CH_2Cl_2)				
1	13.33 ± 0.47	10.67 ± 0.81	11.67 ± 0.47	17.67 ± 2.05
0.5	12.67 ± 0.47	11.00 ± 0.47	10.67 ± 0.47	16.00 ± 2.16
0.25	10.33 ± 0.81	9.67 ± 0.81	9.67 ± 0.47	14.67 ± 2.05
0.125	9.33 ± 0.47	10.00 ± 0.81	9.67 ± 0.47	11.67 ± 2.05
Ethyl acetate (EA)				
1	13.67 ± 0.94	13.33 ± 0.47	11.33 ± 2.05	20.33 ± 0.47
0.5	12.23 ± 0.47	12.00 ± 0.81	12.33 ± 0.47	17.00 ± 0.81

0.25	10.67 ± 0.47	11.00 ± 0.81	9.67 ± 0.47	15.00 ± 0.81
0.125	13.33 ± 0.47	10.67 ± 0.94	7.67 ± 0.47	13.00 ± 0.81
Methanol (MeOH) 1	11.67 ± 0.47	13.67 ± 1.24	11.67 ± 0.94	13.67 ± 1.24
0.5	11.00 ± 0.81	11.00 ± 1.64	10.00 ± 0.81	13.33 ± 0.94
0.25	10.00 ± 0.81	12.33 ± 0.47	10.33 ± 0.94	13.33 ± 0.47
0.125	9.00 ± 0.81	9.67 ± 1.24	10.00 ± 0.81	12.33 ± 0.47
Water (H_2O) 1	13.67 ± 0.47	11.67 ± 0.94	11.67 ± 0.47	14.67 ± 0.47
0.5	10.00 ± 0.00	9.00 ± 0.81	10.00 ± 0.81	11.00 ± 0.81
0.25	8.00 ± 0.00	8.67 ± 0.47	8.33 ± 0.47	10.67 ± 0.88
0.125	8.00 ± 0.81	7.67 ± 0.47	7.00 ± 0.81	9.00 ± 0.81
Standard streptomicyn 1	33.33 ± 1.25	32.33 ± 1.70	31.33 ± 0.47	40.66 ± 4.92
0.5	28.67 ± 0.47	31.67 ± 0.47	26.33 ± 1.24	31.67 ± 4.64
0.25	25.67 ± 0.94	29.33 ± 0.94	24.67 ± 0.47	30.00 ± 2.45
0.125	23.67 ± 0.47	28.33 ± 0.58	23.33 ± 1.25	27.00 ± 2.16

The antibacterial activity of hexane and CH_2Cl_2 extract of CNS (Cashew Nut Shell) against *B. subtillis. P. aeruginosa, E. coli,* and *S. aureus bacteria* were done in these research, the result showed that all extracts have antibacterial activity to *B. subtillis. P. aeruginosa, E. coli and S. aureus bacteri*. The CNSO studied by JS.D.S. Jebapritha was from India, while in this study cashew nut shells were studied from Wonogiri – Centre of Java-Indonesia. The activity of the resulting antibacterial was quite different, perhaps this was influence of the growing place and the chemical content of the cashew nut. All extracts have strong antibacterial activity against *B. subtillis. P. aeruginosa, E. coli,* and *S. aureus)*, while EA extract is very active compared to other extracts. This study showed the majority of CNS extracts exhibited high antibacterial activity.

3.3 TLC results

3.3.1 Gas chromatography-mass spectrometry (GC-MS) of cashew nut shell result

The compounds contained in each CNS extract were observed using GCMS. Identification of the compound in hexane and dichloromethane (CH_2Cl_2) extracts was done by comparing the retention time (RT), molecular formula, and molecular weight (MW) of the compound with retention time molecular formula and MW data of the GC/MS system (Table 2). The GC-MS chromatogram of hexane extract and CH_2CL_2 extract of cashew nut shell were shown in Figures 2 and 3, respectively.

Table 2. Chemical constituent in hexane extract of cashew nut shell (CNS)

No	Compound in hexan extract	RT
1	3-Tridecylphenol (Cardanol C13;0)	22.85
2	Ginkol TMS/Cardanol 15:1	24.328
3	phenol,2methyl	24.567
4	Sabinone	25.008
5	[(z)-3-(pentadec-8-en-1-yl)phenol-	25.298
6	z)-3-(pentadec-10-en-1-yl)phenol]	26.054
7	2,4-diamino phenol	26.773
8	1.3-benzene diol , 4,5-dimethyl	27.125

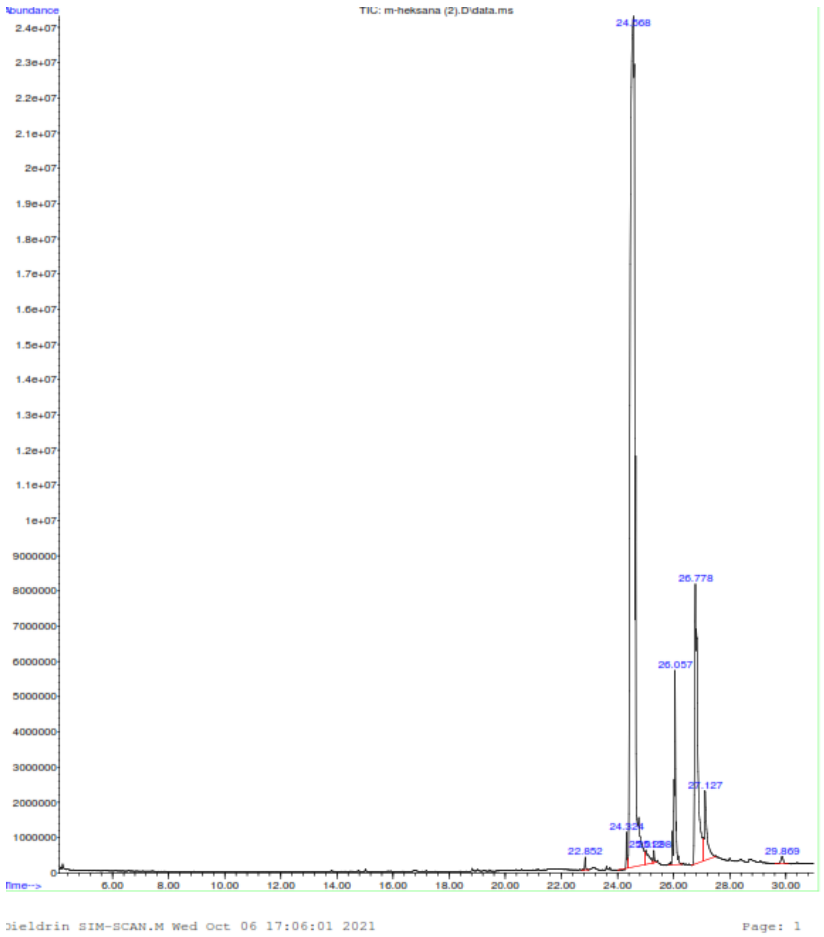


Fig. 2. GC-MS chromatogram profile of cashew nut shell

3.3.2 GC-MS of CNS hexane extract

1. Mass fragmentation of cardanol C13:O

Figure 3 shows the mass fragmentation of cardanol C13:O. We tried to identify cardanol C13:O which should be contained in hexane extract by using these mass fragments of cardanol C13:O, retention time determined by GLC and compared by NIST Mass Spectrometry Data Center in Instrument GC-MS (Table 3) Cardanol C13:O in our sample is very similar to Cardanol C13:O in the NIST library. Base paek [(M-($12CH_2$)]at m/z 108. very small molecular ions appeared at m/z 91 [(M-($12CH_2$+OH] and molecule ion apperaerd at m/z 77 as M-($11CH_2$+1CH+$1CH_3$+1OH]. This are evidence that the compound in hexane extract at Rt 22.85 min is cardanol C13:0.

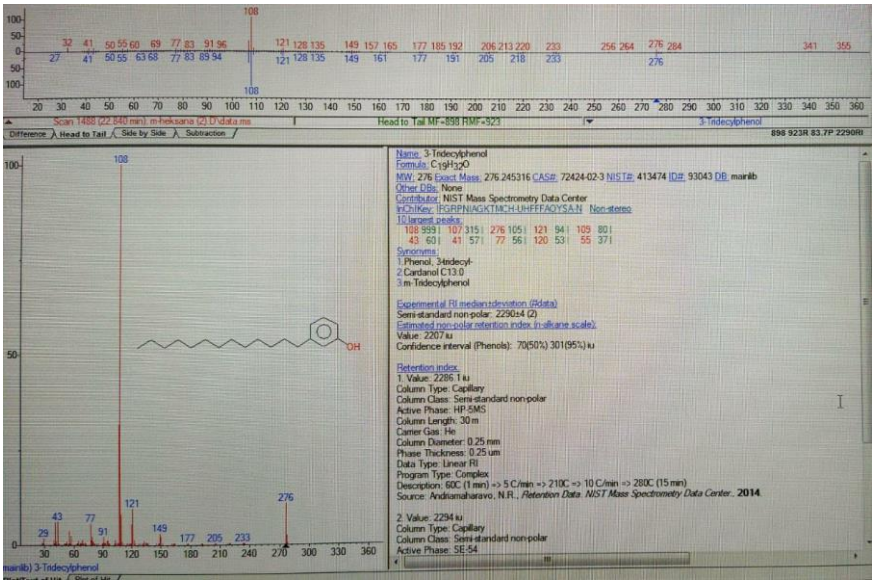


Fig. 3. Fragmentation spectra and structure of cardanol C13:0

2. Mass fragmentation of cardanol C15:1

Figure 4 shows the mass fragmentation of cardanol C15:1. Cardanol C15:1 in hexane sampel showed the same fragment with NIST Mass Spectrometry Data Center in Instrument GC-MS. Hight molecular ions as base peak appeared at m/z 180 = [(M-($12CH_2$+2CH)], another peak at m/z 165 as M - [($12CH_2$+2CH+CH_3)], and also M-[($12CH_3$+2CH+CH3+C6H4+O} appeared at m/z = 73

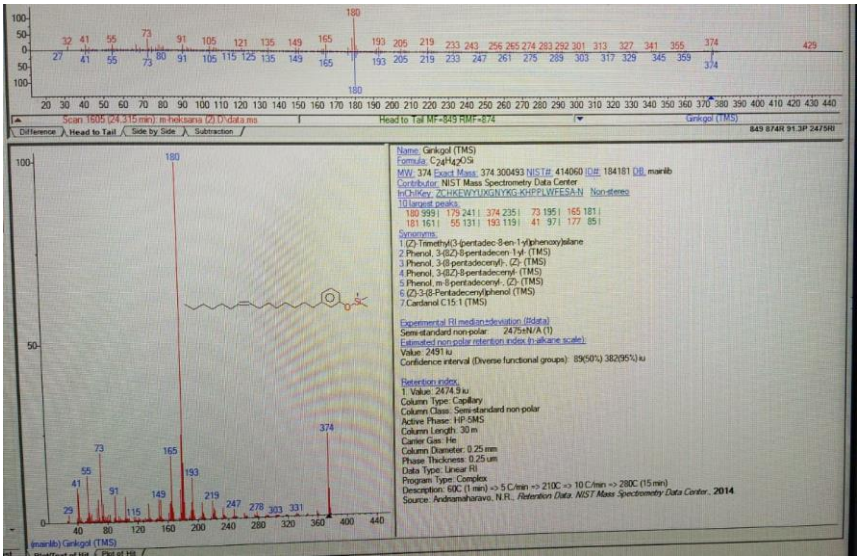


Fig. 4. Fragmentation spectra and structure of cardanol C15:1

3. Mass fragmentation of phenol,2-methyl

Figure 5 showed the mass fragmentation of phenol, 2 methyl synonim 1-hydroxy-2-methyl benzene. With MW – 108. This compound showed the same fragment with NIST Mass Spectrometry library in our instrument GCMS Hight molecular ions as base peak appeared at m/z 108 = (M). Very small moleculer ions appeared at m/z 76 as M-[(CH_3+OH)] and m/z at 91 as (M-OH).

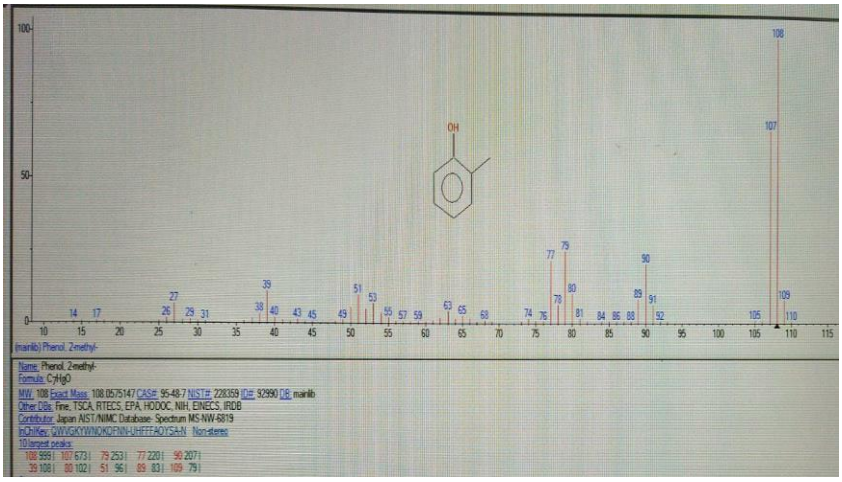


Fig. 5. Fragmentation spectra and structure of -hydroxy-2-methyl

4. Mass fragmentation of cardanol C15:1

In order to identify sabinone was done by GC-MS molecular ion at m/z = 135 as M-(CH_3) and at m/z at 108 as M-[($2CH_3$+C)] (base peak) and molecular ion at m/z 77 appeared as M- [($2CH_3$+C+CH3+O)] see Figure 6.

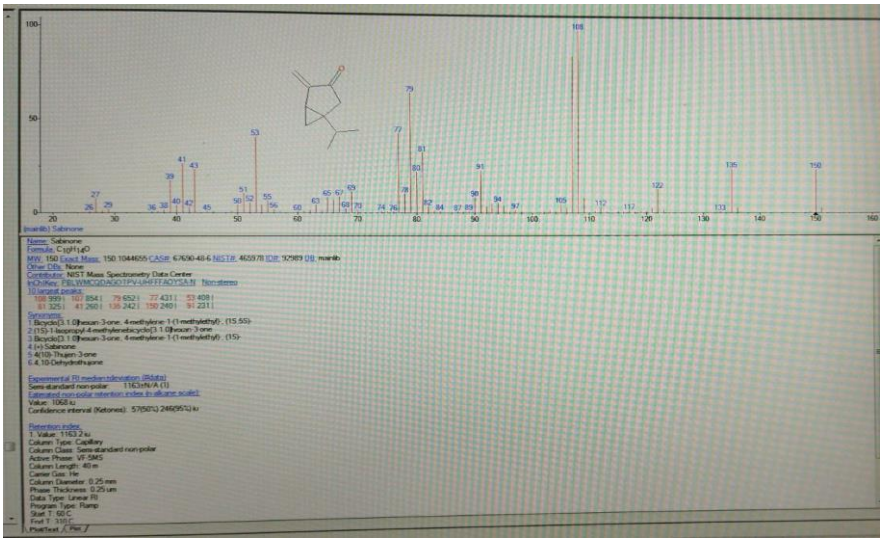


Fig. 6. Fragmentation spectra and structure of –sabinone

5. Mass fragmentation (z)-3-(pentadec-8-en-1-yl) phenol.

Compound (z)-3-(pentadec-8-en-1-yl) phenol) with MW 302, moleculer formula $C_{21}H_{34}O$ give some fragment pattern (Figure **7**). Bigest moleculer ios appeared at m/z 108 as base peak (M-[(12CH2+2CH)] and small peak at (M+H-[(CH3+ $12CH_2$+2CH+OH) at m/z 77.

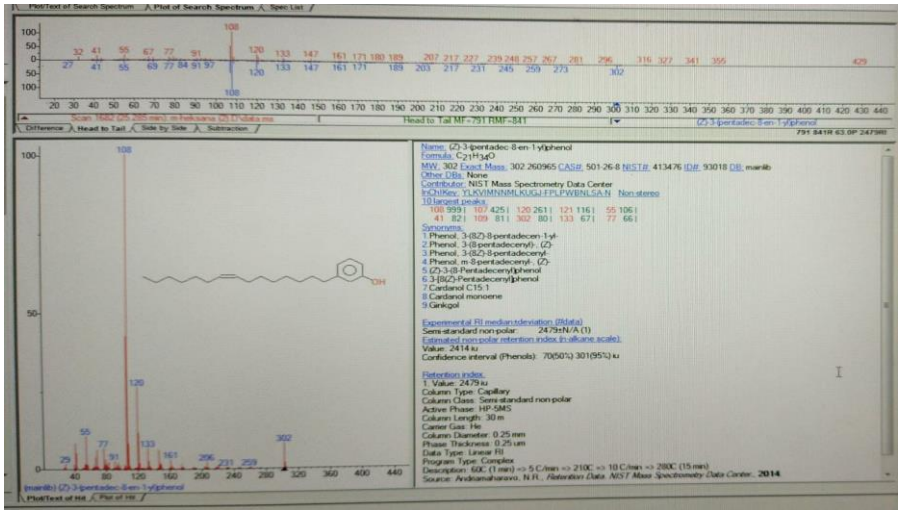


Fig. 7. Fragmentation spectra and structure of (z)-3-(pentadec-8-en-1-yl) phenol)

6. Mass fragmentation of Compound (z)-3-(pentadec-10-en-1-yl) phenol)

Compound (z)-3-(pentadec-10-en-1-yl) phenol) hasMW 330, molecular formula $C_{23}H_{38}O$ gives some fragment pattern (Figure 8). Bigest molecular ions appeared at m/z 108 as M+H-[(CH_3+ $14CH_2$+2CH) and at m/z 147 from M+1- [(CH_3+$9CH_2$+2CH+OH).

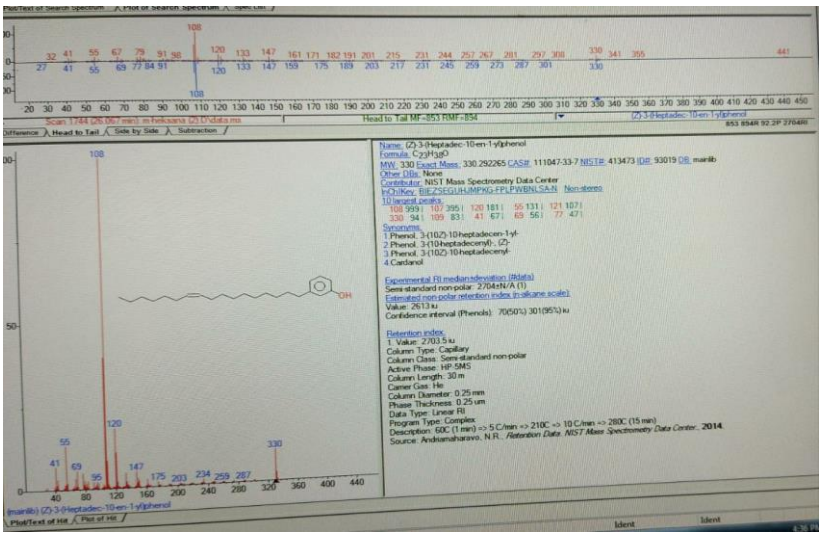


Fig. 8. Fragmentation spectra and structure of (z)-3-(pentadec-10-en-1-yl) phenol)

7. Mass fragmentation of Compound (2.4-Diaminophenol

Compound 2.4-Diaminophenol has MW 124 with structure C_6H_8N2. In order to identify 2.4-Diaminophenol we showed fragmentaion patterns in Figure 9

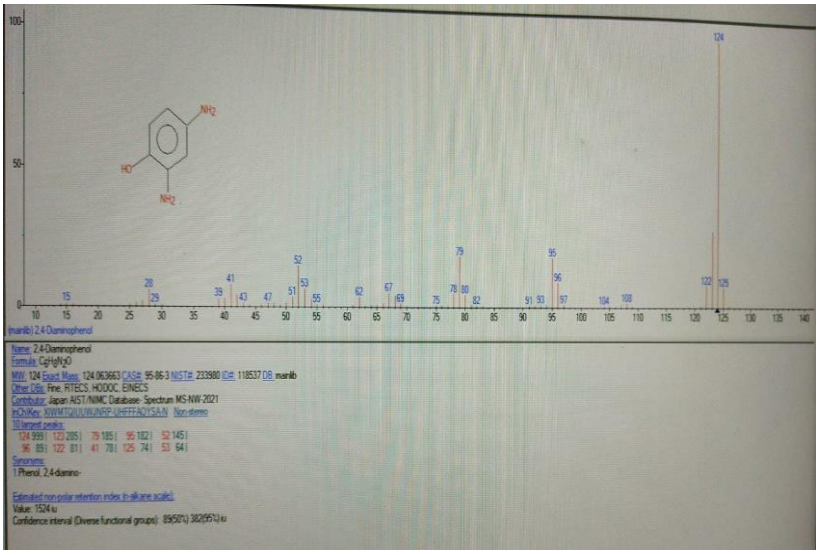
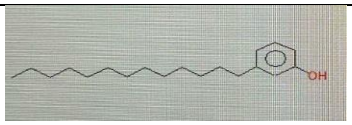
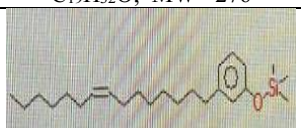
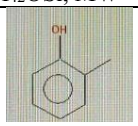
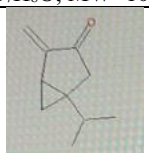
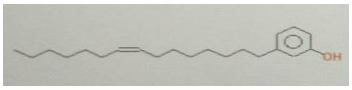
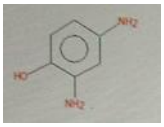
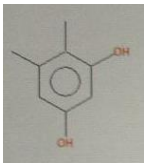


Fig. 9. Fragmentation spectra and structure of 2,4-diaminophenol

Table 3. Chemical constituent in hexane extracts of Cashew nut shell (Structure, Calculate sum formula, moleculer weight (MW)

No	Compound in hexan extract	Structure mocelus, MW
1	3-Tridecylphenol sinonim (Cardanol C13:0) Rt 22.85	 $C_{19}H_{32}O$, MW =276
2	(Cardanol C15:1) Rt =22.85	 $C_{24}H_{42}OSi$, MW =374,
3	phenol,2ethyl Rt 24.567	 C_7H_8O, MW=108
4	Sabinone /sinonim Phenol, 3-methyl RT	 $C_{10}H_{14}O$, MW =150
5	[(z)-3-(pentadec-8-en-1-yl)phenol- Rt 25.298:0) 22.85	 $C_{21}H_{34}O$, MW =302
6	[z)-3-(pentadec-10-en-1-yl)phenol] Rt 26.05	 $C_{23}H_{38}O$, MW =330
7	3-Tridecylphenol sinonim (Cardanol C13:0) Rt 22.85	 $C_6H_8N_2O$ M W =124
8	3-Tridecylphenol sinonim (Cardanol C13:0) Rt = 22.85	 $C_8H_{10}O_2$ =138

3.3.3 GC-MS of CNS dichlorometane extract

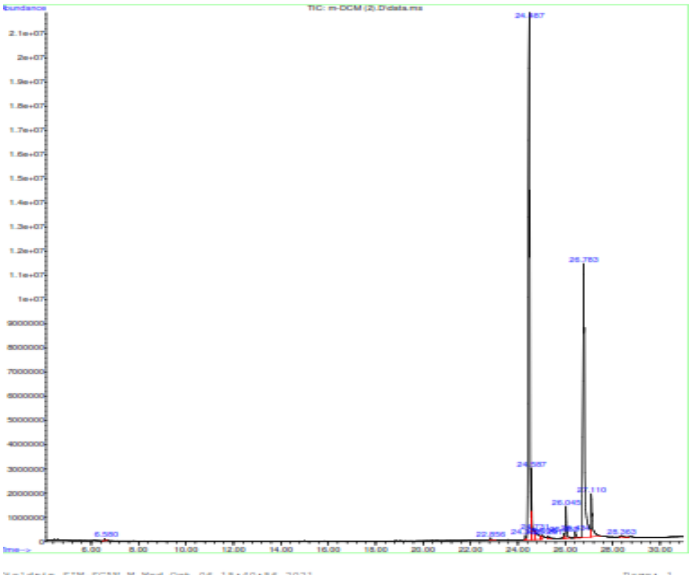


Fig. 10. GC-MS Chromatogram profile of cashew nut shell of CH_2CL_2 extract

Table 4. Chemical constituents in CH_2Cl_2 extracts of cashew nut shell (CNS)

No	Compound in CH_2Cl_2 extract	RT
1	Decane,3,3,4-trimethyl-	6.583
2	3-Tridecylphenol	22.853
3	Ginkgol (TMS)	24.328
4	[(z)-3-(pentadec-8-en-1-ylyl)phenol-	24.492
5	phenol, 3-(pentadecyl	24.592
6	p-cresol	24.731
7	phenol, 3-methyl	25.008
8	Carbomic acid, ethyl 3-methylphenol	25.286
9	3-((4z,7z)-heptadeca-4,7-dien-1	25.953
10	[(z)-3-(pentadec-10-en-1-yl)phenol-	26.042
11	Bilobol C15:1	26.432
12	2,4-idiaminophenol	26.785
13	4,6-diamino-O-cresol	27.113
14	Pregn-4-ene-3.20-dione	26.042

4 Conclusion

GC-MS fragmentation results prove that the hexane fraction of CNSE contains 8 compounds and the dichloromethane fraction contains 14 compounds, and all extracts have antibacterial activity to *B. subtillis. P. aeruginosa, E. coli and S. aureus.*

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