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Chemical investigation of Benzene extract of *Psidium guajava* (leaves)

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ABSTRACT

Benzene extract was separated by column chromatography using silica gel as adsorbent. The column was eluted by different solvents in their increasing order of polarity. Compounds were isolated subsequently purified by repeated reverse phase, silica gel column chromatography and thin layer chromatography. Benzene fraction of Psidium guajava (leaves) yields three compounds in pure form i.e. 17-methoy-β-sitosterol (1), Cholest-5-ene-3, 25-diol (2), 1-hydroxy-3acetoxy-7-methoxy-2'methyl-5'carboxy-(3-carboxy but-2-enyl)-furano (3', 4'5, 6) - xanthone (3).Their structure were determined by spectroscopic and chemical methods.

Keywords: *Psidium guajava* (leaves), I.R., ^{1}HNMR, ^{13}CNMR, and Mass spectroscopy, structure determination.

INTRODUCTION

Psidium guajava commonly Linn. called as "Amrude" belongs to family Myrtaceae, is a low evergreen shrub or tree 6-25 feet hgh wide spreading branches and square, downy twigs.The tree is common throughout the world specially all worms areas of tropical America, West Indies, Asia, Africa and other subtropical countries including India. In Vedic literature its name has been given as Amrood (Amar and Udar) which means a kind of Amrit in all stomach troubles.

P. guajava a widely distributed plant which has several medicinal uses and showed anti filarial[1] antidiarrheal[2] , CNS depressant,[3] anticough,[4] antiamoebic, antispasmodic[5, 6] and antimicrobial activity.[7] Ground leaves are used as poultice, ripe fruits are good laxative and the stem is good astringent, is recommended for gout. The root bark is successfully employed in diarrhea of

children in the form of concentrated decoction. Decoction of fruits are soaked is good for thrust in diabetes[8-12].

Fruits are able to control glycaemia (by reducing the absorption of glucose in the intestine), hypertensive and hypercholesterolemia (by intake of carbohydrate, fiber, vitamin C, lutein and lycopene). High concentration of pectin in guava fruit may play a significant role in reduction of cholesterol and thereby decreases the risk of cardiovascular diseases[13-14].Decoction of leaves applied in rheutism, epilepsy and cerebral affections.Flowers cools the body, used in bronchitis, applied to sore eyes, dry wounds and cools the heated brain. Decoction of the leaves[15] is an efficacious gargle for swollen gums, ulceration of mouth, scurvy and cholera. (for arresting vomiting and diarrheic symptoms)

Previously β sitosterol,[9] oleanolic acid,[10] quercetin and ellagic acid[11] were isolated, ellagic acid is reported to have astringent and haemostatic property. Psidiolic acid is a mixture of four acids viz oleanolic, ursolic, crategolic (maslinic acid) together with a new acid called "guaijavolic acid" ($C_{30}H_{48}O_4$)[14-15], which is 22-hydroxyursolic acid is responsible for the treatment of throat and chest complaints[25]. Guavanic acid, guavacoumaric acid[16] and psidiumoic acid[17] were also isolated by *P. guajava.*

MATERIALS AND METHODS

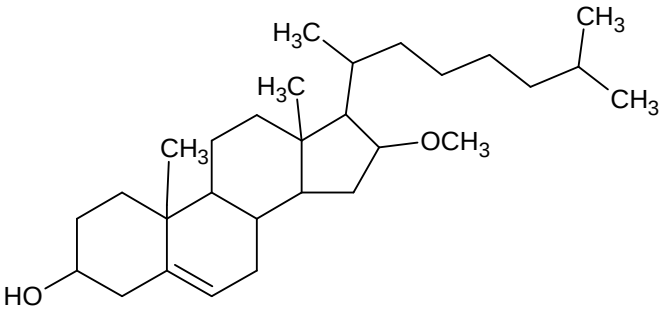
Plant material

The leaves of *P.guajava* were collected from the University campus and were identified by the authorities of Institute of Environment Management and Plant Sciences (IEMPS), Vikram University, Ujjain. A voucher specimen has been deposited atthe Herbarium of Vikram University. The taxonomic identification of the plant material was obtained from the authorities of the Institute of Environment Management and Plant Sciences, Vikram University, Ujjain (M.P.) India.

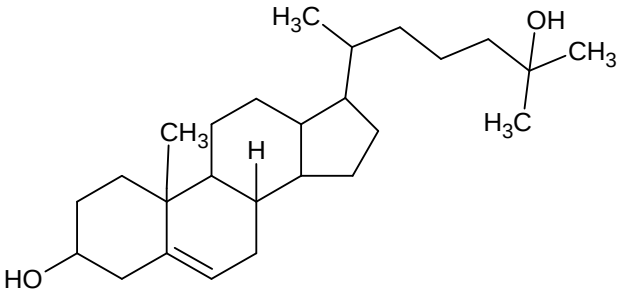
Extraction: The leaves of *P.guajava* were shade dried and crushed with the help of mechanical grinder at room temperature and then extracted in soxhlet extractor with hexane followed by benzene. Solvents were removed in Buchi rotavapour under reduced pressure to yield hexane and benzene extracts. The yield of hexane extract was poor and also work was already been done on hexane extract, we have not processed it further. The yield of benzene extract was found well hence, benzene extract was taken for present study. Benzene extract was qualitatively analyzed by thin layer chromatography using silica gel as adsorbent. The column was eluted by solvents in their increasing order of polarity.

Compound 1: White crystalline solid, M+ 444, mp- 121^{o}C, M.F $C_{30}H_{52}0_2$ IR spectrum showed absorption bands at at 3450 cm^{-1} indicate the presence of hydroxyl group. A broad peak at 1630 cm $^{-1}$ revealed the presence of double bond in a molecule. Bands in the region 2960, 2940, 2870, 1460, 1440 cm $^{-1}$ were due to C-H stretching and bending vibrations ^{1}H NMR showed characteristic signal of steroid molecule two sharp singlets at δ_H 0.80 and δ_H 0.68 each for three protons showing the presence of two angular methyl groups. Another singlet at δ_H 1.10 was assigned to the methyl group at C-14 which is biogenetically possible. A doublet at .82 was δ_H assigned to the methyl group at C-21 position. A sharp singlet at δ_H 3.48 was assigned to the

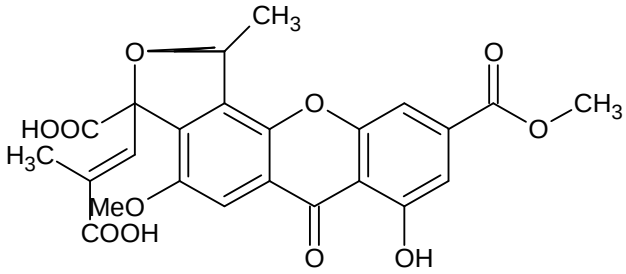
methoxyl group at C-17. The ESIMS showed the molecular ion peak at m/z 444 suggesting its molecular formula as $C_{30}H_{52}O_2$. The peak was observed at m/z 416 which comes from loss of water (M-H20). Other abundant fragments at m/z 323,309,291 and 271 etc.., were in agreement with the proposed structure.Based on the above spectral evidences the compound 1 was characterized as 17 -methoy β sitosterol and being reported for the first time by us.



17 -methoy β sitosterol
(1)



Cholest-5-ene-3, 25-diol
(2)



1-hydroxy-3acetoxy-7-methoxy-2'methyl-5'carboxy-(3-carboxy but-2-enyl)-furano (3',4'5,6)- xanthone
(3)

Compound 2: White amorphous powder, M^+396, mp- 173 ^oC. M.F $C_{27}H_{46}0_2$, I.R. spectrum showed absorption band at 3450 cm $^{-1}$ for the presence of hydroxyl group. A broad peak at 1616 cm $^{-1}$ revealed the presence of double bond in a molecule. Bands in the region 2960, 2940, 2870, 1460, 1440 cm^{-1} were due to C-H stretching and bending vibrations. The other absorption of IR spectrum showed it to be steroidal or terpenoidal molecule[18]. 1H NMR spectrum showed singlet each at δ_H 0.68 and δ_H 1.01 for three protons were assigned to angular methyl groups. The doublet at δ_H 0.93 was due to the presence of methyl group at C-21.Methyl protons of C-26 and

C-27 was resonated at δ_H1.28 as a broad singlet. The deshielding of methyl proton was attributed to the presence of OH group at C-25. A singlet at 3.52 was assigned to methin proton attached to –OH group at C-3 position and doublets at δ_H 1.84 was due to –OH protons. A singlet at 5.35 was due to unsaturation at C-5 in ring B of molecule. The EIMS showed the molecular ion peak at m/z 402 suggesting its molecular formula as $C_{27}H_{46}O_2$.The abundant fragments at 329 (M+-78), 273 (M+-side chain) 231 (M+-side chain + ring D cleavage) and 213 (M+- side chain + ring D cleavage + H_20) indicated the presence of cholestane skeleton. Other abundant fragments at m/z 161, 145 and 81 etc. were in agreement with the proposed structure[19-20].Based on the above spectral evidences the Compound 2 was characterized as Cholest-5-ene-3, 25-diol and being reported for the first time by us from this plant.

Table No-1

Carbon No	^{13}CNMR Value	^{1}NMR Value
1	154.0	3.34,s, OH
2	110.0	7.64 (s)
3	154.0	
4	94.0	
4a	151.0	
4b	141.0	
5	151.0	
6	122.0	
7	153.0	8.01 (d)
7(OCH3)	59.0	4.07 (s)
8	1.09	8.09 (d)
9	177.0	
9(a)	104.0	
10	166.0	
11	22.0	2.24 (s)
12	94.0	4.27 (q)
13	14.2	1.15 (d)
14	91.0	
15	40	3.76, 3.49 (dd)
16	138.0	6.71 (m)
17	134	
18	12.8	1.21 (d)
19	166.0	
20	172	

Compound 3: White amorphous powder, M^+ 498, M.F. $C_{25}H_{42}O_{11,}$ I.R. spectrum showed broad absorption band at 3328 cm^{-1}showed chelated hydroxyl group. The absorption bands at 1652, 1613 and 1581 cm^{-1}suggesting the xanthone skeleton. Presence of band at 1692 cm^{-1} indicated the presence of carbonyl and acetoxy group functions.The ^{1}HNMR Spectrum showed hydrogen bonded hydroxy proton at δ_H 13.31 as singlet was assigned to H-1 proton. Presence of aromatic proton signal at δ_H 8.12 and an O-methyl resonance at δ_H 4.07 as singlet showed the presence of methoxyl group. A singlet for the methyl of acetoxyl moiety was observed at δ_H 2.34 was assigned to be present at H-3 proton. The characteristic signal of 3-carboxy but-2-enyl group was found at δ_H 6.71, 3.76 and 3.49 as a double doublet and 1.21 as doublet was assigned to H-18 protons. The proton showed presence of 2' methyl dihydrofuron ring was showed by the proton resonance at δ_H 4.27 as multiplet (or quartet) and δ_H 1.15 as a doublet confirmed the methylene

and methyl proton of xanthone nucleus was resonated at δH 7.84, 7.39 and 8.09. In the ^{13}CNMR spectra, one hydroxyl substituted at C-1 was showed at δc154.0 ppm. The acetoxyl group was shown at δc 166.0 and 22.0 ppm assigned to C-10 and C-11. The presence of methoxyl was confirmed by δc 153.0 and 59.0 ppm assigned to C-7 position. The characteristic signal at 138.0 and 134.0 ppm for double bond and 166.0 ppm for the carbonyl group confirmed the presence of 3-carboxy-but-2-enyl group. C-12 and C-13 carbon resonance at δc 94.0 and 14.0 confirm the presence of methyl substituted furan moiety. The confirmation of other aromatic signals of xanthone nucleus [21-23] was confirmed using various reference data and are given in table.

Based on the above spectral evidences the Compound 3 was characterized as 1-hydroxy-3acetoxy-7-methoxy-2'methyl-5'carboxy-(3-carboxy but-2-enyl)-furano (3',4'5,6)- xanthone and being reported for the first time by us from this plant.

Experimental

M.P.s are uncorrected. The I.R. spectra were recorded on Perkin-Elmer grating 377 I.R. Spectrometer in KBr phase (range 4000-400 cm-1). ^{1}HNMR spectra were recorded on varian DRX 200 MHz and 400 MHz spectrometer, using TMS as an internal standard and $CDCl_3$ as a Solvent. ^{13}CNMR were recorded 75 MHz spectrometer, using TMS as an internal standard, and Pyridine, as a solvent. TMS In column chromatography alumina Brochmann Gr.III was used. It was made grade III by the addition 7% of distilled water and mixing thoroughly. Silica gel used was purchased from Qualigens, Glaxo and ACME is of 60-120 mesh.

Compound 1: M+ 444**,** $C_{50}H_{86}O_4$. TLC solvent system Benzene: ether (9.5:0.5, v/v) m.p. 121º C Isolated from benzene: ether (9.5/.5 v/v, 19 mg) eluate.

I.R. λ_{max} (KBr): 3425, 2938, 2869, 1621, 1463, 1380, 1055, 1030, 59, 850, 801 cm^{-1}.; ^{1}H NMR (400 MHz, $CDCl_3$, TMS) 0.67 (S, 3H, -CH_3), 1.018 (S, 3H, -CH_3), 0.94 (d, 3H, -CH_3, C-21), 0.82 (d, 6H, 2x-CH_3), 0.84 (t, 3H, -CH_3), C-29), 1.51 (s, 1H, -OH), 3.52 (m, 1H, -CHOH, C-3), 5.35 (bd, 1H, -CH, C-6).; EIMS (m/z, rel, int):M^+444(23.02), 414(63.83), 400 (16.03), 396 (31.44), 382 (20.12), 367 (6.60), 329 (19.50), 315 (8.80), 303 (23.90), 273 (17.61), 255 (26.41), 231 (17.61), 213 (30.18), 199(10.06), 159 (32.70), 145 (39.0), 95 (51.57), 81(58.50), 43 (100).

Compound 2: M+ 402**,** $C_{27}H_{46}O_2$. TLC solvent system Benzene: ether (9.5:0.5, v/v) m.p. 121º C Isolated from Ethyl acetate: benzene (8/2 v/v, 18 mg) eluate.

I.R. λ_{max} (KBr): 3431, 2940, 2852, 1616, 1465, 1379, 1076, 1027, 958, 802 cm^{-1}. ; ^{1}H NMR (400 MHz, $CDCl_3$, TMS δ,): δ 0.65 (s, 3H,-CH_3, C-18), 0.92 (d, 3H, -CH_3, C-21)1.00 s, 3H, 1-CH_3, C-19), 1.3 (s, 6H, 2-CH_3, C- 26 & C-27), 1.48 (s, 1H, OH), 3.8(m, -CHOH), 5.3 (d, 1H, =CH).; EIMS (m/z, rel, int): M^+ 402 (67.0), 392 (20.7), 366 (12.8), 329 (56.7),273 (43.8), 231(43.2),213 (34.5), 161 (22.7), 145 (12.7), 81(97.0),57(100.0), 44(89.0)

Compound 3: M+ 498,$C_{25}H_{42}O_{11,}$ TLC solvent system,EtOAc: CHCl3 (3:2, v/v) M.P.121 ^{o}C Isolated from EtOAc:$CHCl_3$ (6.5/3.5 v/v, 20 mg) eluate.

I.R. λ_{max} (KBr):3328, 1692, 1652, 1613, 1581, 774, 694cm^{-1}. ; ^{1}H NMR (400 MHz, Pyridine, TMS): Table No.1.; ^{13}C NMR **(**75 MHz, Pyridine, TMS): Table No. 1.; EIMS (m/z, rel, int

):M^+502(10.0),498(15.0),465(4.3),414(10.3), 401(3.2), 395(3.6), 364(13.0), 362(3.3), 303(4.7), 264(8.3), 262(33.2),219(25.0),198(5.0),186(20.3),185(4.8),169(6.4),168(4.5),158(3.2),157(3.0), 153(8.7),141(15.9),140(3.2),139(3.3),137(4.1),131 (29.3), 129(5.0),128(4.1),

RESULTS AND DISCUSSION

Compound 1 gave a greenish blue colour with cold Ac_2O and concentrated H_2SO_4. When the compound in $CHCl_3$ treated with Ac_2O and concentrated H_2SO_4 also gave the same result (Libermann-Burchard reaction). Thus Compound 1 to be a steroidal or terpenoidal molecule.

Solution of compound in $CHCl_3$ shaken with conc. H_2SO_4 gave a red colour in the $CHCl_3$ layer, confirming the molecule to be a steroidal or terpenoidal type (Salkowaski reaction). Thus compound 2 to be a steroidal or terpenoidal molecule.

Compound 3, is new, different structure compound isolated by *P. guajava* being isolated first time by us.

CONCLUSION

P. guajava is very useful medicinal plants it's all parts are useful like flower, bark,root and leaves to cure various types diseases so it becomes necessary to isolated the bioactive compounds of the said plant.

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REFERENCES

[1] Temjenmongla, Yadav, A. K. *Proc. Zool. Soc.* **2003**, 56: 57–61.
[2] Lutterodt, G. D. *J. Ethnopharmacol.*, **1989**, 25: 235–47.
[3] Meckes, M., Calzada, F., Tortoriello, Gonzalez, J. L., Martinez, M. *Phytotherapy Res*., **1996**, 10: 600–3.
[4] Jairj, P., Khoohaswan, P., Wongkrajang, Y., Peungvicha, P., Suriyawong, P., Saraya, M. L., et al. *J Ethnopharmacol*., **1999**, 67:203–12.
[5] Lozoya, X., Reyes-Morales, H., Chavez-Soto, M. A., Martinez-Garciam Mde,l C., Soto-Gonzalez, Y., Doubova, S. V. *J. Ethnopharmaco.*, **2002**, 83: 19–24.
[6] Tona, L., Kambu, K., Ngimb, N. *Phytomed.*, **2000**, 7: 31–8.
[7] Qadan, F, Thewaini A.J., Ali, D.A., Afifi, R., Elkhawad, A., Matalka, K.Z. *Am. J. Chin. Med.*, **2005**, 33:197–204.
[8] Nair AGR, Subramanian SS. *Indian J Pharmacy* **1964**, 26: 140-1.
[9] Arthur, H.R. and Hui, W.H., *J. Chem. Soc.*, **1954**, 2782.
[10] Nair, A.G.R. and Subramanian, S.S., *J. Sci. Ind. Res.*, **1962**, 21: 457.
[11] Usan, A. and Gathier, B., *Chem. Abst.*, **1971**, 74:130-358.
[12] Hikino, L., Kiso, Y., Kinouchi, J., Saneda, S. and Shoji, J., *Planta Medica*, **1958**,52:62.
[13] Soliman, G. and Farid, M.K., *J. Chem. Soc.*, **1952**, 134.
[14] Arthur, H.R. and Hui, W.H., *J. Chem. Soc.*, **1954**, 1403.
[15] Osman, A.M., Younes, M. and Sheta, A.E., *Phytochemistry* **1974**, 13:2015- 2016

[16] Begum S.,Imran Hasan S.,Siddiqui Bina S., *Phytochemisty*. **2002**, 61:399-403
[17] Begum S.,Imran Hasan S.,Siddiqui Bina S., *Nat. Prod. Res*., **2007**, 21: 742 - 748.
[18] Ripperger,H and Prozel.,A, *Phytochemistry*,**1992**, 31(2): 725-726.
[19] Wicha, J. et.al, *J. Chem.Commun.*, **1975**, 968.
[20] Jizba, J., Herout, V. and Sorm, F.,*Tetrahed.Lett***, 1967**, 5139.
[21] Yaowapa S, Vatcharin R, Souwalak P. *J Nat Prod*,**2005**,68:1010-1017.
[22] Frederico, G. Cruz, *J. Braz. Chem. Soc.* **2001**, (12), 1: 117-122.
[23] Purev, O., Oyum, Kn. Odentuya, G. Tankhaeva, A.M., Nikolaeva G.G. *Al Z. Natureforsch*, **2002**, 57:331-334.