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Journal of Chemical and Pharmaceutical Research

ISSN No: 0975-7384
CODEN(USA): JCPRC5

J. Chem. Pharm. Res., 2011, 3(2):1-10

Two novel flavonoids from *Bryophyllum pinnatum* and their antimicrobial Activity

Donatus Ebere Okwu* Fred Uchenna Nnamdi

Department of Chemistry, Michael Okpara University of Agriculture Umudike, P.M.B 7267 Umuahia, Abia State, Nigeria

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ABSTRACT

*Chemical investigation of the bioactive constituents from the leaf of a versatile Nigerian medicinal plant Bryophyllum pinnatum resulted in the isolation of two new novel flavonoids; 5^I Methyl 4^I, 5, 7 trihydroxyl flavone **1** and 4^I, 3, 5, 7 tetrahydroxy 5-methyl 5^I-propenamine anthocyanidines **2**. The structure of these compounds were elucidated using NMR spectroscopy in combination with UV, IR and MS spectra data. Antimicrobial studies showed that the isolated compounds successfully inhibited Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli, Staphylococcus aureus, Candida albicans and Aspergillus niger. The antimicrobial observation of the above compounds could be responsible for the activity of Bryophyllum pinnatum and its use in herbal medicine in Nigeria.*

Keywords: *Bryophyllum pinnatum*, antibacterial activity, antifungal activity, bioactive compounds, herbal medicine.

INTRODUCTION

Nigeria is richly endowed with indigenous plants which are used in herbal medicine to cure diseases and heal injuries [1]. Some of the plants are used as food or medicine. These plants exhibit a wide range of biological and pharmacological activities such as anti-cancer, anti-inflammatory, diuretic, oxytocic, laxative, antispasmodic, antihypertensive, anti-diabetic, and anti-microbial functions. The secondary metabolites of plants provides humans with numerous biological active products which has been used extensively as drugs, foods, additives, flavors,

insecticides, colorants, fragrances and chemicals[2]. These secondary metabolites include several classes such as saponins, terpenoids, phenolic compounds, steroids, alkaloids and flavonoids. Different naturally occurring flavonoids have been described and subcategorized into flavones, falvans, flavanones, isoflavonoids, chalcones, aurones and anthocyanidines[3,4]. These flavonoids exhibited remarkable biological activities including inhibitory effects on enzymes, modulatory effects on some cell types, protection against allergies, antiviral, anti-malarial, antioxidant, anti-inflammatory and anti-carcinogenic properties [3,4].

Bryophyllum pinnatum (Kalanchoe pinnata; Lamarch Crassulaceae) is an erect, succulent, perennial shrub that grows about 1.5m tall and reproduces through seeds and also vegetatively from leaf bubils [1]. It has a tall hollow stems, freshly dark green leaves that are distinctlvely scalloped and trimmed in red and dark bell-like pendulous flowers [6]. *Bryophyllum pinnatum* can easily be propagated through stems or leaf cutting. It is an introduced ornamental plant that is now growing as a weed around plantation crop [1, 5]. *Bryophyllum pinnatum* is used in ethnomedicine for the treatment of earache, burns, abscesses, ulcers, insect bites, whitlow, diarrhea and cithiasis [5]. In Southeastern Nigeria, this herb is used to facilitate the dropping of the placenta of new born baby [5]. The lightly roasted leaves are used externally for skin fungus and inflammations. The leaf infusions is an internal remedy for fever [6].

In ethnomedicine, *Bryophyllum pinnatum* is used to induce vomiting of blood, cut, umbilical cord in new born baby, expel worms, cure acute and chronic bronchitis, pneumonia and other forms of respiratory tract infections. The plant is considered a sedative wound-healer, diuretic, anti-inflammatory and cough suppressant [6]. It is used for the treatment of all sorts of respiratory ailments such as asthma, cough and bronchitis [6]. it is employed for the treatment of kidney stones, gastric ulcers and edma of the leg [8,9]. The juice from the leaf is used to treat boils and skin ulcers. *Bryophyllum pinnatum* is widely used in herbal medicine to cure diseases and heal injuries. The various phytoconstituents of this versatile medicinal plant have not been fully documented. Herein we report isolation and structural elucidation of two bioactive flavonoids 5^I Methyl 4^I, 5, 7 trihydroxyl flavone **1** and 4^I, 3, 5, 7 tetrahydroxy 5-methyl 5^I-propenamine anthocyanidines **2**. In addition, we investigate the antimicrobial activity of two novel compounds 1 and 2 isolated from the leaves of *Bryophyllum pinnatum*.

EXPERIMENTAL SECTION

General Experimental Procedure

The IR spectrum was determined on Thermo Nicolet Nexus 470 FTIR spectrometer. The ^{1}H and ^{13}CNMR spectra were recorded on a Bruker Avnce 400FT NMR spectrometer using TMS as internal standard. Chemical shift is expressed in part per million (ppm).

LC-ESIMS spectra were determined in the positive ion mode on PE-Biosynthesis API 165 single quadruple instruments. HRESIMS (Positive ion mode) spectrum was recorded on a thermo Finniga Mat 95XL mass spectrophotometer. Column chromatography was carried out with silica gel (200-300 mesh) and to monitor the preparative separations analytical thin layer chromatography (TLC) was performed at room temperature on pre-coated 0.25mm thick silica gel $60F_{254}$. aluminum plates 20 x 20 cm Merck Darmstadt, Germany. General UV spectrum was recorded on Shimadzu 160A spectrophotometer. Reagents and solvents like ethanol, chloroform,

diethyl ether, hexane were all of analytical grades and procured from Merck. TLC aluminum sheets, silica gel $60F_{254}$ was also purchased from Merck. The nutrient agar was purchased form Scharlan Chemic APHA Spain.

Plant materials

Fresh leaves of *Bryophyllum pinnatum* were harvested from the Botanical garden of Michael Okpara University of Agriculture Umudike, Nigeria on 6th April 2007. The plant samples (leaves and stems) were identified by Dr. A Nmeregini of Taxonomy Section, Forestry Department of the University. A voucher specimen No BP/122 has been deposited at the forestry Department Herbarium of the University.

Extraction and Isolation of Plant Material

Plant materials were treated and analyzed at the Chemistry laboratory, Michael Okpara University of Agriculture Umudike, Nigeria. Mature leaves (1kg) of *Bryophyllum pinnatum* were dried on the laboratory bench for 10 days. The dry samples were milled and ground into powered (860g) using Thomas Willey machine (Model 5 USA). The powdered plant samples (500g) were packed into a Soxhlet apparatus (2L) and extracted exhaustively with 1000 ml ethanol for 24h. The ethanol extract was concentrated using rotary evaporator at $45^{O}C$ and left on the laboratory bench for 2 days to obtain a dry dark green pigment (68g). The column was packed with silica gel and the dark green pigment (40g) of the isolated plant material was placed on top of silica gel and eluted with methanol: chloroform: petroleum ether (20:30:50) to afford three fractions comprising compound 1 (dark green pigment 0.52g Rf 0.2965); Compound 2 (dark green pigment 0.48g Rf 0.3906) and compound 3 (yellow pigment 0.45g Rf 0.3012). Compound 1 was crystallized from hexane to afford (0.45mg Rf 0.2965) IR Vmax $3412cm^{-1}$, (OH); $1713cm^{-1}$ (C=O), $1403cm^{-1}$ (C=C aromatic) and 1085 cm^{-1} (C-O ether) HREIMS m/z 285.2751 [M+] calculated for $C_{16}H_{13}O_5$ (m/z 285) and m/z 73.0292 base peak calculated for m/z 74 (C_6H_2). ^{1}H NMR and ^{13}CNMR were determined. Compound 2 was crystallized from hexane (038mg Rf 0.3906); IR Vmax 3400 cm^{-1} (OH); 1483 (C=C aromatic), 1171 (C-O ether) HREIMS m/z 340.3336 [M+] calculated for $C_{19}H_{18}O^5N$ (m/z 340) and m/z 73.0288 base peak calculated for m/z 73 (C_6H). ^{1}H and ^{13}CNMR were also determined.

Bioassay

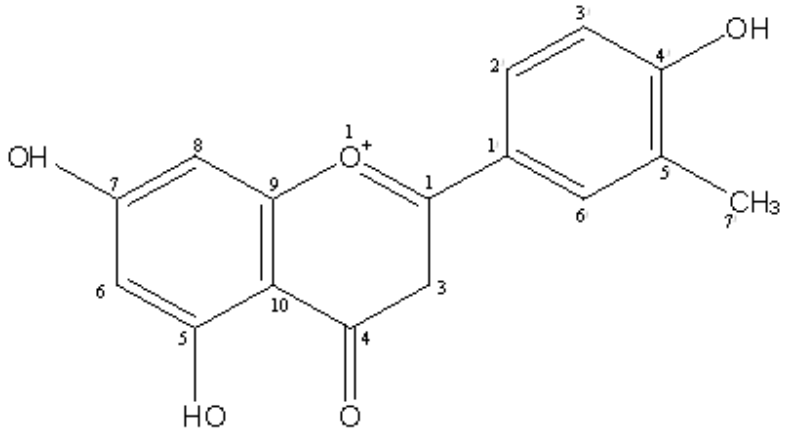
The *in vitro* antimicrobial activity of compounds 1 and 2 were carried out for 24 hrs culture of four selected bacteria and two fungi. The bacteria used were three gram-negative organism comprising *Escherichia coli, Pseudomonas aerugonosa* and *Klebisella pneumonia and a gram positive Staphylococcus aureus*. The two fungi used were *Candidia albicans* and *Aspergillus niger*. All the test organisms are clinical isolates of the pathogens obtained from Federal Medical Centre (FMC) Umuahia, Nigeria. Cultures were brought to laboratory conditions by resuscitating the organism in buffered peptone broth (Scharlan Chemie) and thereafter nutrient agar (Peptone 5g/l and meat 3g/l) and inoculated at $37^{O}C$ for 24 hrs. The antimicrobial activity was performed by filter paper disc diffusion technique. The medium (7g nutrient agar in 250ml distilled water was autoclaved at $115^{O}C$. 20ml of the medium was poured into a sterile Petri dish and allowed to solidify. It was observed for contamination. The sterility of the medium was tested using autoclave at $121^{O}C$ 15Psi for 15 mins. Nutrient agar (Scharlan Chemie) was used for bacteria while subourands agar (Scharlan Chemie) was used for fungi. The isolated samples (Compound 1 and 2) were respectively dissolved in 1ml of absolute ethanol and each made up to 10ml with

distilled water to give a concentration of 100 mg/ml (10% dilution). A colony of each organism was sub-cultured on nutrient broth which contains peptone (5g/l) and meat extract (3g/l) and incubated aerobically at $37^{O}C$ for 8 hrs. 30 mls of the nutrient broth was used to flood the agar plates. A sterilized Whatman No 1 filter paper disc soaked differently in compound 1 and 2 (0.02ml) was used to test for the sensitivity or antimicrobial effect of each of the compounds. The plates were incubated at $37^{O}C$ for 24 hrs. After incubation, plates were observed for zones of inhibition (in mm diameter). The minimum inhibitory concentration was determined. Plates containing agar medium without the addition of compounds1 and 2 were used as control. Each test tube was replicated three times.

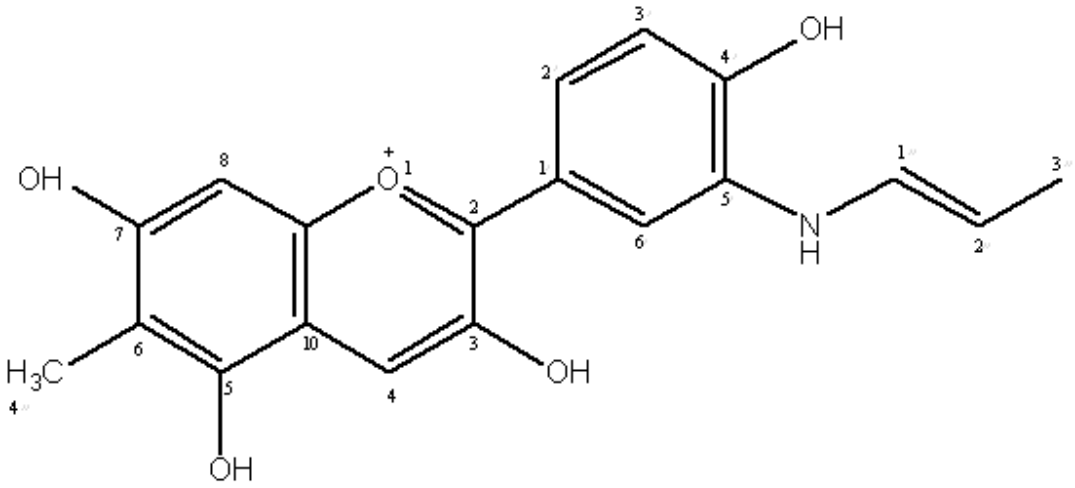
RESULTS AND DISCUSSION

The ethanolic extract of dried leaves of *B. pinnatum* was purified by repeated column chromatography using silica gel. The eluants were subjected to crystallization using hexane to afford compounds 1 and 2. These compounds were elucidated on the basis of their NMR, MS, UV and IR analysis. Compound 1 identified as 5^{I} methyl 4^{I}, 5, 7 trihydroxyflavone was isolated as a dark green pigment from methanol-chlorofoam-petroleum ether (20:30:50). Its IR spectrum showed characteristics absorption bands for hydroxyl ($3412cm^{-1}$), carbonyl ($1713cm^{-1}$), aromatic (1403 cm-1) and ether (1085cm-1) functional group.

The UV absorption bands which occurred at 270nm was classified as a flavone [3]. The electron impact mass spectrum of 1 displayed a molecular peak at m/z 285.2751 [M+] corresponding to a flavone nucleus having hydroxyl groups with molecular formular $C_{16}H_{13}O_5$ (m/z 285 calculated). The mass spectrum of 1 had intense ion peaks at m/z 284.2720 [M-1] which resulted due to proton migration. Detachment of $C_8H_5O_3$ from the parent ion produces the peak m/z 149.0244 with base peak at m/z 73.0292 calculated for C_6H_2 (m/z 74). The pattern of fragmentation of Compounds 1 shown in Figure **1**.



1



2

The ^{1}HNMR spectrum of compound 1 showed the presence of methyl group at δH 2.76378 (3Hs) and four aromatic protons at δH 7.2601 (1Hs). The spectrum also showed the saturated secondary methylene protons at δH 3.6001 (2Hs).The three hydroxyl groups attached to C_4^I, C_5 and C_7 appeared at δH 5.3667, 5.09351 and 5.33894 respectively. The ^{13}CNMR spectrum of compound 1 showed the carbonyl group at δC 178.391 and the aromatic carbons at δC 129.812 and 130.102 while the methyl and methylene carbons appeared at δC 14.187 and δC 29.398 respectively. The above spectroscopic data suggest that compound 1 was a flavonoid and was identified as 5^I methyl 4^I, 5, 7-trihydroxy flavone.

Compound 2 was obtained as a dark green pigment from chlorofoam-methanol (3:1) eluents. The IR spectrum exhibited characteristic absorption bands for hydroxyl (3400cm^{-1}), aromatic (1483 cm^{-1}) and ether (1171 cm^{-1}) functional groups. The UV data was obtained at λmax 540nm indicating that compound 2 may be anthocyanidines. It has a molecular ion peak at m/z 340.3336 corresponding to $C_{19}H_{18}O_5N$ (m/z 340 calculated). The mass spectrum also displayed a large number of fragment ions. Detachment at the amine portions produces the peak at m/z 57.0706 calculated for C_3H_6N (m/z 56) and the major fragment portion produces the peak at m/z 284.2707 calculated for $C_{16}H_{12}O_5$ (m/z 284). Other prominent peaks appeared at m/z 285.2755 due to proton migration from the major peak and m/z 41.0396 calculated for C_3H_5 (m/z 41) occurred due to the elimination of the nitrogen and proton groups for propene amine fragment. The base peak (the most intense peak) was observed at m/z 73.0288 which occurred due to detachment of all the compounds attached to the heterocyclic aromatic ring (figure 2).

The ^{1}HNMR spectrum of compound 2 displayed five aromatic protons at δH 7.2623 (1Hs) and 7.2615 (1Hs). The H_2 olefin proton signal is reported to be multiplet and appeared at δH 5.3722 (1Hm), which correlates to the ^{13}CNMR of olefin peaks at δC 123.998. The methyl proton signals of C_3^{II} appeared as a doublet at δH 1.6120 (3Hd) while the ^{13}CNMR of the methyl groups appeared at δC 24.957. The other aromatic methyl protons appeared at δH 2.7702 (3Hs). The four hydroxyl protons appeared as a broad singlet peak at δH 5.3284, 5.3379, 5.3436 and 5.3518 respectively. Combining the information obtained from UV, MS, IR and NMR. Compound 2 is

identified as 4^{I}, 3, 5, 7 tetrahydroxy 5-methyl 5^{I}-propenamine anthocyanidine. The conjugation of the electrons of the hetrocyclic ring within the aromatic system may be responsible for the dark green colour of the flavonoids. The presence of the phenolic compounds in *Bryophyllum pinnatum* indicates that this plant may be antimicrobial agent since phenols and phenolic compounds are extensively used in disinfections and remains the standard with which other bactericides are compared[10]. The phenolic compounds in *B. pinnatum* may be responsible for the therapeutic, antiseptic, antifungal or bactericides as well as anti-viral and antitumor activities of *B. pinnatum* [5]. The phenolic compounds may undergo oxidation and form phenolates ion or quinine. The phenolate ions are able to scavenge and trap micro organisms [11]. The bioactive phenolic compounds act as a radical scavengers and singlet oxygen quenchers. They react with peroxyl radicals and thus bringing about the termination of radical reaction generated within the system. The antioxidant, anti-inflammatory and anticancer activities of *B. pinnatum* may be attributed to the presence of the flavoniods, flavones and anthiocyanidines. These compounds isolated from the leaves of *B. pinnatum* showed potent inhibition on some micro organisms. The compounds successfully inhibited *Staphyllococus aureus, Pseudomonas aeroginosa, Klebsiella pneumonia, Aspergillus niger* and *Candida albicans* (Tables 2 and 3).

However compound 1 could not inhibit *Escherichia coli*. Many of these organisms are natural flora of the skin and also known etiologic agent of several skin and mucous membrane infections of man (Esimone et al 2008). The result obtained from this study showed that the isolates from *B. pinnatum* inhibited pathogenic fungi (*Candida albicans* and *Aspergillus niger*). The inhibition of these fungi confirm the traditional, therapeutic claims for the use of this herb in the treatment of skin fungus and inflammations [6]. The inhibition of Candida albicans has confirmed the use of *B. pinnatum* in herbal medicine for the treatment of Candida infections. Wounds and boils involve a variety of organisms such as *Pseudomonas aeruginosa, Staphylococcus aureus* and *Klebsiella pneumonia*.

Evaluation of the effects of compound 1 and 2 isolated from *B. pinnatum* leaves on these clinically isolated microbial contaminant of wounds and boils showed varying levels of inhibitory activity against these pathogens (Table 2 and 3). Microbial infection of wound delays healing [12] and causes a more pronounced acute inflammatory reaction [12], which can lead to tissue injury and damage. The antimicrobial activity of these compounds isolated from *B. pinnatum* leaves on wounds pathogens may contribute to wound healing, eliminate infections, thereby resulting to cell proliferation [12]. These findings supported the use of *B. pinnatum* in ethnomedicine in Southeastern Nigeria for the treatment of burns, abscesses, ulcers, boils and the healing of the placenta of newly born baby 1,5]. The inhibition of these pathogens, supported the use of the herb for the treatment of asthma, cough and bronchitis. These findings justifies the use *of B. pinnatu*m in the treatment of skin infections such as boils, carbuncles, abscess, sores and wounds treatment in herbal medicine. If judiciously isolated and processed, *Bryophyllum pinnatum* could provide raw material for the pharmaceutical industries in the country.

Table 1: ^{1}H (400 MHz) and ^{13}CNMR (75 MHz) of compounds 1 and 2

	1				2			
Position	**δC**		**δH**		**δC**		**δH**	
1								
2	129.812	C			77.418	C-O		
3	29.398	CH_2	3.6001	CH_2 2Hs	123.792	C-OH	5.3284	Brs OH
4	178.391	C=O			129.809	C-H	7.2623	1Hs CH
5	130.102	C-OH	5.09351	OH brs	123.792	C-OH	5.3379	Brs OH
6	129.812	C-H	7.26017	C-H 1Hs	130.100	C		
7	130.102	C-OH	5.33894	OH brsC-H 1Hs	123.792	C-OH	5.3436	Brs OH
8	129.812	C-H	7.2601		130.100	C-H	7.2615	1Hs CH
9	130.102	C			127.989	C		
10	130.102	C			127.987	C		
1′	129.812	C			128.934	C		
2′	129.822	C-H	7.26017	CH 1Hs	129.809	CH	7.2615	1Hs CH
3′	130.102	C-H	7.26017	CH 1Hs	128.934	CH	7.2623	1Hs CH
4′	129.812	C-OH	5.36670	1H brs	123.792	C-OH	5.3518	Brs OH
5′	130.812	C-H			129.809	C-H		
6′	129.812	C-H	7.26017	C-H	128.792	C-H	7.2615	1Hs CH
7′	14.187		2.76378	CH_3 3Hs				
1′′					123.798	CH	5.3602	1Hs CH
2′′					123.798	CH_3	5.3722	1Hs CH
3′′					24.957	CH_3	2.7702	3Hs CH_3
4′′					24.788	CH_3	1.6120	
NH							4.3789	NH 1Hs

Table 2: Diameter of zones of inhibition and Mic values of compound 1

	Concentration of compound 1 mg/ml					
	100.0	**50.0**	**25.0**	**12.5**	**6.25**	Mic mg/ml
pathogens	Zone diameter of inhibition (mm)					
Staphyllococus aureaus	7.0	6.0	2.0	-	-	12.5
Escherichia coli	-	-	-	-	-	-
Pseudomonas aeruginosa	8.0	5.0	2.0	-	-	2.5
Klebsiella pnuemonia	8.0	3.0	-	-	-	5.0
Aspergillus niger	3.0	-	-	-	-	100
Candida albicans	7.0	5.0	1.0	-	-	.25
Values are mean of three determinations - = No inhibition						

Table 3: Diameter of zones of inhibition and MIC values of compound 2

Pathogens	Concentration of compound 2 mg/ml					
	100.0	**50.0**	**25.0**	**12.5**	**6.25**	Mic mg/ml
	Zone diameter of inhibition (mm)					
Staphyllococus aureaus	10	5	2	-	-	25
Escherichia coli	3	-	-	-	-	100
Pseudomonas aeruginosa	10	6	2	1	-	12.5
Klebsiella pnuemonia	7	2	-	-	-	50
Aspergillus niger	10	5	2	-	-	25
Candida albicans	11	5	-	-	-	50
Values are mean of three determinations - = No inhibition						

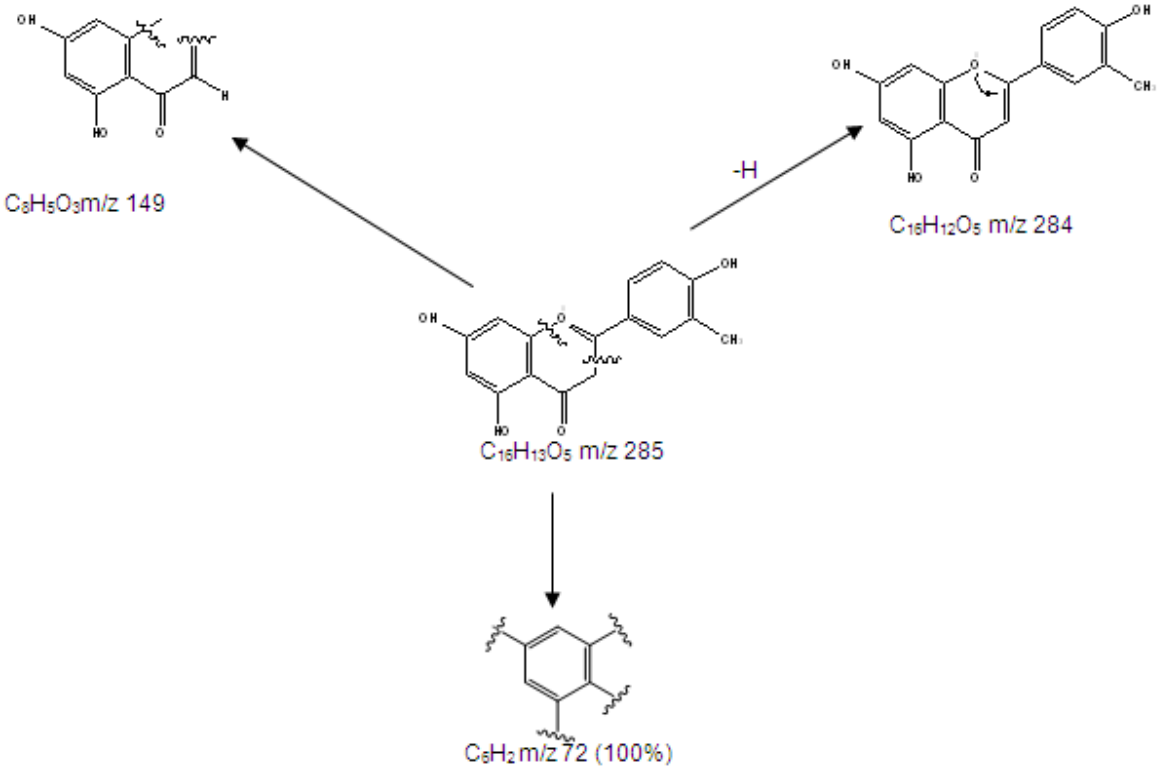


Figure 1: Fragmentation pattern of compound 1

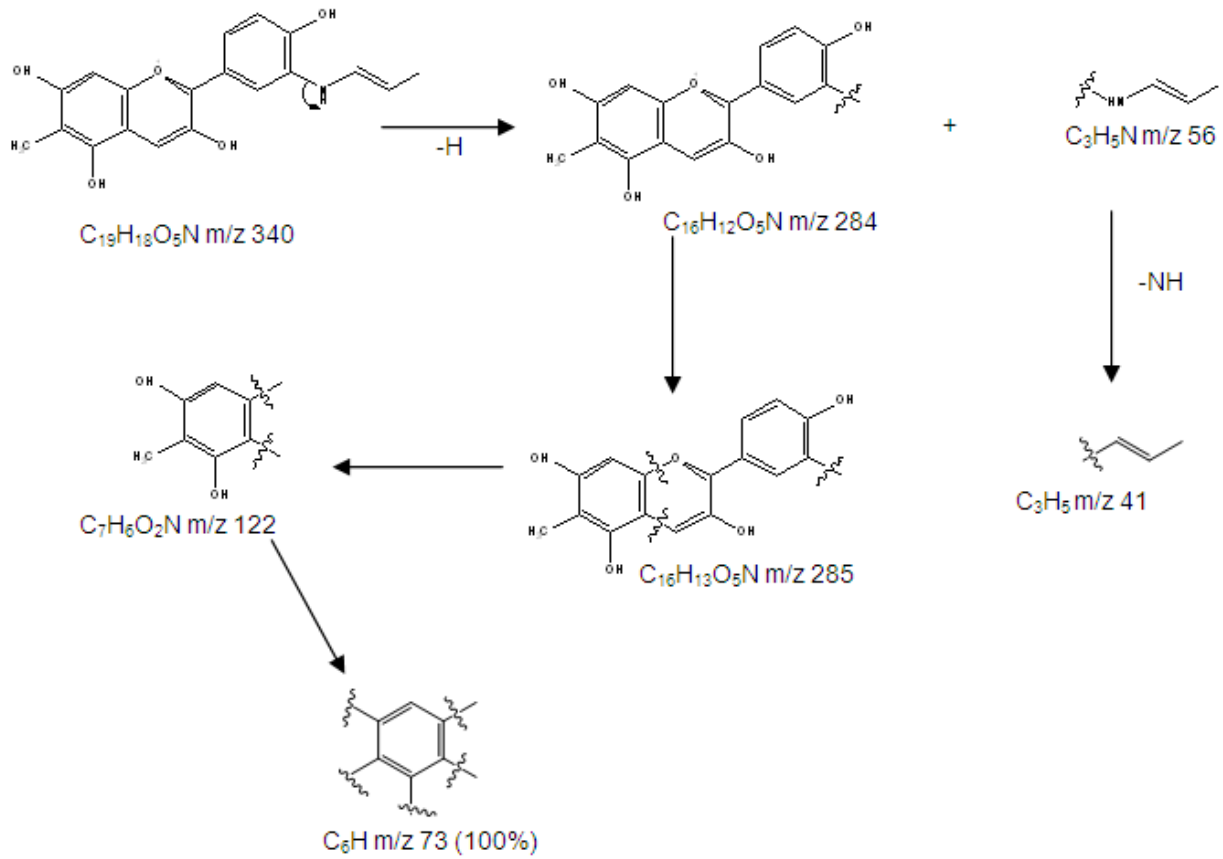


Figure 2:Fragmentation pattern of compound 2

Acknowledgments

We are indeed grateful to Miss Nkechi Ibisi for her kind assistance in running the spectra in China and to Dr. A. Nmeregini for authenticating the plant samples.

REFERENCES

[1] Okwu DE Med. *Aromat Plant Sci Biotechnol*. **2007** 1(1):90-96
[2] Okwu DE, Ezenagu V; *Int. J. Chem Sci*. **2008** 6(2): 705-716
[3] Amnlou M; Ariae S; Farsam H. *J med Aromat. Plant Sci* **2005** 27: 469-475
[4] Veitch NC, Grayer RJ *Natural Product Reports* **2007** 21: 539-573
[5] Okwu DE; Josiah C. *African Journal of Biotech* **2006** 5(4): 357-361
[6] Egereonu UU, Mokwe NR J. *Chem Soc. Nigeria* **2005** 30(2): 192-196
[7] Agoha RC Medicinal plants of Nigeria. Offset Arakkenji faculficider wiskunde. The Netherlands Pp 33,41
[8] Mudi SY; Ibrahim H. *Proceesings, Chem. Sco* Nigeria P
[9] Nassi CC, *Brazilian J. Med Sci* **1992** 14(3-4) 199-202
[10] Okwu DE, Morah FNI *J. Med Aromati Plant Sci* 2007 29: 23-30
[11] Okwu DE, Morah FNI *J. App Sci* **2007** 7(2) 306-309
[12] Okoli C.O, Akah PA and Okoli AC **(2001)** Potentials of the leaves of *Aspiliaa africana* (Compositae) in wound care, and experimental evaluation. *BMC Compliment Allen Med* 7:24-30

[13] Esimone CO, Nworu CS, Ekong US, Okereke BC **(2008)** *International J. Alter. Med*. 6(1):1-8