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Flavonol glycosides with insecticidal activity from methanol extract of *Annona mucosa* Jacq. leaves

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ABSTRACT

Three flavonoids, quercetin 3-*O*-β-D-glucoside (**1**), quercetin 3-*O*-α-D-arabinoside (**2**) and kaempferol 3-*O*-β-D-galactoside (**3**) were isolated from the methanol extract of the leaves of *Annona mucosa* Jacq. Their structures were determined by using physical and spectroscopic methods. The characterized compounds showed promising insecticidal activities against *Sitophilus zeamais* and *Prostephanus truncatus*, insect pests of stored maize.

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1. Introduction

Due to the unpleasant side effects and ineffectiveness of many conventional drugs, the search for new drugs from natural origin has gained momentum in recent years (Mohammadhosseini et al., 2019). Plants have developed secondary metabolites mainly as a defense mechanism against their natural enemies (Wink, 2003). The study of secondary metabolites in plants has led to the discovery of important bioactive molecules of great interest for humankind (Ramesha et al., 2011). A diversity of plants have been established to possess secondary metabolites that have a variety of biological activities. Among the herbal plants, *Veronica cristagailli* and *Veronica persica* which are traditionally used for the treatment of cancer and other diseases related to oxidative stress contain secondary metabolites that have both antioxidant and antiproliferative activities (Mohadjerani and Asadollahi, 2019). *Mimosa caesalpiniifolia* Benth, whose bark is widely used in traditional medicine, as a bleeding stauncher, wound washing, antibacterial and anti-inflammatory agent (Silva et al., 2016) has antileukemic activity (Mororó et al., 2018). Plants in the genus *Ziziphora* whose active ingredients are administered as infusions to address disorders including infections, hemorrhoids, hypertension and gastrointestinal problems (Senejoux et al., 2012), had more than forty natural compounds isolated, most of which are bioactive flavonoids, flavones and their derivatives (Mohammadhosseini, 2017).

Annonaceae is a group of aromatic trees, shrubs and lianas that occur mainly in tropical and subtropical regions (de Lima et al., 2012). It exhibits a pan tropical distribution with 40 genera and 900 species in the Neotropical region (Ribeiro et al., 2016). Despite its diversity, it is one of the lesser phytochemically studied tropical plant families (Ribeiro et al., 2013). *Annonaceae* is one of the best sources of natural compounds with insecticidal properties, especially due to the high diversity of allelochemicals that are synthesized and accumulated in different parts of its herbal plant (Ribeiro et al., 2016). Several species of the genus *Annona* have been traditionally used in the treatment of various ailments and for insect pest control. *Annona muricata* L. leaves are extensively used in the treatment of arthritis and diarrhea (Moghamtousi, 2015), *Annona reticulata* Linn whole plant is used to treat ulcers,

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control of fever and diarrhoea (Pathak and Zaman, 2013). *Annona squamosa* L. leaves, roots and fruits are used to overcome hysteria and fainting, relief for vomiting, sedative to heart, expectorant and spinal disease treatment (Akanksha, 2015), while *Annona mucosa* Jacq. leaves are widely used against fleas and other insects (de Lima et al., 2012). *Annona mucosa* [synonym *Rollinia* mucosa (Jacq.)], (Fig. 1) a medicinal species from the family *Annonaceae*, is a fruit tree that grows well in different habitats though native in Amazon and Atlantic forests (Ferreira et al., 2010). The plant grows to a height ranging from 1 m to 4 m, has brown, hairy twigs and 1 to 3 flowers formed together in the leaf axils. The fruit is conical or heart-shaped and is 15 cm in diameter (Morton, 1987).

Fig. 1. The photograph of *Annona mucosa* trees.

The plant has been shown to have promising impacts on the control of *Plutella xylostella* L. (Souza et al., 2015), *Chrysodeixis includens* (Massarolli et al., 2016) and *Spodoptera frugiperda* (Ribeiro et al., 2016). Previous phytochemical evaluation of various parts of the plant has led to the isolation of a set of flavonoids and flavonol glycosides (Nawwar et al., 2012), some of which possess significant insecticidal properties (Padvamati and Reddy, 1999). This raised the interest in search of compounds in *Annona mucosa* Jacq. with insecticidal properties for control of the stored maize insect pests; *Sitophilus zeamais* and *Prostephanus truncatus*. This search may lead to the development of new insecticidal molecules.

In the current study, the phytochemical screening of *Annona mucosa* Jacq. methanol leaf extracts led to the isolation of three known compounds characterized as quercetin-3-*O*-β-D-glucoside (**1**), quercetin-3-*O*-α-D-arabinoside (**2**) and kaempferol 3-*O*-β-D-galactoside (**3**). However, compounds **1** and **2** are being reported for the first time in this plant. The three isolated and characterized compounds exhibited moderate contact toxicities and antifeedant activities against *S. zeamais* and *P. truncates*, as well.

2. Experimental

2.1. Instrumentation, fine chemicals and solvents

Melting points were determined using Gallenkamp melting point apparatus (Manchester, UK). UV spectra were analyzed using a Shimadzu UV-2401A spectrophotometer (Shimadzu corporation, Kyoto, Japan). IR data were recorded on a Bruker Tensor 27 FTIR spectrophotometer (Bruker Corporation, Bremen, Germany) as KBr pellets. NMR data were measured in DMSO-d_6 on a JOEL NMR instrument operating at 360 and 90 MHz, respectively. The mass spectral data were obtained using a Varian MAT 8200A instrument. Silica gel 60 (63-200 µm, Merck, Darmstadt, Germany) was used for gravity column chromatography (CC). TLC was performed on precoated DC Alufolien 60 F254 sheets (Merck, Darmstadt, Germany) and detected by exposure to UV light, iodine vapour and concentrated ammonia vapor. Paper chromatography was performed on standard Whatman No. 1 chromatography paper. Test reagents were prepared according to the method of Mabry et al (1970). Solvents used were of analytical grade.

2.2. Plant material collection and identification

The leaves of *Annona mucosa* (Jacq.) were collected

from Kitale, Tranzoia County (Lat: 131'0"S and Long: 37^0 16'0"E), Kenya, where the plant grows naturally. The plant was identified by Mr. Mutiso of Botany Department, University of Nairobi, Kenya where voucher specimens are deposited (Reference No.MU/EA/2015).

2.3. Plant material preparation and solvent extraction

The air dried and pulverized leaves (2.5 kg) of *A. mucosa* were sequentially soaked in *n*-hexane (3 x 4.5 L), CH_2Cl_2 (3 x 4.5 L) and MeOH (3 x 4.5 L), each lasting two days at room temperature. The extracts were separately filtered and evaporated under vacuum using a rotary evaporator to afford green (23.0 g), dark green (45.0 g) and brownish-green extracts (126.0 g), respectively.

2.4. Isolation of phyto-constituents of MeOH leaf extract of *Annona mucosa* Jacq.

A portion of the methanol extract (40.0 g) was mixed with 20.0 g of silica gel (200-400 mesh), dried and then subjected to column chromatography [6.0 x 80.0 cm; SiO_2 300.0 g (200-400 mesh) (2.0% oxalic acid deactivated); pressure ≈1 bar]. Elution was started with EtOAc followed by CH_2Cl_2-MeOH mixture with increasing concentration of the more polar solvent (increment 10%) and concluded with pure MeOH. A total of 150 fractions, each 100.0 mL were sampled and their homogeneity determined by TLC [eluent: CH_2Cl_2-MeOH, 95:5, 4:1 and 1:1; *n*-BuOH-HOAc-H_2O (BAW), 4:1:5]. Those fractions exhibiting similar TLC profiles were combined into three major pools **I**-**III**. Pool **I** (fractions 20-35, 4.0 g) eluted with EtOAc gave non flavonoid compounds and therefore was not considered. Fractions 38-79 (Pool **II**, 4.3 g) afforded one major spot with R_f value 0.37 (solvent system: CH_2Cl_2-MeOH, 1:1). However, this pool was contaminated with impurities. Therefore, it was further purified by repeated crystallization using aqueous MeOH: 5% to give compound **3** (80.5 mg). Fractions 84-135 (≈8.0 g) that were eluted using CH_2Cl_2-MeOH (1:1) followed by pure MeOH constituted pool **III**. This pool was further repeatedly purified by low pressure column chromatography over a column (4.0 x 80.0 cm, SiO_2 160.0 g, pressure ≈ 1.5 bar) using solvent system CH_2Cl_2-MeOH (1:1) to give compound **2** (Rf =0.34, 44.0 mg with solvent system: *n*-BuOH-HOAc-H_2O, 4:1:5) and compound **1** (R_f = 0.21, 30.3 mg with solvent system: *n*-BuOH-HOAc-H_2O, 4:1:5).

2.5. Acid hydrolysis of compounds **1**-**3**

Hydrochloric acid solutions (2.0%) containing 10.0 mg each of compounds **1**-**3** were separately heated under reflux for 2 hrs and thereafter the solvent was removed under vacuum. The residues were separately dissolved in MeOH (10.0 mL) and neutralized with a drop of NaOH solution. The resulting solutions were extracted with acetone and the excess solvent removed under vacuum. The residues were then separately crystallized in aqueous MeOH (5.0%). A comparative analysis on 2% oxalic acid deactivated silica gel TLC (solvent system: CH_2Cl_2-MeOH, 4:1) of the aglycones with authentic quercetin and kaempferol afforded R_f values of 0.46 and 0.50, respectively. Similarly, the aqueous residues when compared with authentic samples of glucose, arabinose and galactose on TLC using solvent system consisting of EtOAc-MeOH-H_2O-HOAc (6:2:1:1) gave R_f values of 0.50, 0.60 and 0.53, respectively. This was after spraying the TLC plate with aniline hydrogen phthalate followed by heating on hot plate for one minute.

2.6. Physical and spectral data of compounds **1**-**3** isolated from the methanol extract of Annona mucosa Jacq. leaves

2.6.1. Quercetin 3-*O*-β-D-glucoside (**1**):

A pale yellow amorphous powder with R_f = 0.21 (solvent system: BAW, 4:1:5), mp > 250 °C; UV λ_{max} (MeOH) nm: 358 (band I), 302, 258 (band II); (MeOH + $AlCl_3$): 434 (band I), 312, 274 (band II); (MeOH + $AlCl_3$/HCl): 400 (band 1), 360, 300, 270 (band II); (MeOH +NaOMe): 410 (band I), 328, 272 (band II); (MeOH + NaOAc): 382 (band I) , 322, 274 (band II); (MeOH + NaOAc/H_3BO_3): 378 (band I) , 262 (band II) IR υ_{max} (KBr) cm^{-1}: 3385.5, 3019.4, 1631.1, 1565.4, 1215.3; ^1H and ^{13}C (DMSO-d6) δ ppm: see Tables 1 and 2; EI-MS (70 ev): *m/z* (rel. Int.): 303.2 (100), 289 (11), 245 (2), 216 (8) 153 (10), 136 (15), 108 (3).

2.6.2. Quercetin 3-*O*-α-D-arabinoside (**2**):

Amorphous yellow powder, R_f = 0.34 (solvent system: BAW, 4:1:5); mp > 250 °C; UV λ_{max} (MeOH) nm: 352 (band I), 302, 258 (band II); (MeOH +$AlCl_3$): 438 (band I), 316, 270 (band II); (MeOH+$AlCl_3$/HCl: 400 (band I), 310, 272 (band II); (MeOH + NaOMe): 396 (band I), 266 (band II); (MeOH + NaOAc): 376 (band I), 318, 272 (band II); (MeOH + NaOAc/H_3BO_3): 374 (band I), 300, 260 (band II): IR υ_{max} (KBr) cm^{-1}, 3888.4, 2946.8, 1720.9, 1630.9, 1567.0, 1457.7, 1376.3, 1255.2, 1215.9, 1181.7, 1033.5, 986.9, 910.1; ^1H and ^{13}C NMR (DMSO-d_6) δ ppm: see Tables 1 and 2; EI-MS (70ev): *m/z* 301 (*rel. Int.*): (100), 289 (11), 245 (2), 216 (8) 153 (10), 136 (15), 108 (3).

2.6.3. Kaempferol 3-*O*-β-galactoside (**3**):

was isolated as a yellow amorphous powder, mp > 250 °C; UV λ_{max} (MeOH) nm: 354 (band I), 302, 262 (band II); (MeOH +$AlCl_3$) nm: 406 (band I), 304, 272 (band II); (MeOH + AlCl3/HCl) nm: 400 (band I), 352, 300, 272 (band II); (MeOH + NaOMe) nm: 396 (band I), 324, 272 (band II); (MeOH + NaOAc) nm: 366 (band I), 272 (band II); (MeOH + NaOAc/H_3BO_3) nm: 362 (band I), 264 (band II); ^1H NMR (DMSO-d_6) δ ppm: see Tables 1 and 2; EIMS (70 ev):*m/z* (rel. int) 287.6 [M+H]+ (100), 253.6 (23), 244.8 (25).

2.7. Contact toxicity assay

This experiment tested the hypothesis that topically

applied pure isolate solutions exhibit contact toxicity versus *S. zeamais* and *P. truncatus*.

The contact toxicity of *A. mucosa* Jacq leaf pure isolates were monitored according to a known method (Tapondjou et al., 2005) by topical application using 3rd instar larvae. The pure isolate solutions were prepared immediately prior to the assays by dissolving in acetone to obtain solutions of concentrations 10 µg/mL, 30 µg/mL, 50 µg/mL and 100 µg/mL. The experiment was carried out in three replicates and for each replicate, 10 larvae were transferred onto a Whatman No. 1 filter paper disc in a 90 mm disposable Petri dish. The larvae were each treated topically with a 0.5 µL droplet of each solution, applied onto the pronotum, using a Hamilton's syringe (700 series, Microliter TM Hamilton Company, USA). In the control treatment, larvae were treated with 0.5 µL of acetone and 0.5 µL of deltamethrine. After topical application, the insects were confined in Petri dishes within metal rings. They were then provided with 5 corn kernels and maintained at 26 ± 2 °C and 60 ± 5% relative humidity on a 16:8 (L:D) photocycle for 48 h, after which mortality was assessed. Insects that were treated with acetone and deltamethrine served as negative and positive controls, respectively. The percentage mortalities were subjected to analysis of variance (ANOVA). The lethal concentration 50 (LC_{50}) values, the confidence upper and lower limits, regression equations and chi-square (χ^2) values were calculated using probit analysis (Finney, 1971).

2.8. Feeding deterrence assay

The activities of the compounds were studied using leaf disc no choice bioassay method (Arrivoli and Tennyson, 2013). This experiment tested the hypothesis that larval feeding is deterred by compounds incorporated in fresh maize flour leaf discs (1350 sq.mm). The compounds were tested at concentrations of 10, 20, 50 and 100 µg/mL. For the negative control treatment, 100 µL of HPLC grade acetone was added to the diet, while for the positive control 100 µL of azadirachtin was added. Each concentration treatment combination was tested individually versus the control. The larvae were inserted into Petri dishes individually on a piece of Whatman No. 1 filter paper (1 x 1 cm) and placed centrally on portions of both treated and control diet pieces. The larvae were presented with flour leaf discs treated with the respective compounds versus an equal amount of acetone solvent treated (negative control) and azadirachtin treated (positive control) diet in the 50 mm round Petri dishes. Larvae were incubated at 25 ± 2 ºC for 24 h and on a 16:8 (L:D) photocycle. The insects were allowed to be fed on treated and untreated flour discs for twenty-four hours. At the end of the experiment, unconsumed area of leaf disc was measured with the aid of a leaf area meter. Percent antifeedant activity (Eqn. 1) was calculated (Singh and Pant, 1980) and data subjected to analysis of variance. Each experiment was repeated three times.

$$\text{Antifeedant activity (\%)} = \frac{\text{Leaf disc consumed by the insects in control-leaf disc consumed by the insect treated}}{\text{Leaf disc consumed by the insect in control + leaf disc consumed by the insect in treated}} \times 100 \quad \text{(Eqn. 1)}$$

The antifeedant index 50 (AFI_{50}), the confidence upper and lower limits, regression equations and chi-square (χ^2) values were calculated by subjecting the data to probit analysis (Finney, 1971).

2.9. Data analysis

For the initial screening bioassay, data were corrected for mortality in the controls using Abbott's formula (Abbot, 1926) and then normalized using an arcsine transformation. Transformed data were submitted to a randomized complete block analysis of variance (ANOVA) ($p < 0.05$) and differences between treatments were compared using Tukey's test ($p < 0.05$).

3. Results and Discussion

3.1. Structural elucidation of compounds **1**-**3**

Compound **1** was isolated as greenish-yellow amorphous powder with mp > 250 °C. It appeared as deep purple on paper chromatography under UV light and turned yellow with conc. ammonia vapour suggesting that the compound is a flavonoid derivative (Mabry et al., 1970). The compound showed a green color when reacted with aqueous ferric chloride indicating the presence of 5-hydroxyl group (Wolbis and Krolikowa, 1988) and this was supported by its UV spectrum (Suppl 1) which exhibited a bathochromic shift of 42 nm in band I with shift reagent $AlCl_3$/HCl (Mabry et al., 1970). The compound dissolved in aqueous NaOAc suggesting the presence of free hydroxyl groups at C-7 and C-4' positions (Wolbis and Krolikowa, 1988). The presence of 7-hydroxyl group was further supported by a bathochromic shift of 16 nm in band II with NaOAc relative to the methanol spectrum, while the presence of 4'-OH group was substantiated by bathochromic shifts of 52 nm and 20 nm in band I with NaOMe and NaOAc/H_3BO_3, respectively (Mabry et al., 1970). The rapid decomposition of the compound in NaOMe could be attributable to the alkali sensitive 3',4',5-hydroxylation pattern in the compound (Howard and Mabry, 1970). In fact, the foregoing evidences suggested glycosylation at C-3 position (Manguro, 1994). The IR spectrum (Suppl 2) of compound **1** gave absorption bands due to hydroxyl groups (3888.4 cm^{-1}) and an α, β-unsaturated ketone (1631.1, 1565.4 cm^{-1}). Acid hydrolysis (2% HCl) yielded quercetin and glucose confirmed by TLC co-chromatography with authentic samples. Quercetin structure was further confirmed by mass spectrum (70 ev) (Suppl 3) which showed a peak at *m/z* 303.2 $[M\text{-}C_6H_{12}O_6+H]^+$. The downfield part of the ^1H NMR spectrum (Table 1, Supp 4, obtained in DMSO-d_6)

showed the characteristic aglycone pattern of quercetin derivative; namely 5-OH group which displayed a peak at δ 12.64. In addition, in the spectrum, a 3H ABX systems was observed with peaks being exhibited at δ 7.59 (d, *J* = 2.0 Hz), 7.52 (dd, *J* = 9.0, 2.0 Hz) and 6.86 (d, *J* = 8.5 Hz) attributable to H-2'', H-6" and H-5", respectively. Together with these, there were meta-coupled doublets at δH 6.41 (d, *J* = 2.1 Hz) and 6.20 (d, *J* = 1.8 Hz) which represented H-8 and H-6, respectively. The anomeric proton signal appeared at δ 5.48 as a doublet with coupling constant *J* = 7.1 Hz which is in accordance with diaxial coupling between the proton on C-1" and C-2" in a β-linked D-glucopyranoside (Markham, 1982; Anderson et al, 1991). Therefore, from physical, chemical and spectroscopic data, compound **1** was concluded to be quercetin 3-*O*-β-D-glucoside.

Compound **2**, from the methanol leaf extract, afforded an R_f value of 0.34 in BAW (4:1:5) and 0.20 in HOAc). It was isolated as a yellow amorphous powder being crystallized from aqueous MeOH (5%). Its melting point determined with Gallenkemp melting apparatus gave an m.p. Value > 250 °C. The compound on exposure to conc. ammonia vapor appeared on TLC (both cellulose and silica gel) as dark UV absorbing spots suggesting a flavonol with substituted 3- hydroxyl and free 5-hydroxyl groups (Markham, 1982). The compound exhibited two major UV absorption bands MeOH: band I at 352 nm and band II at 258 nm (Suppl. 5), which suggested a flavonol nucleus with substituted 3-hydroxy group (Markham et al., 1970). Addition of shift reagent NaOAc/H_3BO_3 caused a bathochromic shift of 22 nm (band 1) relative to the spectrum in methanol, which indicated the presence of ortho-dihydroxyl groups in the B-ring. This was in turn supported by the absorption spectrum in NaOMe/MeOH which gave a bathochromic shift of 42 nm which rapidly decreased in intensity confirming the presence of free 3'.4'-dihydroxyl groups. Similarly, a bathochromic shift of 14 nm (band II) was noted in the presence of NaOAc/MeOH relative to MeOH indicated flavonols with free 7-OH, hydroxyl group (Markham, 1982), whereas the presence of free 5-OH group was ascertained by a bathochromic shift of 42 nm in band I obtained with $AlCl_3$/HCl relative to MeOH (Mabry et al., 1970). Thus, the UV data of compound **2** suggested that this compound is a flavonol with free hydroxyl groups at C-7, C-5, C-4' and C-3'. The presence of free C-5 OH group was confirmed by a singlet at δH 12.45 (Suppl. 6). The ^{1}H and ^{13}C NMR data (Tables 1 and 2) of the aglycone were similar with those of quercetin 3-*O*-β-D-glucoside (1), implying that this compound is quercetin derivative. This was confirmed by acid hydrolysis of the compound (2% HCl, reflux for 2 h) which released the aglycone quercetin and a sugar moiety identified as α-arabinose after comparison with standard samples of arabinose, glucose and galactose using silica gel TLC in a solvent system consisting of EtOAc-MeOH-H_2O-HOAc (6:2:1:1). The ^{13}C NMR spectrum (Suppl. 7) corroborated the acid hydrolysis and resulted by exhibiting the presence of 20 carbons in the molecule of which five carbon signals in the glycosidic region corresponded to a pentose moiety and the remaining 15 carbons were due to the aglycone. The assignment of all the carbon signals due to the aglycone was done by comparison with reported data for quercetin 3-*O*-α-arabinoside (Park et al., 2015). The aromatic proton signal of the sugar moiety appeared as doublet at δH 5.29 (*J* = 5.1 Hz) with corresponding ^{13}C NMR peak at 101.92. The coupling constant of *J* = 5.1 Hz signified axial-equatorial interaction which is characteristic of α-sugar conformers (Arima and Danno, 2002). The position of attachment of arabinose on the aglycone was suggested to be at C-3 due to a chemical shift value of the aglycone at δ 134.04, a fact that was further supported by HMBC correlation (Suppl. 8) between the anomeric hydrogen (δ 5.29, *J* = 5.1 Hz, H-1") and C-3. In the mass spectrum (70 eV) (Suppl 9), a peak at *m/z* 302 is consistent with the quercetin aglycone $C_{15}H_{10}O_7$ indicating the loss of arabinose unit from the molecule. Thus, on the basis of accumulated evidences compound **2** was established as quercetin 3-*O*-β-D-arabinoside.

Kaempferol 3-*O*-β-galactoside (**3**) was isolated as a yellow amorphous powder with R_f = 0.37 (solvent system: BAW, 4:1:5). The UV spectrum of this compound in MeOH showed absorption maxima at 354 nm (band I) and 262 nm (band II) (Suppl. 9) suggesting substituted hydroxyl group at C-3 (Mabry et al., 1970). The bathochromic shift of band I with $AlCl_3$/HCl (52 nm) is a typical feature of non-transformed hydroxyl group at C-5, whereas the bathochromic shift of band II (10 nm) observed with NaOAc indicated the presence of unsubstituted hydroxyl group at C-7 (Mabry et al., 1970; Manguro, 1994). The foregoing evidences were supported by the ^{1}H NMR spectrum (Table 1, Suppl. 10) which exhibited two meta-coupled protons at δ 6.41 (d, *J* = 2.1Hz) and 6.20 (d, *J* = 1.8 Hz) assignable to H-8 and H-6, respectively. On the other hand, the non-degeneration of the NaOMe UV spectrum with time suggested the absence of the 3'-OH group in the molecule (Mabry et al., 1970), a fact substantiated the ^{1}H NMR two doublet AB peaks at δ 7.57 (*J* = 8.5 Hz) and 6.88 (*J* = 9.0 Hz) attributable to C-2', C-6' and C-3', C-5", respectively (Omar et al., 2015). On hydrolysis in an acidic medium (2% HCl), the compound yielded kaempferol and galactose. The identity of kaempferol as aglycone was confirmed by comparison of its TLC, UV and ^{1}H NMR with authentic sample, while galactose was identified by comparison on TLC (solvent system: EtOAc-MeOH-H_2O-HOAc, 6:2:1:1) with authentic galactose, confirmed by ESI-MS $[M+H]^+$ 449.4 representing $C_{21}H_{20}O_{11}$. In the sugar region, the ^{1}H NMR spectrum displayed only one resolved doublet at δ 5.48 (*J* = 7.2 Hz) assigned to anomeric proton H-1". Thus, on the basis of physical, chemical and spectroscopic data, compound **3** was confirmed to be kaempferol 3-*O*-β-D-galactoside.

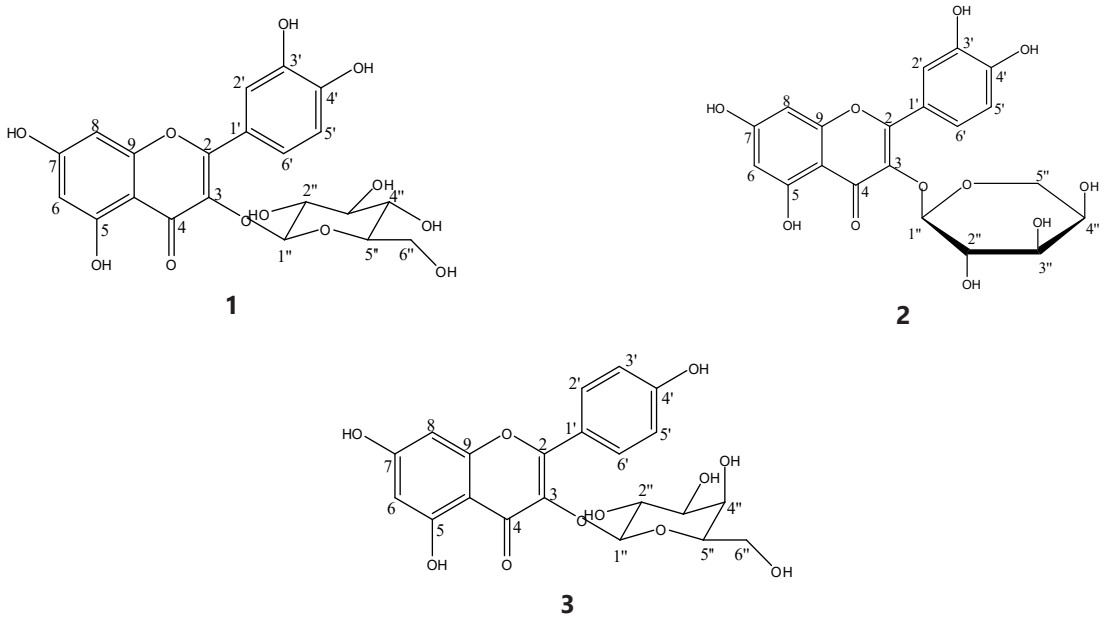

Fig. 2. Compounds 1-3 from MeOH extract of *Annona mucosa* leaves.

Table 1
^{1}H NMR (DMSO-d_6) of compounds **1**-**3**.

C #	Compound 1	Compound 2	Compound 3
2			
3			
4			
5			
6	6.20 (d, *J* = 1.8 Hz)	6.18 (d, *J* = 1.4 Hz)	6.20 (d, *J* = 1.8 Hz)
7			
8	6.41 (d, *J* = 2.0 Hz)	6.40 9d, *J* = 1.8 Hz)	6.39 (d, *J* = 2.1 Hz)
9			
10			
1′			
2′	7.59 (d, *J* = 2.0 Hz)	8.05 (d, *J* = 2.4 Hz)	7.57 (d, *J* = 8.5 Hz)
3′			6.88 (d, *J* = 9.0 Hz)
4′			
5′	6.85 (dd, *J* = 9.0, 2.0 Hz)	6.88 (d, *J* = 9.18 Hz)	6.88 (d, *J* = 9.0 Hz)
6′	7.57 (d, *J* = 8.5 Hz)	7.60 (dd, *J* = 8.46, 2,3 Hz)	7.57 (d, *J* = 9.0 Hz)
1″	5.48 (d, *J* = 7.1 Hz)	5.29 (d, *J* = 5.1 Hz)	5.48 (d, *J* = 7.2 Hz)
2″	4.50-2.95 m, overlapping	3.78-3.40 m, overlapping	4.60-3.00 m, overlapping
3″	4.50-2.95 m, overlapping	3.78-3.40 m, overlapping	4.60-3.00 m, overlapping
4″	4.50-2.95 m, overlapping	3.78-3.40 m, overlapping	4.60-3.00 m, overlapping
5″	4.50-2.95 m, overlapping	3.78-3.40 m, overlapping	4.60-3.00 m, overlapping
6″	4.50-2.95 m, overlapping		4.60-3.00 m, overlapping

Table 2
^{13}C NMR (DMSO-d_6) of compounds **1**-**3**.

C#	Compound 1	Compound 2	Compound 3
2	158.70	156.66	161.82
3	136.00	134.04	134.80
4	179.40	177.85	178.20
5	163.00	161.53	164.50
6	99.90	99.05	98.42
7	166.00	164.47	164.50
8	94.70	93.93	93.34
9	158.40	156.66	157.14
10	104.60	104.30	104.51
1′	121.70	122.20	121.21
2′	109.96	116.26	130.50
3′	146.40	145.20	115.11
4′	136.10	148.86	160.18
5′	146.40	115.72	115.11
6′	110.00	121.30	130.50
1′′	105.50	101.92	103.43
2′′	73.30	66.71	76.41
3′′	75.10	72.15	75.15
4′′	70.00	71.15	71.04
5′′	77.30	65.01	79.76
6′′	61.90		63.09

3.2. Insecticidal activities

3.2.1. Contact toxicity

When the compounds were tested against *S. zeamais* and *P. truncatus* for their contact toxicities, compounds **1**, **2** and **3** showed promising activities against the two post-harvest insects. The activities were concentration-dependent, increasing with increase in concentration. Quercetin 3-*O*-β-D-glucoside (**1**) had the highest

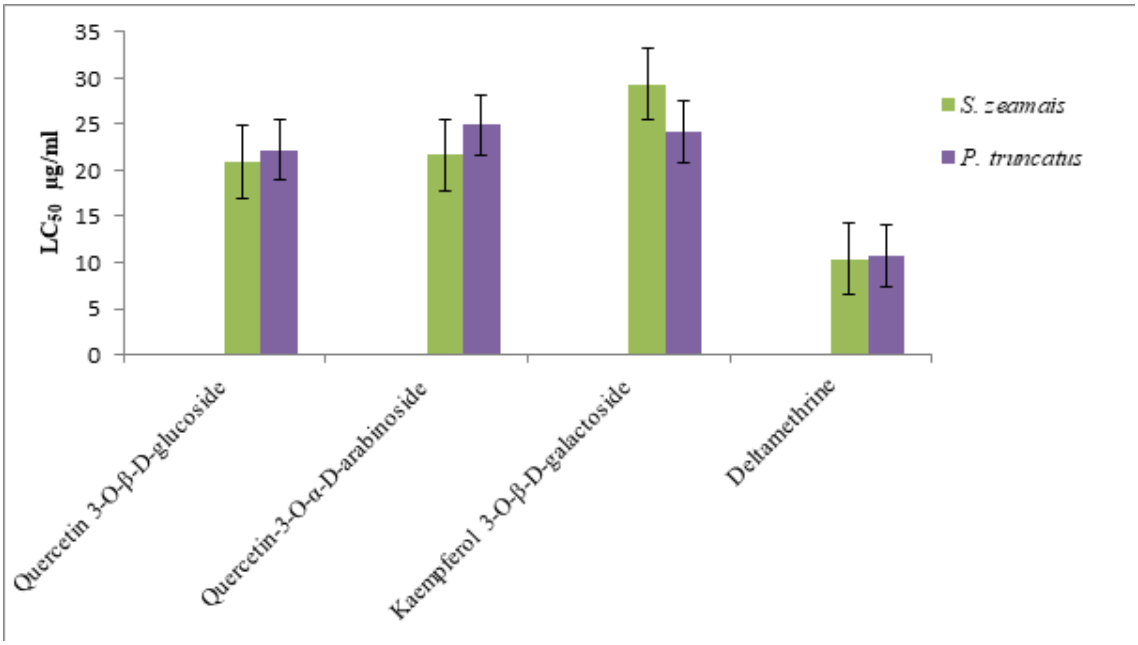

Fig. 3. Contact toxicity activity of *A. mucosa* compounds against *S. zeamais* and *P. truncatus*.

activity against both *S. zeamais* and *P. truncatus* at LC_{50} of 20.9 µg/mL and 22.2 µg/mL, respectively (Fig. 3). This was however lower than the activity of deltamethrine, a commercial insecticide used as a positive control, with LC_{50} values of 10.4 µg/mL and 10.7 µg/mL against *S. zeamais* and *P. truncates*, respectively. Compounds **2** and **3** also exhibited fairly high toxicities with LC_{50} values of 21.7 µg/mL and 24.9 µg/mL for compound **2** and 29.3 µg/mL and 24.2 µg/mL for compound **3** when being tested against *S. zeamais* and *P. truncatus*, respectively.

3.2.2. Antifeedant activity

Compounds **1**, **2** and **3** exhibited interesting antifeedant activities against *S. zeamais* and *P. truncatus*. The activities were concentration-dependent, increasing with increase in concentration. Quercetin-3-*O*-β-D-glucoside (**1**) showed the highest activities with AFI_{50} of 14.9 µg/mL and 16.8 µg/mL against *S. zeamais* and *P. truncates*, respectively (Fig. 4). This activity was lower than the activity of azadirachtin, a commercial antifeedant that had an activity of AFI_{50} =12.2 µg/mL and AFI_{50} =12.4 µg/mL against *S. zeamais* and *P. truncatus*, respectively. Compounds **2** and **3** also exhibited relatively high antifeedant activities with AFI_{50} values of 17.3 µg/mL and 20.0 µg/mL for compound **2** as well as 21.6 µg/mL and 20.2 µg/mL for compound **3** when tested against *S. zeamais* and *P. truncatus*, respectively. Flavonoids such as quercetin-3-*O*-β-D-glucoside, quercetin-3-*O*-α-D-arabinoside and kaempferol-3-O-β-D-galactoside are known to have high antifeedant activities (Medeiros et al., 1994). This may be due to the presence of a keto group at C-4 in the pyran ring, a hydroxyl group at C-5, C-7 and a large number of hydroxyl groups in the structure (Medeiros et al., 1994; Nascimento et al., 2013).

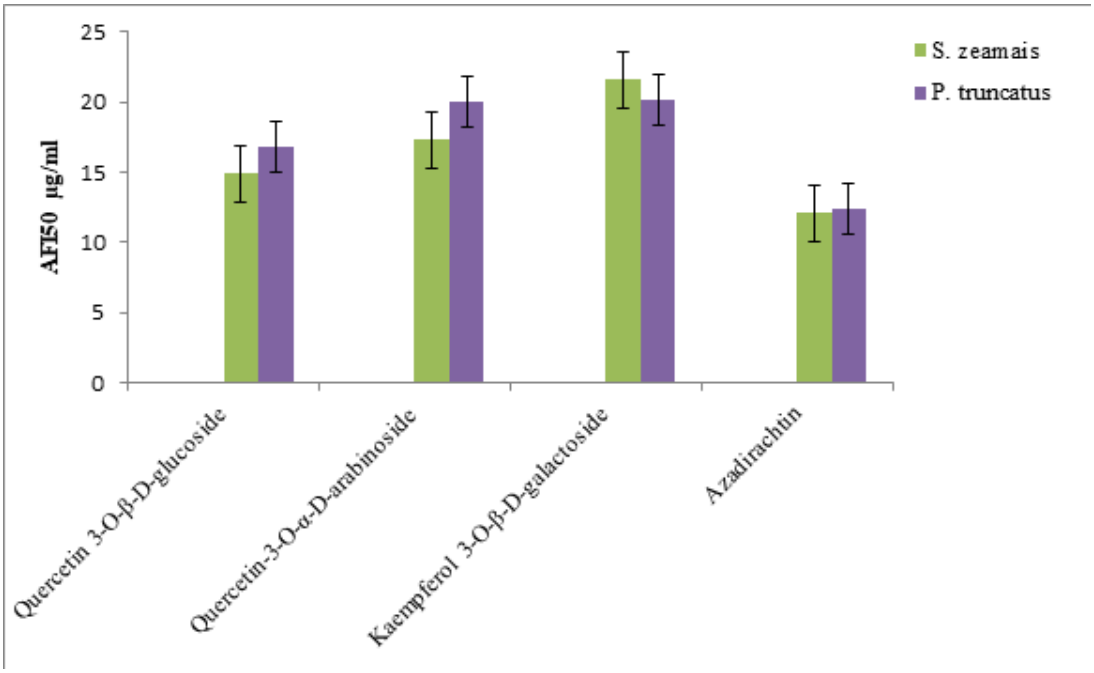

Fig. 4. Antifeedant activity of *A. mucosa* pure isolates against *S. zeamais* and *P. truncates*.

4. Concluding remarks

The current investigation evaluated for the first time the insecticidal potential of *Annona mucosa* Jacq. leaf methanolic isolates against *S. zeamais* and *P. truncatus*, major insect pests of stored *Zea mays* (maize). Destruction of maize by insect pests is a threat to global food security of which maize is a major component. *Annonaceae* is considered as one of the best sources of natural compounds with insecticidal properties, among them alkaloids, acetogenins and lignoids are so prevalent in the corresponding profiles. Our study reports the isolation of three flavonoids for the first time with promising contact toxicity and antifeedant activities against *S. zeamais* and *P. truncatus* from *Annona mucosa* leaves, a species from the genus *Annonaceae*.

Three flavonoids; quercetin 3-*O*-β-D-glucoside (**1**), quercetin 3-*O*-α-D-arabinoside (**2**) and kaempferol 3-*O*-β-D-galactoside (**3**) were isolated from the methanolic leaf extracts of *Annona mucosa* with compounds **1** and **2** being reported for the first time in this species. Results of our investigation demonstrated that compound **1** exhibited the highest antifeedant and contact toxicity activities against *S. zeamais* and *P. truncatus*, respectively. Compounds **2** and **3** also showed promising contact toxicity and antifeedant activities against the two insects. The high insecticidal activities of the flavonoids is likely to be due to the presence of a keto group at C-4 in the pyran ring, a hydroxyl group at C-5, C-7 and a large number of hydroxyl groups in the structure. These results authenticate the traditional use of *Annona mucosa* in stored maize protection against *S. zeamais* and *P. truncatus*. However, further investigation may be carried out on the three compounds to determine their insecticidal efficacy using different

assay methods and the molecules of the compounds may be derivatized to improve their activities. Due to their promising insecticidal activities, the characterized compounds may also be tested against other insect species in the future projects.

Conflict of interest

The authors declare that there is no conflict of interest.

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