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Flavonoids from the leaves of *Physalis angulata* Linn

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ABSTRACT

Plants from the genus Physalis are particularly rich in secondary metabolites.*Physalis angulata* is a widely distributed annual plant belonging to the nightshade family Solanaceae.*Physalis angulata* have been used for centuries as medicinal herbs in the treatment of urinary tract infection,skin diseases,gonnorhea,ulcers,sores and as vermicidal drug.The main purpose of this present study is to screen for phytochemical constituents that may be responsible for some of these biological activity. The dried leaves of *Physalis angulata* were sequentially extracted with dichloromethane and 70 %methanol. The aqueous methanol extract was partitioned between water, ethyl acetate and n-butanol. From the n-butanol fraction, three Flavonoids were isolated through repeated column chromatography over silica gel G and sephadex LH-20. Based on the spectroscopic data which include NMR and mass spectroscopy,the chemical structures were determined as quercetin(1),quercetin 3-O-methyl ether (2) and isoquercetrin(3). These compounds were isolated for the first time from the leaves of this plant.

KEYWORDS: *Physalis angulata*, flavonoids,quercetin,quercetin methyl ether and isoquercetrin

INTRODUCTION

The plant kingdom is vast and the antique use of plant to treat various diseases in human beings is not well known. There is considerable number of natural products used in traditional medical systems in many countries as alternative medicine for the treatment of various diseases [1].Many of these plants provide relieve of symptoms comparable to that obtained from allopathic medicines[1].*Physalis angulata* belonging to the night shade family solanaceae is known by several names including: cut leaf ground cherry,wild tomato,camapu and winter cherry. It is a branch annual shrub and is widely distributed throughout tropical and subtropical regions of the world including Nigeria and its extracts and infusions have been used in many countries in popular medicine in the treatment of a variety of diseases such as malaria,dermatitis,asthma,hepatitis and rheumatism [2,3].The methanol extracts of the leaves of *physalis angulata* has been reported to show cytotoxic activity against P-388,KB-16 and A-549 cell lines with ED50 between 2.5-3.93 µg/ml and from this extract a new flavonol glycoside Myrcetin 3-O-neohesperidoside was isolated with ED50 of 0.048 µg/ml on lymphocytic leukaemia P-388 cells [4].Physalins A,B,C and D have been isolated from the leaves of this plant and these compounds were reported to possess antimicrobial and antiparasitic activity [5,6]. The presence of Withanolides A and B was reported by [7].In this present study, we report here in the isolation of three flavonoids from the n-butanol soluble fraction of the aqueous methanol extract of *Physalis angulata* leaves.

MATERIALS AND METHODS

Instrument

The NMR experiments were recorded in CD_3OD and TMS as internal standard on a Bruker Avance DRX 400MHz and 100MHz spectrophotometer. UV was carried out on Jenway 6505 UV-visible spectrophotometer,while IR was performed on Perkin-Elmer FT infra-red spectrophotometer. Mass spectra were recorded on LC-MS ESI-MS positive mode.Thin layer chromatography was performed on pre-coated silica gel plate(0.2mm)Merck (Germany),column chromatography was carried out on silica gel G(230-400

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MESH,Fluka,Switzerland),while gel filteration was performed on sephadex LH-20 Sigma(Switzerland).

Plant material

The leaves of *Physalis angulata* were collected in Agbarha in Ughelli Noth local government area of Delta state in November, 2011 and authenticated at the herbarium unit,Forestry and Wild life Department,University of Portharcourt were a voucher specimen has been deposited.

Preparation of Extract

The air-dried powdered leaves (278 g) was extracted exhaustively at room temperature for seven days with dichoromethane (2.5 L), the combined dichloromethane extract was concentrated using a rotary evaporator to a dark green sticky mass 15.3 g (5.4 %w/w). The dried marc was then extracted with 70 % methanol (2.5 L) using the same process for seven days and the combined extract concentrated to a brown sticky mass 35.5 g (12.7 %w/w) using a rotary evaporator. A portion of the methanol extract (20 g) was suspended in 100ml of water and fractionated with 1 L each of the following solvents with increasing polarity: ethyl acetate and n-butanol (five times 200ml each) the fractions were concentrated to dryness by rotary evaporator to give 1.2 g of ethyl acetate soluble fraction and 4.2 g of n-butanol soluble fraction and the residual water fraction.

Isolation

2.5 g of the n-butanol soluble fraction was packed in a column of (50x2.0 cm) with silica gel (40 g) and eluted gradiently with dichloromethane(100 %) and dichloromethane: methanol mixtures(99:1,98:2,97:3,96:4,95:5,90:10,80:20,60;40,50;50,30:70,10:90 and methanol(100 %) using flash column chromatography,100 ml aliquots were collected and the elution process was monitored on TLC using the solvent system Ethyl acetate:methanol:water(100:16.5:13.5),fractions 15-17 pooled together (0.25 g) eluted with 5 %methanol in dichloromethane and 10 %methanol in dichloromethane which revealed 3 spots on TLC using the above solvent system was subjected to gel filteration over sephadex LH-20 eluting with methanol to give compound 1 a yellow solid (6 mg) and compound 2 a pale yellow amorphous solid (5.2mg). Fractions 19-21 eluted with 20 % methanol in dichloromethane (0.16 g) was similarly purified over sephadex LH-20 eluting with methanol to give 15 fractions. Fractions 10-13 revealed a single spot on TLC using the same solvent system Rf 0.52 to give a yellowish-brown solid (4mg).

RESULTS.

Compound 1 a yellow amorphous solid (6 mg).
UV(MEOH), λmax(nm): 254,and 368
IR(Nujol) cm^{-1}: 1637.3(C=O),3420(O-H).
^{1}H-NMR (CD_3OD) δ(ppm): 6.13,1H,d,(J=2Hz)H-6,6.34,1H,d(J=2Hz) H-8, 6.83,1H,d,(J=8Hz) H-5',7.58,1H,d,d(J=2,8Hz) H-6' and 7.69,1H,d,(J=2Hz) H-2'.
^{13}C-NMR(see table 1).ESI-MS),M/Z= 302(M^+).
Compound 2,a pale yellow solid (5.2mg).
UV(MEOH):λmax(nm):255 and 356
IR(Nujol) cm^{-1}: 1740(C=O), 3370(O-H)
^{1}H-NMR(CD3OD) δ(ppm):6.20,1H,d,(J=2Hz)H-6,6.40,1H,d,(J=2Hz) H-8,6.90,1H,d,(J=8Hz) H-5',7.60,1H,d,d(J=2,8Hz) H-6',7.71,1H,d(J=2Hz) H-2',3.82,3H,s(OCH_3).
^{13}C-NMR (Table 1).
ESI-MS, m/z: 316(M^+), 302(M-OCH_3+H^+)
Compound 3 a yellowish-brown solid (4mg).
UV(MOH) λmax(nm): 257 and 361
IR(Nujol) Cm-^{1}: 1745(C=O),3370(O-H)
^{1}H-NMR(CD_3OD),δ(ppm): 6.19,1H,d(J=2Hz) H-6,6.40,1H,d(J=2Hz) H-8,6.85,1H,d(J=8Hz)H-5',7.57,1H,d,d(J=2, 8Hz) H-6',7.70,1H,d(J=2Hz) H-2',5.20,1H,d(J=7Hz),H-1'',3.2-3.7(5H),glucose unit.
^{13}C-NMR(see table 1).

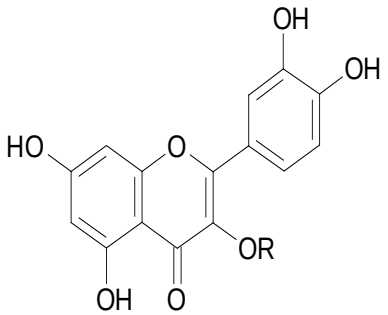

Fig.1 Structure of isolated compounds.

Compound 1 R=H
Compound 2 R=CH_3
Compound 3 R=Glucose.

Table 1 ^{13}C- NMR Spectra (100MHz) of compounds 1-3 in (methanol-d_4)

No. of carbon	compound 1	compound 2	compound 3
2	148.9	157.8	158.1
3	135.8	39.2	135.4
4	175.9	179.4	179.1
5	160.8	163.2	162.7
6	98.3	99.3	99.5
7	164.0	164.7	165.6
8	93.5	94.3	94.2
9	156.2	162.3	156.5
10	103.1	105.8	104.2
1'	122.1	122.3	122.6
2'	115.2	115.7	115.2
3'	145.1	145.2	145.5
4'	147.7	149.0	148.5
5'	115.5	116.2	116.5
6'	120.1	123.0	123.0
1''			04.3
2''			74.3
3''			76.2
4''			70.1
5''			77.5
6''			62.5
OCH_3		59.7	

DISCUSSION

The aqueous methanol extract of *Physalis angulata* was fractionated into ethyl acetate,n-butanol and aqueous layer through solvent fractionation. The column chromatography of the n-butanol fraction over silica gel G and gel filteration over sephadex LH-20 afforded three known flavonoids: compounds 1-3 isolated for the first time from this plant. Structural identification of these compounds were carried out by interpretation of their spectroscopic data and compared with literature.

Compound 1 was obtained as a yellow amorphous powder and gave a molecular ion peak (M^+) at m/z 302. The IR spectrum showed absorbance broad band due to hydroxyl (3420cm^{-1}) and a sharp peak at 1637cm^{-1}) due to ketone functional group. The ^{1}H-NMR spectrum indicated a 5,7-dihydroxylated pattern for ring A (two meta-coupled doublets at δ 6.13 and 6.34 ,J=2Hz) and a 3',4'-dihydroxylation pattern for ring B (ABX system signals at δ =6.83.d,J=8Hz,7.58,d d,J=8.0,2Hz),allowing the aglycone to be recognized as quercetin [8]. The ^{13}C-NMR (table 1) showed fifteen carbon signals of a flavonoid nucleus,the ^{1}H-NMR and ^{13}C-NMR spectral data were in good agreement with that of quercetin [9].This was further confirmed from the MS where the base peak of m/z 302 was observed and this points to a molecular formula $C_{15}H_{10}O_7$ which suggest quercetin. Compound 2 was assigned to be a methoxy derivative of compound 1 by the appearance of a methoxyl protons at δ=3.82,3H,s.The proton NMR showed similar patterns to that of compound 1 except the additional methoxy proton thus suggesting a quercetin nucleus. The ^{13}C-NMR spectra confirm the presence of a methoxy carbon at δ=59.7ppm. The spectral data of compound 2 were in good agreement with the published data for quercetin 3-O-methyl [10]. This was further confirmed from the MS which revealed a molecular ion peak at m/z 316 which points to a molecular formula $C_{16}H_{12}O_7$. Compound 3 was again identical with compound 1 with the exception of a monosaccharide moiety. An anomeric proton signal was observed at δ=5.21 ,1H,d(J=7Hz) and the ^{13}C-NMR spectra revealed the sugar carbons at δ=104.3(1''),74.6(2''),77.5(C-3''),70.5(C-4''),76.8(C-5'') and 62.5(C-6'') suggesting the presence of a β-glucopyranoside unit,the linkage between H-1'' of the sugar unit and C-3 of the quercetin nucleus was evident from the HMBC spectrum, thus compound 3 was found to be quecertin 3-O-β-D-glucopyranoside. The spectral data are in agreement with that reported in the literature [11, 12]. These compounds were isolated for the first time from the leaves of this plant.

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