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Prenylated Benzoquinone and Hydroxylated Benzophenone Glycoside from the Leaf and Stem Bark Extract of *Annona muricata* (Annonaceae)

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Abstract

The EtOAC fraction of the partitioned MeOH portion of the leaf extract of *A. muricata* was subjected to Si-gel column chromatography. Similar fractions where pooled together on the basis of their TLC profile. This was further re chromatographed on silica gel chromatography to afford red crystallized needles as compound 1. The EtOAC from the partitioned MeOH portion of the stem bark extract of *A. muricata*, was also subjected to column chromatography, similar fractions with TLC profiles where pooled together and subsequently subjected to a repeated gel filtration techniques over sephadex L_H 20 to afford compound 2.Their structures were elucidated as2-(1-methoxy-carbonyl-4-6-dihydroxyphenoxy)-3-methoxy-5,6-di-(3-methoxyl-2-butenyl)-1,4-benzoquinone (1) and 1-O-(4''-O-Caffeoyl)-β-glucopyranosyl-4-dihydroxy-2-(3',3'-dimethylallyl) benzene (2) using standard spectroscopic protocols.

Keywords: *Annona muricata*, Prenylated Benzoquinone, Hydroxylated Benzophenone, Leaf, Stem bark, spectral data.

INTRODUCTION

Natural products from plants are a rich resources used for centuries to cure various ailments. The use of plant derived compounds is on the rise, because the main pre occupation with the use of synthetic drugs is the side effects which can be even more dangerous than the disease they claim to cure [1]. In fact plants derived medicines are based upon the premise they contain natural substances that can promote health, alleviate illness and are proven to be safe better patient tolerance, relatively less expensive and globally competitive [2] . The natraceuticals in plants have been a pivotal pipeline for pharmaceutical discovery. The importance of the active ingredient of plants in agriculture and medicine has stimulated significant scientific interest in the biological activities of these substances [3]. Despite these studies, a restricted range of plant species experienced detailed scientific inspection and our knowledge is comparatively insufficient concerning their potential role in nature. Hence, the attainment of a reasonable perception of natural products necessitates comprehensive investigations on the biological activities of these plants and their phytochemical [4].

Annona muricata (Annonaceae). The soursop is a tripti broad leaf flowering, evergreen tree native to central America, the Caribbean and northern south America, Colombia and Brazil, Mexico and Venezuala [5]. This plant is also found to be native to sub- Saharan African countries that lie within the tropics. Other common names it's known with include: guanabana (Spanish), graviola (Portuguese), Brazilian pawpaw and guyabano [6]. The flower has been describe as a combination of strawberry and pineapple with sour citrus flower notes contrasting with an under laying creamy flower reminiscent of coconut or banana. The flesh of the fruit consists of an edible, white pulp and a core of indigestible, black seeds. The species is the only member of its genus that is suitable for processing and preservation. The sweet is used to make juice, as well as candies, sorbets and ice cream flavorings [7]. In folkloric medicine, graviola has been used for the treatment of cancer (all types), as antimicrobial [8], antiparasitic, antitumorous [9] and as a hypertensive agent [10]. Previous chemical studies on this species have reported the isolation of annocatalin, annohexacin, annomonicin, annontacin, annomuricatin, A and B, corrossolone [11], cohibin A thru D, Core poxylone [12] , Coronin, Muracin A thru G, Javaricin [13], Montanacin, Montecristin, Catenol, Muricatetrocin A

and B [14]. The dry ethanolic leaf extract of A. muricata was found to exhibit molluscidal properties against Biomphalaria glabrata [15], while the aqueous leaf extract was found to be used for treatment of diminished or prevented pancreatic oxidative damage produced by Streptozotocin and also showed antioxidant activity [16]. The aqueous extract of A. muricata was also found to exhibit antibacterial effect against Staphylococcus aureus and Vibrio cholera [17]. Muricoreacin, Aurihexocin and Acetogenins obtain from the leaf extract exhibit significant cytotoxicities against six human tumor cell lines with selectivity to the prostate adenocarcinoma (Pc-3) and pancreatic carcinoma (PACA – 2) cell lines [18]. In this paper we reported the isolation and structural elucidation of Prenylated Benzoquinone and Hydroxylated Benzophenone glycoside from the leaf and stem bark extract of A. muricata.

MATERIAL AND METHODS

Plant Material

The leaf and stem bark of A .muricata were collected in August, 2017 in Basawa village outskirt of Zaria, Kaduna State, Nigeria. A voucher specimen (DC. 1902) was deposited at the herbarium of the Biological Science Department Ahmadu Bello University, Zaria, Nigeria.

General Experimental

Optical rotations were measured using a Perkin- Elmer Model 341 LC spectrometer at room temperature. IR spectra were recorded on spectrophotometer shimadzu 8400s. Melting points were determined on XT4A Apparatus and results are uncorrected. ^{1}H NMR and ^{13}C NMR experiment was performed on Bruker spectrometer 500 MHz for ^{1}H and 125 MHz for ^{13}C NMR. NMR spectra were referenced to CD_3OD solvent signals at δ3.30ppm (^{1}H) and 49.00 for (^{13}C) with TMS as an internal standard. Chemical shift value (δ) were reported in part per million (ppm) in relation to the appropriate internal solvent standard (TMS). The coupling constants (J-values) were given in Hertz, HRESI-MS/ FABMS were measured on a mass Autospect-ultima-TOFspectrometer. TLC was carried out on plates precoated with RP-18 gel (merck) and silica gel F254 Qingdao Marine Chemistry Ltd. Spots on the plates were visualized by spraying with 10% H_2SO_4 followed by heating in the oven. Column chromatography was performed on silica gel 60(0.040-0.0653mm) and column (40-63µm, 310mm and 15mm i.d). TLC visualization was done by UV absorption at 254mm. All solvent were distilled before use.

Leaf

Extraction and Isolation

The ground powdered material of the leaf of A.muricata (800g) was exhaustively extracted with MeOH (2x2.0L) using cold maceration technique for 48hrs. The combine extracts were evaporated under reduced pressure to give a brown gummy mass (176g). The gummy mass was subsequently dissolved in H_2O/MeOH (2:1) (600ml) and this was then partition with EtOAc (4x400ml). The EtOAc extract was concentrated under reduced pressure using rotary evaporator to afford a light brownish gummy mass (28g). The extract was then subjected to Si-gel column chromatography and successively eluted with n-hexane, n-hexane/EtOAc (2:1), n-hexane/EtOAc (1:1), n-hexane/EtOAc (1:2) and finally EtOAc (100%) to give $F_{1\text{-}54}$(5ml) fractions. Similar fractions with the same TLC pattern were pooled together to afford 4 subfractions of A=2 – 9, B = 10 – 15, C= 16 – 45, D = 46 - 53. Fraction C (3.8g) was re-chromatographed on a silica gel column chromatography and eluted again with n – hexane/EtOAc (7:3) to afford 30 (10ml) fractions coded as A_1=1 – 12, A_2=16 – 28. Fraction A_2, was further combine and recrystallized with $CHCl_3$/ n – hexane to afford compound 1 (23mg) as red crystallized needles.

Stem bark

Extraction and Isolation

The air dried stem bark powdered material A. muricata [850g] was extracted with methanol [3×2L] for 48 hrs at room temperature and the solvent was recovered under pressure. The methanol extract [162g] was suspended in H_2O (400ml) and partitioned with n-hexane, chloroform and ethylacetate to obtained n-hexane (21g), chloroform (25g) and ethylacetate (33g). The ethylacetate (2.8g) was subjected to column chromatography using Si gel 60-120 [merck] and the column eluted with CH_2Cl_2 / MeOH mixtures and MeOH 100%. The fractions eluted gave eight pooled fractions (1 – 8). Fractionation of the seventh fraction of [1·7g] over silica gel with CH_2Cl_2 /MeOH mixture followed by repeated purification over sephadex LH −20 gave an amorphous reddish brown solid coded as compound 2, (32mg).

RESULTS

The brownish gummy mass of Ethylacetate portion of the leaf extract was subjected to repeated column chromatography to afford a red crystallized needle shape (23mg) coded as compound 1. This was further subjected to infra red spectral analysis exhibiting absorption at 3384 and 1654cm^{-1} for carboxyl and carbonyl functionalities. The ^{13}C NMR spectrum in CD_3OD portrays 25 carbon signals, while signals at δc 165.6ppm and δc 163.4ppm signifying the presence of aromatic moiety in compound 1. The seventh fraction was subjected to repeated purification using LH 20 sephadex to afford a reddish brown amorphous solid coded as compound 2. The FABMS gave m/z 525 $[m+Na]^+$ suggesting the molecular formula of $C_{26}H_{30}O_{10}$ with an intensive fragment at m/z 325 signifying the loss of caffeoyl moiety. The ^{13}C NMR exhibited 26 carbon signals with 11-carbons

corresponding to aglycone, 9-carbons to caffeoyl moiety and 6 to glucopyranose.

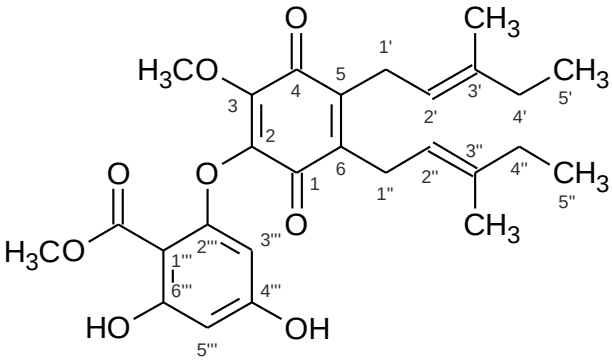

Fig-1: 2-(1-methoxy-carbonyl-4-6-dihydroxyphenoxy)-3-methoxy-5,6-di-(3-methxyl-2-butenyl)-1,4- benzoquinone

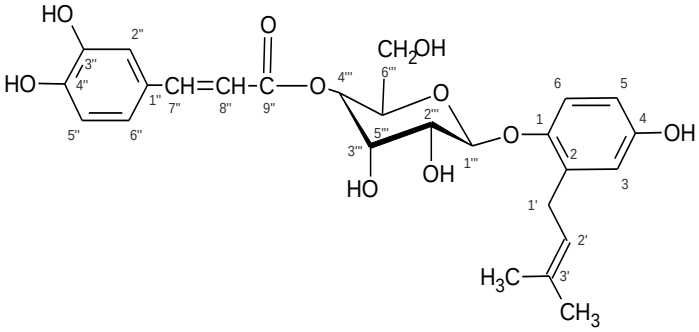

Fig-2: 1-O-(4"-O-Caffeoyl)-β-glucopyranosyl-4-hydroxy-2-(3',3'-dimethylallyl)– benzene

DISCUSSION

Compound 1

The IR spectrum of compound 1 exhibited absorption bands at 3384 and 1654Cm^{-1} for hydroxyl and carbonyl functionalities. The UV spectrum exhibited an absorption band at max 204, 222 and 274nm which were in conformity with the absorptions of 1-4 benzoquinone chromophore [19]. Compound 1 was obtained as red crystals and was assigned the molecular formula $C_{25}H_{28}O_8$ by HRESI-MS.

Table-1: ^{13}C NMR (125MH$_Z$) and 'H NMR (500MH$_Z$) Spectral Data of compound 1 in (CD_3OD)δ in ppm, J in Hz)

Position	δ_C	δ_H
1	183.6[a] (184.2)[b]	-
2	135.8	-
3	147.8[a](148.3)[b]	-
4	182.5 [a] (183.1)[b]	-
5	142.3 [a] (143.7)[b]	-
6	143.1 [a] (141.3)[b]	-
1'	28.6 [a] (26.1)[b]	3.24(2H, brd, J=7.0)
2'	119.7 [a] (120.5)[b]	4.95 (1H, m)
3'	134.6 [a] (134.2)[b]	-
4'	25.8 [a] (26.1)[b]	1.67 (3H, s)
5'	18.3 [a] (18.4)[b]	1.78 (3H,s)
1"	25.6 [a] (26.1)[b]	3.18 (2H,brd, J=7.0)
2"	119.3 [a] (120.4)[b]	4.94(1H,m)
3"	134.3 [a] (135.3)[b]	-
4"	26.2 [a] (25.7)[b]	1.67(3H,s)
5"	18.3 [a] (25.7)[b]	1.70(3H,s)
1"'	97.3 [a] (97.4)[b]	-
2"'	160.1 [a] (161.4)[b]	-
3"'	95.6 [a] (96.6)[b]	5.74(1H,d,J=2.4)
4"'	163.4 [a] (164.2)[b]	5.46
5"'	98.6 [a] (98.5)[b]	6.10(1H,d, j=2.5)
6"'	165.6 [a] (166.3)[b]	-
OCH_3	52.7 [a] (52.7)[b]	3.92(3H,s)
$COOCH_3$	170.5	4.04(3H,s)
OCH_3	61.3	4.04 (3H,s)

The ^{13}C NMR spectrum in (CD_3OD) showed resonances for all the 25 carbons present in compound 1 (Table 1). The carbon signals at δc183.6ppm, δc182.5ppm, and δc170.5ppm, could be attributed to the two P- benzoquinone carbonyls and an ester carbonyl respectively [20]. The ^{13}C- NMR spectrum of compound 1 indicated the presence of three hydroxylated or alkoxylated aromatic carbon signals at δc 160.1ppm, δc 163.6ppm, and δc 165.6ppm. The 'H NMR spectrum and HMQC spectra indicated the presence of two methoxyls from the signals at δ_H4.04ppm (δc 60.3ppm) and δ_H3.92ppm (δc 52.3ppm). The HMBC spectrum further suggested that the former is attached to an olefinic carbon at δc 135.8ppm while the latter is located at a carbonyl carbon atδc 170.5ppm [21, 22]. The 'H NMR and HMQC spectra also exhibited the presence of signals due to two meta-coupled aromatic protons at δ_H6.14ppm /(δc 98.6ppm) and δ_H5.7ppm/(δc95.6ppm).The spectrum further revealed the correlations between C–6‴ (δc165.6ppm), C – 1‴ /(δc97.3ppm) and C – 5‴ /(δc96.6ppm) with the OH - 6‴ proton signal at δ_H11.02ppm while the signals at C – 4‴ /(δc163.4ppm), C – 3‴/(δc 95.6ppm) and C – 5‴/(δc98.6ppm) correlated with the H - 4‴ proton signal at δ_H 5.04ppm, hence suggested the two hydroxyls to be located at C – 4‴ and C – 6‴ respectively[23,24].

In the aromatic region of the spectrum, it was observed that the proton signal at δ_H1.4ppm /(H - 5‴) further exhibited correlations with the carbon signals at δc165.6ppm /(C – 6‴), δc163.4ppm/ (C – 4‴), δc97.3ppm /(C – 1‴), and δc95.4ppm/ (C – 3‴).

The presence of phenyl set of `H and ^{13}C NMR spectral data signals at δ_H3.24ppm/ (δc28.6) δ_H4.95ppm/(δc119.7),(δc134.6),δ_H1.67/(δc25.8),δ_H1.78 ppm/(δc18.3),and δ_H3.18ppm/(δc25.6),δ_H4.95ppm/(δc134.3) δ_H1.67ppm/ (δc26.2) and δ_H1.70ppm/(δc18.3) has unambiguously assisted in the structural determination of compound 1 [25/26]. The HMBC spectrum further showed that, the proton signal at δ_H3.24ppm(H – 1`) correlated with the carbonyl carbon at δc 183.6ppm (C – 1), olefinic carbon signals at δc 143.6ppm(C – 5) with those at δc134.6ppm(C – 1`) and δc119.3ppm(C – 1``). In this respect it was confirmed that the two prenyl units are located or situated adjacent to each other on the 1, 4 – benzoquinone ring of compound 1 [27,28]. The correlations of the methoxy protons resonating at δ_H4.04ppm (3H,s) with a carbon signal at δc135.8ppm(C – 2) was also observed.

Compound 2

Compound 2 gave an ion in FABMS at m/z 525 $[M+Na]^+$, suggesting the molecular formula to be $C_{26}H_{30}O_{10}$. An intensive fragment ion at m/z 325 indicated the loss of Caffeoyl moiety. The UV max (MeOH) displaced absorption at 331, 243, 380, 312, 238 and 234nm while the IR V max cm^{-1} displayed absorption at 3400, 2925, 2858 and1695cm^{-1} respectively [29, 30].The ^1H NMR spectrum showed signal at δ_H 6.58/ H-3, δ_H 6.77ppm/H-5 and δ_H 7.04ppm/H-6 indicating an aromatic protons [31, 32]. The proton signal at δ_H 3.6ppm could be attributed to an oxymethylene proton of the sugar moiety. The ^1H NMR spectrum displayed signal pattern typical of an alkyl hydroquinone [33, 34]. The proton signals at δ_H1.70/H-4' and δ_H 1.72 indicates the presence of methyl groups arising from theprenyl residue [35,16]. An aromatic proton signal at δ_H4.7ppm could be observed on H-1" coupled with two protons of the sugar moiety at δ_H 3..62ppm and δ_H 3.74ppm indicating the oxymethylene protons of a β–glucopyranose residue as observed on Table 2 [36].

Table-2: ^1H NMR (500MHz) and ^{13}C NMR (125 MHz), Spectral data for Compound 2 in (CD_3OD; δ in ppm, J in Hz).

Position	δ_C	δ_H
1	149.6	-
2	134.2	-
3	112.5	6.58(1H, d, j=2.6)
4	154.0	
5	116.4	6.51 (1H, dd, j=3)
6	117.8	7.04 (1H, d, j=8.6)
1'	28.7	3.61 (2H, d, j=7.4)
2'	123.6	5.8 (1H, d, j=7.6)
3'	133.4	
4'	18.4	1.71(3H, s)
5'	26.5	1.74 (3H, s)
1"	103.5	4.82 (1H, d, j=7.5)
2"	74.6	3.41 (2H, m)
3"	75.0	3.43 (2H, m)
4"	71.9	3.30 (1H, m)
5"	76.3	3.56 (1H, m)
6"	61.8	a=3.63(2H,m)b=3.70(2H, m)
1'"	168.3	-

Position	δ_C	δ_H
2'''	117.1	-
3'''	148.0	-
4'''	123.6	-
5'''	114.7	-
6'''	149.8	-
7'''	146.7115.6	-
8'''	115.6	-
9 '''	127.2	-

The ^{13}C NMR spectrum exhibited 26 carbon signals with 11-carbon signals corresponding to aglycone 9-carbon signals to Caffeoyl moiety and 6 to glucopyranose residue (Table 2). The NOESY experiment has confirmed the correlations between H and H-1" .confirming the location of the prenyl moiety with sugar residue. HSQC and HMBC correlation has also allowed the identification of Caffeoyl moiety at C-4" -O substitution of the glucose residue . The C-4" signal of the sugar at δc 71.9ppm was found to be 0.8ppm down field while C-3 and C-5 where found to be shifted up field, Table 2 [28] .

CONCLUSIONS

In conclusion, on the basis of spectral analysis (FTIR, HRESI-MS, ID and 2D NMR) and comparison with ^{1}H NMR and ^{13}C NMR for reference data, compound 1 was determine as 2-(1-methoxy-carbonyl-4-6-dihydroxyphenoxy)-3-methoxy-5,6-di-(3-methxyl-2-butenyl)-1,4- benzoquinone. The spectral analysis (FTIR, FABMS, ID and 2D NMR) of compound 2 and comparison with ^{1}H NMR and ^{13}C NMR for reference data, compound 2 was determine as 1-O-(4"-O-Caffeoyl)-β-glucopyranosyl-4-hydroxy-2-(3',3'-dimethyl allyl) – benzene.

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REFERENCES

1. Soheil Zorofehehian Moghdamtousi, Mehran Fadaeinasab, Sonia Nikzad, Gokula Mohan, Hapipah Mohd Ali and Habsah Abdulkadir (2015).Anona muricata (Anonaceae): A Review of its traditional uses, Isolated Acetogenins and Biological Activites. International journal of Molecular Sciences. 16: pp 15625-15658.
2. Moghadamtousi, S.Z; Kamarudin, M.N.A., Chan C.K, Goh, B.H.andKadir H.A (2014).Phytochemistry and biology of Loranthus parasiticusmerr, a commonly used herbal medicines. Am. J. Chin. Med.42. pp23-35
3. Sofowora, A (2008). Medicinal plants and Traditional Medicine in Africa,Spectrum Books Limited Nigeria. P 10-37
4. A.A Baskar, S. Ignacimuthu, G.Paulrag and K.Numair (2010). BMC Comp. Alternative to med. 10, 24.
5. Watt JM and Breyer – Brandwijk MG (1963). Medicinal and Poisonous plants of Southern and Eastern Africa. E. S Living Stone Edinburg publishers. P. 1046 – 1048.
6. G.H.F. Viera, J.A.Mourao, R. Costa and R. Viera (2010). Rev. Inst. Med. Trop. Saupolo. 52.129-132.
7. Gajalakshmi S., Vijayalakkshmi S. and Rajeshwwari Devi V. (2012). Phytochemical and Pharmacological properties ofAnona muricata: A.Review, International jounal of Pharm Sci. 4(2): 3-6.
8. W.Li, Y. Lu, Q.T Zheng, N.H Tan, C – M. Li, J. Zhao. (2007). J . Model, Structure, 827. 145 – 8.
9. Luna, J. S., et al. (2006) "Acetogenis in Annona muricata L (Annoceae) leaves are potent molluscicides". Nat. Prod.; 20(3): 253 –7 Jaramillo, M.C., et al. "Cytotoxicity and antileishmanial activity of Annona muricata pericarp." Fitoterapia 2000; 71(2): 183 – 6.
10. Consolacion Y. Ragasa, Richard F.Galian and Chien Chang Shen (2014). Chemical Constituents of Anona muricata. Der Pharma Chemica 6(6): 282-387.
11. C.Y Ragasa, G.S Lorena, E.H Mandia, D.D Raga and C.C Shen (2013). American Journal of Essencial oils. Nat. Prod. 1(2, 7-10).
12. C.Y Rigasa, J.L Caro, L.G. Lino, C.C, Shen (2014). J.Pharm.Biol. Chem. Sci. 5(6), 344-248.
13. A.R Tapas, D.M sakarkar and R.B KAKDE (2008). Flavonoids a Nutraceuticals: A Review. Tropical Journal of pharmaceutical Research. 7(3): 1089-1099.
14. S.A Adewole, E.A. (2006.). Caxton-Martins, Afr. J.Biomed. Res. 9, 173-187.
15. A.F, Dos santos and A.E.G Sant Ana (2001). Phytomed. 9 pp. 173-187.
16. Yu L, Huz, Ding H, Maz and Zhao X (2014). Novel prenylated bichalcone and Chalconefrom humulus and their guinone reductose induction activities. Fitoterapia (93) pp 115 –120.
17. Yuan, S.S., et al. 2013 " Annonacin, a mono-tetrahydro furan acetogenin, arrests cancer cells at the G 1 phase and causes cytotoxicity in a Bax and caspase- 3 – related pathway" Life Sci. May; 72(25): 2853-61.

18. 18. Kemp W (1991). Organic Spectroscopy Macmillan Education Ltd. Sound Mills Basing Stole, Hampshire.
19. M. Ilyas, U.U Pateh, A.M. Musa and M.Muhammed (2014). Saponins from the stem bark extract of Stachytarpheta angustifolia mall (Vahl) Verbenaceae 3(2). P 1-10.
20. Zeng, L., et al(2012) "five new mono-tetrahydrofuran ringacetongenins from the leaves of Annona muricata.
21. Bashir H.S Mohammed,A.M., Magsound, A.S and Shaoub, A.M (2013). Isolation of Three flavonoids from Withanza- Halizer Sonimfera leaves (Solanacea4.) and their Antimicrobial Activities.J. Forest product and industries 2(5), 39-45.
22. M. Mohammed*,I. M. Bugaje and M. A. Garba (2015). Three iridoid glycosides from the Root extract of Stachytarpheta angustifolia Mill (Vahl) Verbenaceae. American Research Institute for Policy Development (Journal of Chemistry and Biochemistry). Vol.3. 1.47-62.
23. 23. Hu Yu-rong, Wang Qing Hu, Han Jing – Jing and Bao Baiyingmuquier (2015). Two new Terpenoids from ArtemisiaSacrorumladeb. Journal of Med. Plant Research Vol. (38) 981 – 985.
24. Richard, J. P. C. (1998). Natural Product Isolation. Glaxo Welcome Research and Development Steven age Hertz U. K. Humana press Totowa New Jersey U.S.A. P. 243 – 361.
25. N. Negreira I. Rodriguez, M. Ramil, E. Rubi and R. Cela (2009). Solid – Phase extraction followed by liquid chromatography – Tandem mass spectrometry for the determination of hydroxylated benzophenone U.V absorbers in environmental water samples J. andchimicaActaVol. 654 (2) pp. 162 – 170.
26. Suha Tarazone, Alberto Chisvert, Zacariasleos and Amparo Salvador (2010). Determination of hydroxylated Benzophenone UV filter in sea water samples by disperses liquid – liquid micro extraction followed by gas chromatography – mass spectroscopy J. of chroma A. (05) 47 pp 4771 – 4778.
27. Samarya Krishna and Sarin Renal (2013).Isolation and identification of flavonoids from Cyperusrotundus LINN.Invo and In vitro. J. Drug Delivery and Therapeutics. Vol. 3 (2), 109-113.
28. Zheng – Xiang Xiat, Dan – Dan Zhang, Shuang Liang Yuan – ZhiLaol, Hong Zhang, Hong – Sheng tant, Shi – Lin Chnet, Xin – Hong wangt and Hong – Xi xut (2012). Bioassay – Guided isolation of prenyiatedXanthones and polycylic Acylphloroglucinols from the leaves of Garcinia nujiangensis J. Nat. Prod. 75 (8) pp 1459 – 1464.
29. Robert M.S (1989). Spectroscopic Identification of OrganicCompound. Third End John Wiley and sonInc New York London Sydney Torento. P. 196 - 230
30. Zhi Yong Xiao, Yi Han Zeng, Qing Mu, Winnie Ka Po Shiu, Simon Gibbsons (2010). Prenylated Benzophenone peroxide Derivatives from Hyperium sampsoni J. of Chemistry and BioDiversity 7(4) 953 – 958.
31. Brown G.D (2003). ^{13}C-^{2}H Correlation NMS spectroscopy studies of the In vivo transformation of Natural products from Artemisia annua. J. Phytochem. Res. 5. 45 – 59.
32. Weirong CAL, Xiaohong, Gu and Jian TANG (2010). Extraction, Purification and characterization of the flavonoids from Opuntia milpalta skin.Chech. J. Food Sci. Vol.28 2: 108-116
33. 33. P. Persick, J. Kerschbaumer, S. Helling, B. Hildmann, B. Wibbeling and G. Haufe (2012) Org. Lett. 14(22) 5628 – 5631.
34. Kithsiri Herath, Hiranthi Jayasuriya, John G. Ondeyka, Ziquiang Guan, Robert. Borris Eiristijfhoorn, Dennis Stevenson jianhuwang, Neelam Sharma, Karen MacNaul, John G. Menke, Aisha Ali, Marvin J. Schulman and Sheo B. Singh. (2005). Guttiferone I, a new prenylated Benzophenone from Garciniahumilis as a Liver X Receptor Ligand, J. Nat. Prod. 68 (4), pp 617 – 619.
35. Yao Zhang, Jun – Song Wang, Dan – Dan weit, Yu – Cheng Gu, Xiao – Bing Wang and Ling Kong (2015). Bioactive Terpenoids from the fruits of Aphanamixisgrandifolia J. Nat. Prod. 76 (6) 1191 – 1195.
36. Christophe Gleye, Alain Laurens, Oliver Laprevote, Laurent Sereni and Reynald Hocquemiller (1999).Isolation and structure of Sabadelin, an acetonin, an actonin from roots of thnonamurica, phytochemistry (58) 8.Pp 1403 – 1408.