MERIT RESEARCH JOURNALS
www.meritresearchjournals.org

Merit Research Journal of Environmental Science and Toxicology (ISSN: 2350-2266) Vol. 1(8) pp. 147-155, October, 2013
Available online http://www.meritresearchjournals.org/est/index.htm

Full Length Research Paper

Isolation and identification of bioactive compounds from twigs of *Artocarpusaltilis*

Muhammad Nor Omar[1]*, Ahmad Muzammil[2], Naik T. Khan[3], Muhammad Zahid[4] and Tara Kamal[1]

[1]Research management center, Office of the Campus Director, International Islamic University Malaysia (IIUM), Jalaln Sultan Ahmad Shah, Bandar InderaMahkota, 25200 Kuantan, Pahang, Malaysia

[2]Department of Biotechnology, Kulliyyah of Science, International Islamic University Malaysia (IIUM), Jalaln Sultan Ahmad Shah, Bandar InderaMahkota,25200 Kuantan, Pahang, Malaysia

[3]Research Officer (Tenure Track), H.E.J Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan

[4]Science Officer Centre for Research and Instrumentation National University of Malaysia

***Corresponding Author's E-mail: noromar@yahoo.com, tarajalal@ymail.com**

Abstract

The present study focuses on the bioassay guided isolation of antimicrobial compounds from *Artocarpusaltilis*. The hexane, dichloromethane and methanol extracts were obtained from the dried (twigs of *A. altilis*) by using Soxhlet extraction. The plant crude extracts were evaluated for *in vitro* antimicrobial activities revealed that twigs part of *A. altilis* had potent antimicrobial activities, further separation had been done through column chromatography (CC) of dichloromethane and hexane extracts of *A.altilis*, which were afforded stigmasterol and cycloartenyl acetate respectively, the two compounds exhibited *in vitro* antimicrobial activity against *S. aureus* value of (11±0.5mm) and (11.3±0.5mm). Whereas minimum inhibition concentration (MIC) and minimum bactericidal/minimum fungicidal concentration (MBC/MFC) for isolated compounds were ranged from 62.5-500 µg/ml. Their structures were identified by ^{1}H-NMR, ^{13}C-NMR, IR and the spectra obtained were compared with previous data. This study forms a strong basis for declaring *A. altilis*as antimicrobial potential source to treat pathogenic microorganisms.

Keywords: *Artocarpusaltilis*, antimicrobial, cycloartenylacetat, stigmasterol, MIC, IR, NMR

INTRODUCTION

Many of the modern medicines are produced indirectly from medicinal plants. Plants are directly used as medicines by a majority of cultures around the world, Chinese medicine and Indian medicine. Many food crops have medicinal effects, for example garlic. Medicinal plants are resources of new drugs. It is estimated there are more than 250,000 flower plant species. Cultivation and preservation of medicinal plants protect biological diversity, such as metabolic engineering of plants. The medicinal effects of plants are due to metabo-lites especially secondary compounds produced by plant species, many valuable drugs were isolated from medicinal plants through scientific studies which include; aspirin, atropine, morphine, reserpine and quinine among others and all were proved for the activity. Due to the general awareness of the widespread toxicity and harmful effects associated with the long use of synthetic drugs (Ziegler and Facchini, 2008). Tropical plants may present a rich source of such compounds from which novel antibacterial and antifungal, antioxidants and chemotherapeutic agents may be obtained which can potentially be developed as alternative promising agents.

Hence, many tropical plants from Malaysia are being and has been extensively studied as well as their biological activity. One of them is the *Artocarpus* species of the family Moraceae which consists of about 55 species and is widely distributed throughout subtropical and tropical regions of the World from Indian subcontinent south of the Himalayas, SriLanka, Burma, Thailand, Indo-China, Southern China, Taiwan and Malay Peninsula (Kochummen and Go, 2000). *Artocarpusaltilis*(breadfruit) have been found to exhibit biological activity including inhibition of including inhibition of platelet aggregation(Wang et al., 2006), antibacterial (Khan et al., 2003), anti-oxidant, antifungal (Amarasinghe et al., 2008), inhibition of leukemia cells and anti-tumor agent (Nomura et al., 1998). Terpenoid compound with a cycloartan model was successfully isolated from *Artocarpu*s plants such as cycloartenol from fruits of *A. altilis* (Achmad et al., 1996).

Experimental

Collection and preparation of plant materials: The plant was collected from Kuantan, Pahang, Malaysia.

Extraction and isolation

The fresh plant material was 1 kg, the twigs of *A. altilis* was prepared for extraction by washing off all dirt and soil residues. They were left todry in the oven at 40˚C for 72h. After drying, the powder of *A. altilis* (700 g) was extracted using various solvents were applied in a sequence of*n*-hexane, dichloromethane and methanol using Soxhlet apparatus according toGreen, (2004). It is to be highlighted that after each solvent, the thimble was left to air-dry to remove all residues from previous solvent. Upon obtaining all the crude extracts, the extracts were completely dried in the vacuum rotary evaporator (BÜCHI Rotavapor R-200) at 60˚C. The extracts were stored at 4˚C until further analyses.

Chromatographic separation

Column chromatography of the dichloromethane extract of *A. altilis*

Based on the antimicrobial activity (Kamal et al., 2012) of DCM (dichloromethane) extract of *A. altilis,* it was further subjected to column chromatography over silica gel (30 g) (the isolation process was repeated four times) packed using mixture of (Hexane: DCM 9:1). One g of DCM extract was fractionated into three sub-fractions f (1-5), f (6-12) and f (13-23) using a gradient mixture of n-hexane and DCM of increasing polarity. Fraction f (13-23) in ratio of (4:6; n-hexane: DCM) was washed with hexane and DCM to afford pure white crystalline compound DEC (10 mg).

Column chromatography of hexane extract of *A. altilis*

One g of hexane extract was subjected to silica gel CC (the isolation process was repeated four times) with increasing polarity of DCM in hexane to obtain two sub-factions f (2-6) and f (7-12). Fraction f (7-12) in ratio of (7:3; n-hexane: DCM) afforded a pure crystalline compound HFC (20 mg).

Antimicrobial activities for isolated compounds DEC and HFC

Disc diffusion method

The antimicrobial activity for isolated compounds DEC and HFC from dichloromethane and hexane extracts of *A. altilis* were separately applied for disc diffusion assay (Murray et al., 1999). Isolated compounds were dissolved in methanol. The solution of compounds were applied to sterile filter paper discs (Whatman No. 1; 6 mm in diameter) to give a final concentration of 30 µg/disc and placed on the surface of the inoculums media using Nutrient Agar (NA) for bacteria and Potato Dextrose Agar (PDA) for fungi. The petri dishes were incubated at 37˚C for 24 h for bacteria and 32˚C for 48 h for fungi. Antimicrobial activities were observed by measuring the inhibition zone around the discs in mm.

Determination of Minimum Inhibitory Concentration (MIC) of isolated compounds

The MIC assay was performed by a broth micro dilution technique, using 96-well microliter plates according to Sarker et al. (2007). Isolated compounds were prepared in MeOH to make 1000 µg/ml as a stock solution; this was then serially diluted by adding to the broth media in a 96-wells microlitre to obtain two fold serial dilutions ranging from 3.9 to 500 µg/ml. The MIC of DEC and HFC were tested against pathogenic microorganisms; the growth media NB and PDB were prepared and sterilized by autoclaving. The assay plates were filled with Nutrient Broth (NB) and Potato Dextrose Broth (PDB) media and contained different dilutions of the samples needed to be evaluated. The bacterial inoculate contained approximately (108 CFU/mL) and fungi (106 CFU/mL) in a final volume of 200 µl/well. Streptomycin and Nystatin were used as a positive control for bacteria and fungi, while the methanol was used as negative control. The cultured micro plates were incubated for 24 h at 37˚C for bacteria and 32˚C for 48 h for fungi. The turbidity was

Table 1. Antimicrobial activities of extraction compounds as zone of inhibition.

	Extracted compounds	
	DEC	HFC
S. aureus	11±0.5*	11.3±0.5*
B. cereus	10.0±1.0*	9.0±0.5*
E. coli	8.0±1*	7.8±0.6*
P.aeruginosa	UD	UD
C.albicans	9.3±0.5*	10±0.5*
C.neoformans	9.1±1.4*	9.7±0.6*

* Significant at $p < 0.05$ level, ns = not significant, UD = Undetected DEC =Stigmasterol, HFC = Cycloartenyl acetate *S.aureus= Staphylococcus aureus,E.coli = Escherichia coli,B. cereus = Bacillus cereus,P.aeruginosa = Pseudomonas aeruginosa,C.albicans= Candida albicans, C. neoforman= Cryptococcus neoformans.*

Table 2. Minimum Inhibitory Concentration (MIC) (µg/ml) of isolated compounds

	DEC	HFC
Gram-Positive		
S. aureus	125	125
B. cereus	125	250
Gram-Negative		
E. coli	250	250
P.aeruginosa	UD	UD
Fungi		
C. albicans	125	125
C.neoformans	125	125

DEC = Stigmasterol, HFC = Cycloartenyl acetate, UD = Undetected *S.aureus= Staphylococcus aureus,E.coli = Escherichia coli,B. cereus =Bacillus cereus, P. aeruginosa = Pseudomonas aeruginosa,C.albicans= Candida albicans, C. neoforman = Cryptococcus neoformans.*

observed in the wells to exhibit the bacteria and fungi concentration.

Minimum Bacterial Concentration (MBC/MFC) of isolated compounds

The MBC and MFC assay were determined by a broth micro dilution technique, using 96-well microliter plates according to Sarker et al. (2007). Further sub culture was performed on NA and PDA as described by Vollekova et al., (2001) and Usman et al., (2007). In this technique, the contents of the wells resulting from MIC was streaked using a sterile wire loop on agar plate. The lowest concentration of the isolated ccompounds which showed no visible growth on the agar was noted and recorded as the MBC/MFC.

RESULTS

The antimicrobial activity of isolated compounds the DEC and HFC in vitro against the selected pathogenic microorganisms are shown in Table 1. The results revealed that the compounds were active against microbe. The two isolated compounds were active against the tested microbial strains *S. aureus*, *B. cereus*, *E. coli*, *C. albicans*, *C. neoformans*. The compounds were found to be generally more active on the Gram-positive bacteria followed by fungal strains and lastly the Gram-

Table 3. Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) (µg/ml) of Isolated Compounds

	MBC/MFC (µg/ml)	
	DEC	HFC
Gram-Positive		
S. aureus	500	500
B. cereus	500	500
Gram-Negative		
E. coli	500	500
P. aeruginosa	UD	UD
Fungi		
C. albicans	500	500
C.neoformans	500	NA

DEC = Stigmasterol, HFC = Cycloartenyl acetate, UD = Undetected, NA = Not active *S.aureus= Staphylococcus aureus, E.coli = Escherichia coli,B. cereus = Bacillus cereus, P.aeruginosa = Pseudomonas aeruginosa, C.albicans= Candida albicans, C. neoforman= Cryptococcus neoformans.*

Table 4. ^{1}H- and ^{13}C-NMR chemical shift of compound DEC (Stigmasterol) (δ ppm), 600MHz inCDCl3 $^{a-d}$exchangeable assignments in a column, "o" denotes overlapping signals.

DEC (Stigmasterol)				Stigmasterol (Abiodun et al., 2008)	
Position	**^{13}C δppm**	**Multiplicity**	**^{1}H δppm (J Hz)**	**^{1}H δ**	**^{13}C δ**
1	37.3	CH_2	1.86; 1.08, o		37.5
2	31.7[a]	CH_2	1.83; 1.50, o		31.8
3	71.8	CH	3.53, m	3.28	71.9
4	42.3	CH_2	2.30, ddd (13.2, 5.4, 2.4); 2.24 td (13.2, 2.4)		42.2
5	140.8	C	-		140.9
6	121.7	CH	5.35, dd (5.4, 3.6)	5.33	121.7
7	31.9[a]	CH_2	1.99; 1.55, o		31.9
8	31.8[b]	CH	1.53, o		32.2
9	50.2	CH	0.94, o		50.3
10	36.5	C	-		36.6
11	21.1	CH_2	1.54-1.47, o		21.0
12	39.7	CH_2	1.99; 1.18, o		39.7
13	42.2	C	-		42.5
14	56.9	CH	0.96, o		57.0
15	24.4	CH_2	1.51; 1.07, o		24.4
16	28.9	CH_2	1.72; 1.26, o		28.9
17	56.0	CH	1.15, o		56.0
18	12.2[c]	CH_3	0.70, s	0.65	12.4
19	19.4	CH_3	1.01, s	0.80	19.4
20	40.4	CH	2.04, o		40.5
21	21.2[d]	CH_3	1.03, d (6.6)	0.90	21.1
22	138.3	CH	5.16, dd (15.0, 8.4)	5.15	138.4
23	129.3	CH	5.02, dd (15.0, 8.4)	5.02	129.4
24	51.2	CH	1.54, o		51.3
25	31.9[b]	CH	1.52, o		32.0

Table 4. Continue

26	18.9	CH_3	0.84, d (6.0)	0.83	19.0
27	21.0[d]	CH_3	0.80, d (6.0)	0.81	21.2
24’	25.4	CH_2	1.41; 1.17, o		25.4
24”	12.0[c]	CH_3	0.81, o	0.84	12.0

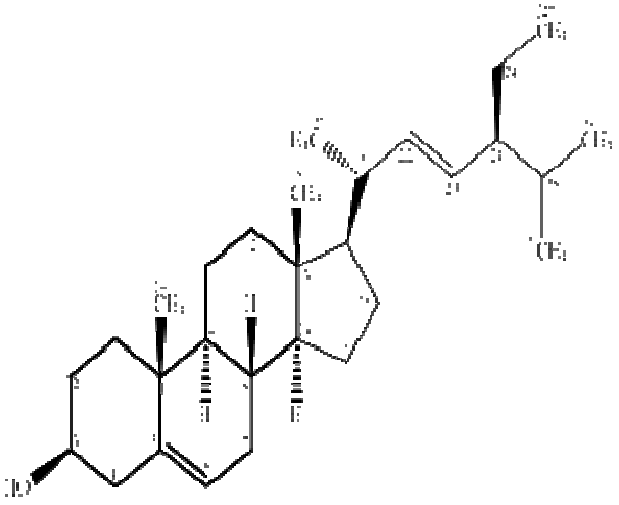

Figure 1. Structure of compound DEC(Stigmasterol)

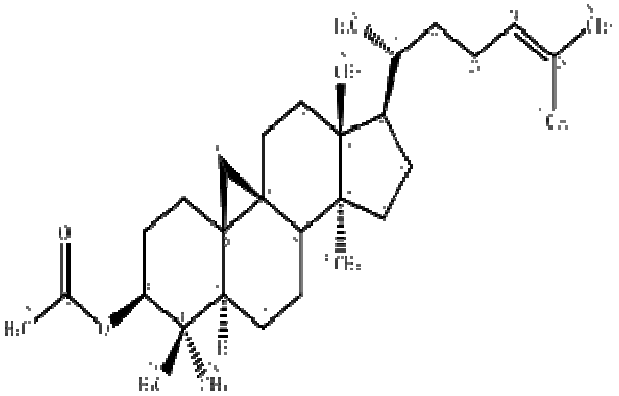

Figure 2. Structure of compound HFC (Cycloartenyl acetate)

negative bacteria. In all, the activities of isolated compounds were moderate with zone of inhibition ranging from 7.8±0.6 to 11.5±0.2mm. In this study compound DEC and HFC possessed spectrum activities because they were all potent on at least one bacterial and fungal strain. Table 2 and 3 show antimicrobial activities of compounds DEC and HFC against both bacterial and fungal strains. All isolated compounds showed moderate activity with MIC value of 125 and 250 µg/ml for the tested microbial strains causing human pathogens. Compounds DEC and HFC exhibited MIC ranged from 125-250 µg/ml on Gram-positive, Gram-negative and fungal strains. In the present study, the lowest MIC of 125 µg/ml against *S. aureus* was observed by the two isolated compounds.

Structure elucidation of compounds

DEC and HFC

Compound DEC

DEC was a crystalline solid (10 mg). The physical and

Table 5. ^{1}H- and ^{13}C-NMR chemical shift of compound HFC cycloartenyl acetate (δ ppm), 600 MHz in CDCl3 [a, b] exchangeable assignments in column,"o" denotes overlapping signals

	HFC cycloartenyl acetate			Cycloartenyl acetate(De Pascul et al., 1987)	
Position	**^{13}C δ ppm**	**Multiplicity**	**^{1}H δ ppm (J Hz)**	**^{1}H δ**	**^{13}C δ**
1	31.6	CH_2	1.61; 1.27, o		31.6
2	26.8	CH_2	1.78; 1.62, o		26.8
3	80.7	CH	4.56, dd (10.8, 5.4)	4.56	80.7
4	39.4	C	-		39.5
5	47.2	CH	1.38, dd (18.6, 3.0)		47.2
6	20.9	CH_2	1.56; 0.82, o		20.9
7	28.1	CH_2	1.91; 1.28, o		28.1
8	47.8	CH	1.52, dd (11.4, 4.8)		47.8
9	20.2	C	-		20.2
10	26.0	C	-		26.0
11	25.8	CH_2	1.32; 1.08, o		25.8
12	35.5	CH_2	1.37; 1.28, o		35.6
13	45.3	C	-		45.3
14	48.8	C	-		48.8
15	32.9	CH_2	1.62, o		32.9
16	26.5	CH_2	1.98; 1.58, o		26.6
17	52.3	CH	1.61, o		52.3
18	17.9	CH_3	0.97, s	0.95	18.0
19	29.7	CH_2	0.34; 0.58, d (4.2)	0.34, 0.57	29.7
20	35.9	CH	1.43, o		35.9
21	18.2	CH_3	0.88, d (7.0)	0.88	18.3
22	36.4	CH_2	1.43; 1.36, o		36.4
23	24.9	CH_2	2.05; 1.85, o		25.0
24	125.3	CH	5.12, t (6.6)	5.10	125.3
25	130.8	C	-		130.8
26	17.6	CH_3	1.62, s	1.60	17.6
27	25.4	CH_3	1.69, s	1.68	25.7
28	19.3	CH_3	0.84, s	0.88	19.3
29	15.1	CH_3	0.89, s	0.89	15.1
30	25.7	CH_3	0.89, s	0.84	25.4
1'	170.9[a]	C	-		170.9
2'	21.3[b]	CH_3	2.06, s	2.04	21.3

spectroscopic data of DEC was in agreement with the reported data of stigmasterol. The IR spectrum indicated absorption bands at 3410 and 1055 cm-1 for a hydroxyl functionality. The presence of double bond (1634 and 917 cm-1) was aslo inferred from the IR analysis. A close analysis of the NMR spectra revealed that DEC could be aphytosterol. The 1H-NMR (Table 4) of DEC showed methyl proton signals at δ 1.03, 1.02, 0.84, 0.0.81, 0.80 and 0.70. Four distinct one-proton signals at δ 5.35 (dd, J = 5.4, 3.6 Hz), 5.16 (dd, J = 15.0, 8.4 Hz), 5.02 (dd, J = 15.0, 8.4 Hz) and 3.53 (1H, m, H-3) were also observed in the 1H-NMR spectrum. The ^{13}C-NMR spectrum of compound DEC exhibited a total of twenty-nine carbon signals (Table 4) which can be differentiated as six methyl, nine methylene, eleven methine and three quaternary carbons. The signals δ 5.36/δ 121.7 and 140.8 were due to a trisubstituted double bond, while the NMR signals at δ 5.16/δ 138.3 and 5.02/129.3 were due to a disubstituted double bond. Moreover, the signals at δ 3.53/71.8 were indicative of the presence of a hydroxyl group. The spectral data of DEC was in agreement with the reported data for stigmasterol (Abiodun et al., 2008),

(Table 4) (Figure 1).

Compound HFC

Compound HFCwas an UV inactive like DEC, crystallineneedle (20 mg). The physical and spectroscopic data suggested that HFCwas a cycloartane type triterepenoid. The IR spectrum indicated absorption bands at 1736 and 1041 $cm^{-1}$for an ester ($_3$HC-COO-) functionality. The presence of double bond was also inferred from the absorptions 1652 and 887 cm^{-1}.A close analysis of the NMR spectra also supported that HFC was a cycloartane type triterepene. The ^{1}H-NMR (Table 5) of HFC showed upfield methylene proton doublets at δ 0.34 and 0.58 (J = 4.2 Hz), characteristic of cycloartane type triterpenoids (De Pascul et al., 1987). Seven methyl proton singlets were also resonated at δ 2.06, 1.69, 1.62, 0.97, 0.89 and 0.84, along with a double t δ 0.88 (J = 7.0 Hz). A methine proton triplet at δ 5.12 (J = 6.6 Hz) and doublet at δ4.56, (J = 10.8, 5.4 Hz) were also observed in the ^{1}H-NMR spectrum of HFc_3. The ^{13}C-NMR spectrum of compound HFC exhibited a total of thirty-two carbon signals (Table 5) as eight methyl, eleven methylene, six methine and seven quaternary carbons. The NMR signals at δ 170.9, δ 4.56/80.7 and δ 2.06/21.3 indicated the presence of an acetate ester group. Similarly, the two methyl signals at δ 1.62/17.6 and 1.69/25.4 along with a quaternary carbon δ 130.8 and methane δ 5.12/125.3 also indicated a trisubstituted double bond. The unusually upfield methylene signals at δ 0.58, 0.34/29.7 was characteristic for a disubstitutedcyclopropane ring. Comparison of the spectroscopic data of HFC with reported data confirmed that HFC was cycloartenyl acetate (De Pascul et al., 1987), (Table 5) (Figure 2).

DISCUSSION

The isolated compounds were tested by the disc diffussion method for antimicrobial activities against four bacteria (two Gram-positive and two Gram-negative) and two fungal strains, all causing human pathogens.

From previous study first compound that had been isolated in this study was Stigmasterol, it is a known compound and previously was isolated from Moringa oleifera (leaf) and the antimicrobial activity was ranged (14-17mm zone of inhibition) against *S. mitis*, *S. salivarius*, *S. aureus*, *L. acidophilus* and *L. fermentum* strains (Koteswara et al., 2011), anthor finding by Nazia et al. (2010) MIC for stigemasterol isolated from *Haloxylon salicornicum* against *Mycobacterium tuberculosis* was 100 µg/ml.

As for the second compound (cycloartenyl acetate) also known but there are not many tests where Dennis et al., (2011) reported that cycloartenyl stearate which is similar to cycloartenyl acetate but different with number of methyl group that connected to ester group exhibited potent analgesic and anti-inflammatory activities at effective doses of 6.25 mg/kg body weight and 12.5 mg/kg body weight, respectively. Maximum activity was exhibited by compound HFC against *S. aureus* (11.3±0.5mm) and the lowest value was (7.8±0.6mm) also by HFC against *E. coli* (Table 1).

Previous studies on antimicrobial activities of medicinal plants have indicated that inhibition zones of 10mm or greater were taken to represent good activity of such plants (Najihah et al., 2012). MIC of compounds DEC and HFC is shown in (Table 2). MIC values for compounds DEC and HFC ranged from 125-250 µg/ml; the highest (250 µg/ml) occurred in compound DEC and HFC on the Gram-positive and Gram-negative strains tested (*B. cereus* and *E. coli*) whereas, the lowest MIC (125 µg/ml), indicating most potent activity, was exerted by compoundsDECand HFC on*S. aureus,C. albicans* and *C. neoformans*. The results show that compounds DEC and HFC have similar activity with some differences on the selected pathogenic microorganisms. From the results, the activities of the compounds were more remarkable on the Gram-positive followed by fungi and finally the Gram-negative strains. From these findings, it can be indicated that compound DEC and HFC isolated from hexane and dichloromethane extracts were in principle, able to control diseases especially on the Gram-positive bacteria and fungi. All the isolated compounds had a moderate activity on Gram-positive strains, while they were weak against Gram-negative strains that were used in this study. This result is in line with Alonso et al. (2000) who reported that Gram-negative bacteria are frequently reported to have developed multi drug resistance to many of the antibiotics currently available in the market. Therefore, it is not surprising to learn that *P.aeruginosa* showed the least response to bacteria strains tested on the plant etracts and isolated compounds.

The isolated compounds demonstrated moderate antimicrobial activities on the tested pathogens microorganisms by disc diffusion method and also from MIC results. Therefore, this study shows that these isolated compounds have the potential therapeutic. It supports World Health Organization (WHO) which encourages countries to evaluate traditional medicine with a view of identifying and utilizing concepts that provide safe and effective healing for diseases (Akinyemi et al., 2005).

CONCLUSION

The present study has been done for the first time on the antimicrobial activities of twigs part of *Artocarpus altilis,* respectively. In line with other studies, it showed that many diseases are known to be treated with herbal remedies throughout the history of mankind. Even today,

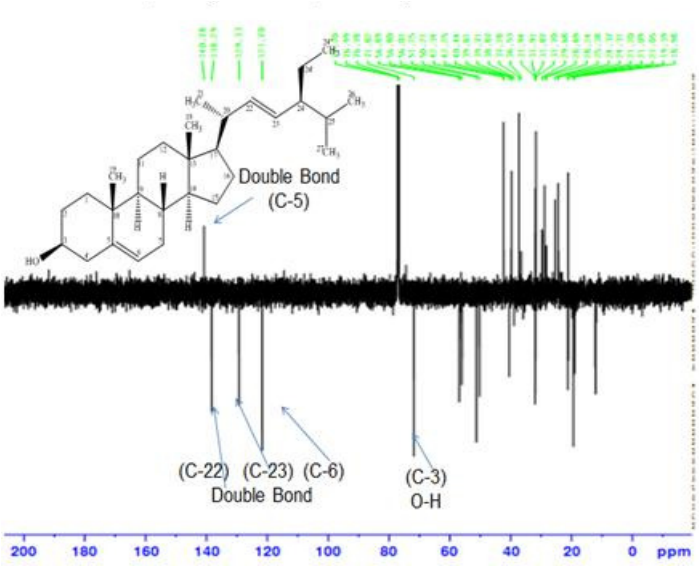

Figure 3. ^{13}C-NMR ($CDCl_3$, 150 MH_Z) of Compound DEC

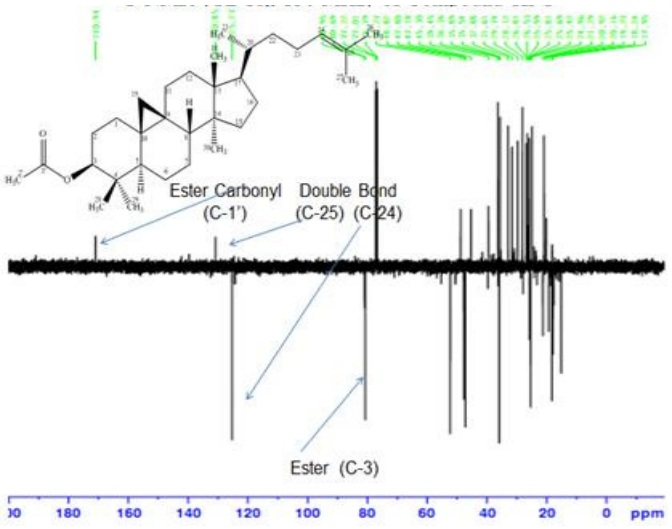

Figure 4. ^{13}C-NMR ($CDCl_3$, 150 MH_Z) of Compound HFC

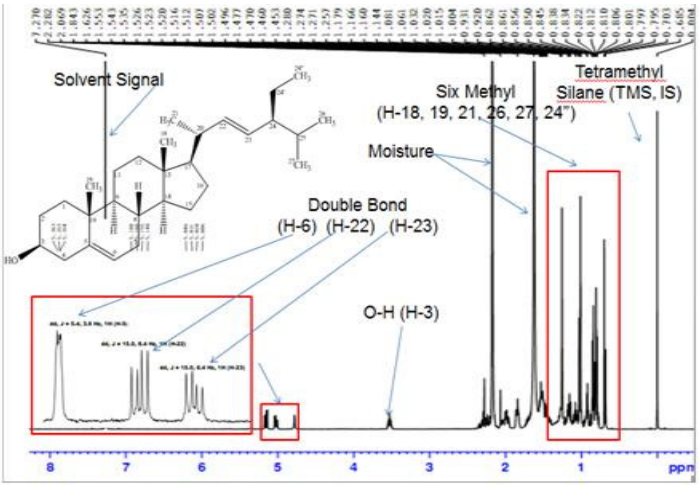

Figure 5. ^{1}H-NMR ($CDCl_3$, 600 MH_Z) of Compound DEC (Stigmasterol)

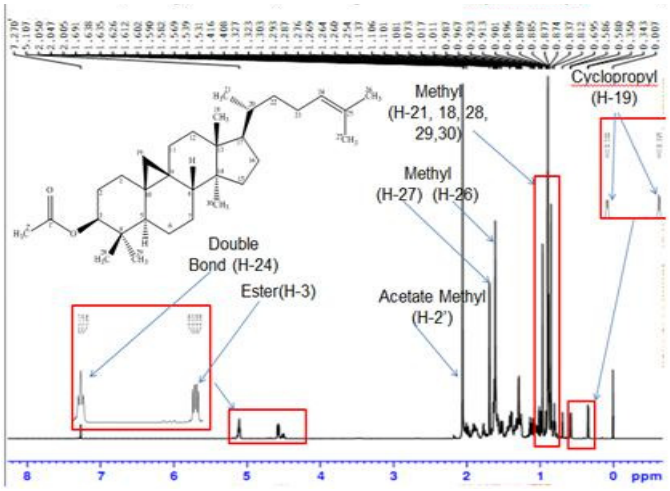

Figure 6. ^{1}H-NMR ($CDCl_3$, 600 MH_Z) of Compound HFC (Cycloartenyl Acetate)

plant materials continue to play a basic role in primary health care as an exclusive source of drugs for the majority of the world's population. Even today, plants material continue to play a basic role in primary health care as almost the exclusive source of drugs for the majority of the world's population. The two isolated compounds (Stigmasterol and Cycloartenyl acetate) obtained broad spectrum activities against the tested bacteria and fungi strains making them suitable antimicrobial properties could be possible leads for the development of antibacterial pharmaceuticals as an antibiotic. The indication of antimicrobial activities by *A. altilis* may lead to new choices for the treatment of infectiousdiseases. Overall, the isolated compounds had a clear antibacterial against gram-positive, Gram-negative and antifugal strains activities.

REFERENCES

Abiodun F, Sajjad A, Irfan MQ, Iqbal MIC (2008). Phytochemical and Biological Investigation of Chroroform and Ethylacetate fractions of *euphorbia heterophlla* leaf (Euphorbiaceae). J. Med. Plants Res. 2(12), 365-369.

Achmad SA, Hakim EH, Juliawaty LD, Makmur L, Suyatno Aimi N (1996). A new prenylated flavone from *Artocarpuschampeden*. *J Nat Prod,* 59, 878-879.

Akinyemi KO, Oladapo O, Okwara CE, Iba CC, Fasure KA (2005). Screening of crude extracts of six medicinal plants used in South-West Nigeria unorthodoxx medicine for anti-methicillin ressistant*S. aureus*acivity. *Complementery and Alternative Medicine,* 5, 6.

Alonso R, Fernandez-Aranguiz A, Colom K, Herreras A, Cisterna R (2005). Profile of Bacteria isolates and Antimicrobial Susceptibility, Multicenter Study Using a One-dayCut-off. *Revista Espanola. Quimioter Journal,* 13(4), 384-393.

Amarasinghe NRL, Jayasinghe N, Hara, Fujimoto Y (2008). Chemical constituents of the fruits of *Artocarpusaltilis*. *Biochemical Systematics and Ecology*, 36(4), 323-325.

De Pascul TJ, Urones JG, Marcos SI, Basabe P, Sexmer CMJ, Fernandez MR (1987). Triterpenes from *Euphorbia broteri.Phytochemistry,* 26(6), 1767-1776.

Dennis DR, Candice LC, Chenga K, Catherine ICL, Wileen ZPO, Vernon JADG, Chien-Chang S, Francisco CFJ, Consolacion YR (2011). Bioactivities of triterpenes and a sterol from Syzygium samarangense. Verlag der Zeitschrift für Naturforschung, Tübingen, 6, 235-244.

Green RJ (2004). Antioxidant Activity of Peanut Plant Tissues. North Carolina State University, USA.

Khan MR, Omoloso AD, Kihara M (2003). Antibacterial activity of *Artocarpusheterophyllus.Fitoterapia,* 74, 501-505.

Kochummen KM, Go R (2000). Moraceae. In *Tree FloraSabah and Sarawak*; Ampang Press:Kuala Lumpur, Malaysia, pp. 181-212.

Koteswara PR, Bhaskar DR, Ravi CHK, Ravindra MN, Madhavi Y, Raghava TR (2011). In vitro antibacterial activity of Moringa oleifera against dental plaque bacteria. Journal of Pharmacy Research, 4(3), 695-697.

Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolke RH (1999). Manual of clinical microbiology. *ASM, Washington, DC 6*.

Najihah MH, Mawardi R, Gwendoline CLE, Mohd AS, Maizatulakmal Y, Muhamad AMA, Ali AM, Rusea G (2012). Antioxidant, antimicrobial and tyrosinase inhibitory activities of xanthones isolated from Artocarpus obtusus F.M. Jarrett. Molecules, 17, 6071-6082.

Nomura T, Hano Y, Aida M (1998). Isoprenoid-substituted flavanoids from *Artocarpus* plants (Moraceae). *Heterocycles,* 47(2), 1179-1205.

Sarker SD, Nahar L, Kumarasamy Y (2007). Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. *Methods,* 42, 321-324.

Usman H, Abdulrahman FI, Ladan AH (2007). Phytochemical and Antimicrobial Evaluation of Tribulusterrestris L. (Zygophylaceae).Growing in Nigeria. *Res. J. Bio. SciMedwell J.* 2(3), 244-247.

Vollekova AD, Kostalova, Sochorova R (2001). Isoquinoline Alkaloids from Mahoniaaquifolium stem bark is active against *Malassezia Sp. Folia. Microbiol.,*46, 107-111.

Wang Y, Deng T, Lin L, Pan Y, Zheng X (2006). Bioassay-guided isolation of antiatherosclerotic phytochemicals from *Artocarpusaltilis*. *Phytotherapy Research,* 20(12), 1052.

Ziegler J, Facchini PJ (2008). Alkaloid biosynthesis: metabolism and trafficking. *Annual Reviewof Plant Biology*,59,735-769.