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Electrocardiography effects of *Abelmoschus esculentus* fruit extract and its isolated compounds using isolated frog heart perfusion

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Abstract

This study evaluated the electrocardiograph effects of Abelmoschus *esculentus* and its isolated compounds on *Rana temporaria* (frog) heart. Cardiovascular disease is one of the major causes of mortality which contribute to about 57 million deaths as disclosed by WHO in 2002. There have been many therapeutic approaches to reduce the death rate from cardiovascular disease which include the use of herbal drugs from plants. The whole fruit of *Abelmoschus esculentus* (Okra) fruit has been used for culinary purposes in Africa. Medicinal properties of *Abelmoschus esculentus* reported in literature include its use as anti-diabetics, antimicrobial, diuretic agent, and for plasma replacement. However, there have been little or no report on the cardiovascular activities of the extract or isolated compounds from *A. esculentus*.

A. esculentus (Okra) fruits were collected, identified, dried, and powdered. The powdered was extracted using cold maceration with 100% methanol for 72 hours. The crude extract was subjected to repeated fractionation to isolate four (4) compounds. The cardiovascular activities of the crude extract and its isolated compounds were investigated using isolated heart perfusion technique on *Rana temporaria* (frog) heart. The effects of the extract and isolated compounds on the rate (frequency) and force of contraction (amplitude) of the heart beat were evaluated.

The isolated compounds were subjected to spectroscopic analysis using ultraviolent (UV), nuclear magnetic resonance (NMR) (1D and 2D experiments), and mass spectrometry. The compounds were elucidated as quercetin glycoside, quercetin diglycoside, urs-12-ene-3-O-β-D-glucopyranoside and 3-hydroxy-2,3-dihydroimidazo [1,5-a] pyridin-8(5H)-one-5-β-glucopyranoside (esculentoside). The extract and the isolated compounds showed positive inotropic effect on the heart (increase in amplitude of the heart) while they all also possess negative chronotropic effect (decrease in frequency) except compound 4 which possess positive chronotropic effect.

This study isolated three different classes of compounds from *A. esculentus* namely Flavonoids (Quercetin glycoside), Triterpene (Ursolic acid) and pyridine-imidazole (esculentoside). This study also demonstrated that *A. esculentus* extract and compounds isolated from it has negative chronotropic effects and also possess positive inotropic effects on the heart of *R. temporaria*.

Keywords: Electrocardiography; Cardiovascular; Flavonoids; Triterpene; Esculentoside; Inotropic; Chronotropic; *Rana temporaria* Heart

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1. Introduction

Cardiovascular diseases are one of the main contributors to morbidity and mortality worldwide [1]. Drug therapies historically have concentrated on the endpoint components of the disorder, congestion and myocardial dysfunction (heart failure), with treatment strategies emphasizing the use of cardiac glycoside [1]. In view of the efficacy of cardiac glycosides in the management of cardiac diseases the biological standardization is vital provided that, the drugs used were plant extracts [2]. Cardiac glycosides are one of the important medications used in the therapeutic sections of congestive heart failure (CHF) [3]. The mechanism of action is anonymous; on the other hand it is acknowledged that an increase in the amount of intracellular calcium is observed which react with the contractile proteins [4]. In calcium homeostasis of cardiac tissue, the most significant regulator is sarcoplasmic reticulum (SR), which functions as a sink for calcium ions during relaxation and as a calcium source during contraction [5]. Cardiac glycosides produce the positive isotropic action by inhibiting Na-K ATPase pump and hence facilitating the calcium influx [3]. It is widely known that a number of inotropic intervention share a common mechanism that governs the availability of calcium ions at same sites critical for cardiac contraction.

Okra (*Abelmoschus esculentus* (L) Moench) or bhendi also known as Ladies Finger is an imperative vegetable being native of tropical Africa. Okra (*Abelmoschus esculentus)* (L) moench is an annual dicotyledonous crop. Okra is also known as Kacang Bendi, Qiu Kui, Okra, okura, Okro, Quiabos, Ochro, Quiabo, Okoro, Gumbo, Quimgombo, Bamieh, Bamya, Quingumbo, Bamia, Gombo, Bhindi, Kopi Arab[6]. It grows wild along the river Nile in Egypt as well as Ethiopia French colonialist carried okra to the new world soon after 1700[6]. The presence of cardiac glycosides, flavonoids, phenolics, saponins and tannins in high concentrations was reported by Ashidi, *et al*., (2013). The crude extract of fruit of okra possesses lipid regulating ability and inducing cholesterol rats [8], It was also reported that the leaves of *A. esculentus* helps in the therapy of heart condition [7].

2. Material and methods

2.1. Isolation of Compounds

Abelmoschus esculentus (Okra) fruit was collected at the Obafemi Awolowo University Farm. A. A. Ogunlowo, the Herbarium Supervisor of Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Ile – Ife was accessed for its identification. The plant was air-dried and crushed. The milled samples which weighed 1.0 kg was extracted using maceration method with 100 % methanol (5 L) for 72 hours (3days). The resulting filtrates were concentrated *in vacuo* to obtain 164.8 g of the crude extract (CE).Purification (chromatographic processes) of several stages were done. At the ending stage, compound 1 (0.085 g, Rf -0.52 and a yellow powder), compound 2 (0.034 g, Rf - 0.44 and a brown powder), compound 3 (0.024 g, Rf - 0.58 and a white amorphous powder) and compound 4 (plain sticky substance were achieved.

2.2. Identification and Elucidation

Structural elucidation and characterization of active phytochemical compounds in *A. esculentus* were achieved by using chromatography (open column and size exclusion). Sephadex LH-20 (Pharmacia) and Silica gel (ASTM 230–400) mesh were used for size exclusion and open column chromatography's respectively. Column eluates were studied by Thin Layer Chromatography (at room temperature). Mobile phase was made of EtOAc: MeOH: H_2O: AcOH, in the ration of 10:2:1:0.2. The resulting spots on TLC plates were visualized under UV lamp (254 nm wavelength) and detected with the use of 10% H_2SO_4 in CH_3OH. 1H and 13C NMR spectra (for both 1D and 2D experiments) were obtained on the Bruker AV400 (IconNMR) Spectrometer on both 300 and 75 MHz for 1H and 13C spectra respectively at the School of Pharmacy and Biomolecular Sciences, Faculty of Science, Liverpool John Moores University, UK. Mass Spectrometry analyses were obtained from an Agilent TOF spectrometer at the School of Chemistry and Physics, Faculty of Science, Pietermaritzburg Campus, Kwazulu-Natal University, South Africa.

2.3. Isolated heart perfusion techniques on *A. esculentus* extracts and isolated compounds on *R. temporaria*

Frogs weighing between 100 to 140 g were used for this study. The frogs were paralyzed by the pitting method, pinned to a frog dissecting board and opened up. The heart was suspended by the heart clip. Ringer solution, a physiological solution isotonic with the amphibian visceral environment was applied to the heart followed by the solution of extract or isolated compounds or the reference drug after a period of wash off. The electrocardiographs of the drugs/extracts were recorded by a Startling Heart Lever writer with the rate (frequency) and the force height (amplitude) of the heart beat recorded on paper wrapped round a drum and mounted on the kymograph paper.

3. Results and discussion

3.1. Spectroscopic data of isolated compounds

3.1.1. Compound 1

The compound was isolated as a yellow powder. The absorption of UV was observed at spectrum 257.78 and 356.35 nm which is a characteristic of quercetin flavonoid. HRTOFMS in the negative mode displayed a signal at *m/z* 463.0880 $[M-H]^+$ corresponding to a molecular formula $C_{21}H_{20}O_{12}$ (cal. 463.0877).

^{1}H NMR (300 MHz, MeOD-*d4*): δ 7.75 (1H, d, *J*=2.1 Hz), 7.58 (1H, dd, *J*= 8.5, 2.1 Hz), 6.85 (1H, d, *J*=8.6 Hz), 6.44 (1H, d, *J*=2.1 Hz), 6.24 (1H, d, *J*=2.1 Hz), 5.23 (d, 1H, *J* = 7.6 Hz).

^{13}C NMR (75 MHz, MeOD): δ 179.4 (C-4), 166.2 (C-7), 163.3 (C-5), 159.4 (C-2), 158.1 (C-9), 149.7(C-3′), 145.6 (C-4′), 135.7 (C-3), 123.5(C-6′), 123.3 (C-1′), 117.9 (C-2′), 116.5 (C-5′), 105.7(C-10), 104.5 (C-1″), 99.7 (C-6), 94.7 (C-8), 78.2 (C-5″), 78.1 (C-3″), 75.7 (C-2″),71.2 (C-4″),62.6 (C-6″). On comparison of the NMR data with the literature values of Liao, *et al.*, (2012), the structure of compound 1 was determined as Quercetin glycoside.

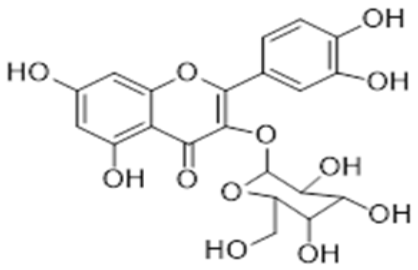

Figure 1 Structure of Compound 1 (Quercetin glycoside)

3.1.2. Compound 2

The Compound was isolated as a brownish powder. Its UV spectrum displayed strong absorption at 257 and 357 nm, which is characteristic of quercetin flavonoid. The high resolution TOF ES MS gave a signal at m/z 649.1378 [M+Na] + for a molecular formula $C_{27}H_{30}O_{13}Na$ (649.1381).

The 1H NMR (300 MHz, MeOD-d4): δH: 7.74 (1H, d, J=2.0), 7.65 (1H, dd, J=8.4, 2.0 Hz), 6.86 (1H, d, J=8.2 Hz), 6.44 (1H, d, J=1.9 Hz), 6.24 (1H, d, J=1.8 Hz), 5.26 (1H, d, J=7.3 Hz), 4.15(1H, d, J=7.6 Hz).

The 13C NMR (75 MHz, CD3OD): δ 156.9 (C-2), 135.4(C-3), 178.5 (C-4), 161.9(C-5), 98.7 (C-6), 166.3 (C-7), 94.3 (C-8), 158.6 (C-9), 104.7(C-10) 122.7 (C-1′), 115.8 (C-2′), 145.9 (C-3′), 146.8 (C-4′), 116.6 (C-5′), 104.9 (C-1″), 77.0 (C-5″), 76.5 (C-3″), 75.8 (C-2″),71.9 (C-4″),68.9 (C-6″), 103.6 (C-1‴), 81.5 (C-2‴), 76.6 (C-3‴), 71.8 (C-4‴), 73.8 (C-5‴), 62.4 (C-6‴). On comparison of the NMR data with the literature values of Liao, *et al*., (2012), the structure of compound 2 was determined as Quercetin diglycoside.

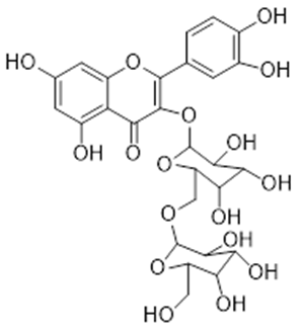

Figure 2 Structure of Compound 2 (Quercetin diglycoside)

3.1.3. Compound 3

The Compound was isolated as a pale white amorphous powder. The NMR spectra result displayed 30 carbon atoms with a unit of sugar indicating the presence of a triterpenoid compound. In the QTF MS, a base peak of m/z 599 was detected, which is 11 amu higher than the mass for urs-12-ene glycoside and the molecular formula of compound 3 was determined to be $C_{36}H_{60}O_6$ by TOF ES MS analysis and m/z of [2M]+ is 1176

1H NMR (300MHz, CD3OD); δH: 0.96 (3H, d, J=6.2Hz H-7), 0.99 (3H, d, J=6.4Hz, H- 5), 1.01 (3H, s H-14), 1.02 (3H, s, H-16), 1.21 (3H, s H-13), 1.43 (3H, s H-15), 2.63 (1H, d, 5=11.2Hz, H-18), 4.04 (1H, d, J=9.4 Hz, H-10), 4.23 (lH, d, J= 9.4, Hz, H-2) 5.47 (lH, s, H-12), 9.65 (lH, s, H-3), δH: 0.64 (3H, s, H-4), 0.65 (3H, s H-6), 0.78 (3H, s, H-9), 0.80 (3H, s, H-1), 0.82 (3H, d, J=1.67 Hz, H-11), 0.88 (3H, s, 19), 0.93 (3H, s, H-20) and 0.94 (3H, s, H-17).

13C NMR (75 MHz, DMSO-d6): δ: 140.9 (C-13), 121.7 (C-12), 101.3 (C-1'), 77.3 (C-3), 77.3 (C-2'), 77.3 (C-4'), 73.9 (C-5'), 70.6 (C-3'), 61.6 (C-6'), 55.9 (C-5), 50.1 (C- 18), 49.1 (C-17), 45.6 (C-9), 42.3 (C-14), 40.2 (C-4), 39.7 (C-8), 39.4 (C-20), 39.2 (C-19), 38.8 (C-1), 36.9 (C-10), 35.9 (C-22), 31.9 (C-7), 29.7 (C-21), 29.1 (C-15), 25.9 (C-23), 25.9 (C-2), 23.1 (C-16), 23.1 (C-27), 21.1 (C-30), 19.6 (C-28), 19.6 (C-6), 19.4 (C-29), 19.1 (C- 26), 12.3 (C-24), 12.1 (C-25). Based on comparison with literature values of Maeda *et al*., (1994), the compound 3 was determined as Ursene-3-O-β-D-Glucopyranoside. This is the first time triterpenoid of ursane skeleton will be reported in *Abelmoschus esculentus*.

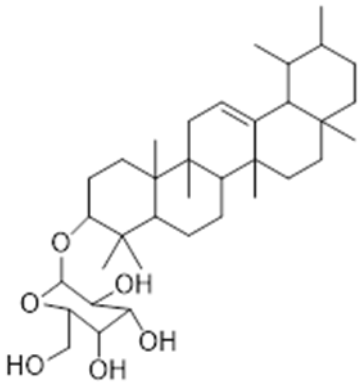

Figure 3 Structure of Compound 3 (Ursene-3-O-β-D-Glucopyranoside)

3.1.4. Compound 4

The compound was isolated as a plain sticky substance. The UV spectrum gave a Lamda maximum at 267.11 nm with a retention time of 2.13 minutes. The TOF ES MS gave a peak at m/z 360 [MNH3] +. The proton NMR spectrum gave three olefinic signals at δH 7.84 (1H, d, J=8.1 Hz, H-7), 7.42 (1H, s, H-1), 5.86 (1H, t, H-6), aliphatic signals at 4.44 (1H, d, J=7.7 Hz, H5), 4.37 (1H, s, H-3) and 3.49 (3H, s, H-11). An anomeric proton was observed at δH 4.40 (1H, d, J=7.6 Hz), with the high coupling constant indicating a β-orientation of the glycoside. The remaining signals were observed between 2.98-4.57 for the sugar protons. The signal at 7.86 was observed to have a proton proton correlation with the triplet signal at δH 5.86 in the COSY spectrum while having a long range correlation with the signal at δc 166, 151, 101 and 90. The signal at δH 3.49 displayed a long range correlation with the signals at δc 101, 73 and 62.5 which allowed the placement of the methoxy group on the glycoside and a methoxy glycoside has been reported before in *A.esculentus* [9]. Other signal are; 13C NMR (75MHz, CD3OD): δ 128.6 (C-2), 141.8 (C-3), 166.1 (C-4), 128.8 (C-5), 151.6 (C-6), 79.8 (C-7), 90.2 (C-8), 101.3 (C-1′), 73.8 (C-2′), 75.9 (C-3′), 70.4 (C-4′), 75.8 (C-5′), 62.5 (C-6′) and 60.7 (C-7′). The compound is therefore identified as 3-hydroxy-2, 3-dihydroimidazo [1, 5-a] pyridin-8(5H)-one-5-β-glucopyranoside (esculentoside). This is a new compound been reported for the first time.

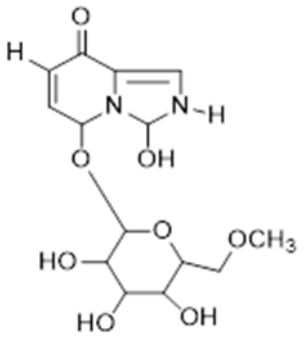

Figure 4 Structure of Compound 4 (3-hydroxy-2,3-dihydroimidazo [1,5-a] pyridin-8(5H)-one-5-β-glucopyranoside)

3.2. Electrocardiography Results

Table 1 Result of Crude Extract (AE) on frog

Doses	Frequency (Heart rate)	Average amplitude (forces height)
Normal Heart rate	20	0.30 cm
Adrenalin (1 mg/mL)	9	0.50 cm
1 mg/mL	10	1.00 cm

Table 2 Result of isolated heart perfusion technique on compound 1

Doses	Frequency (Heart rate)	Average amplitude (forces height)
Normal heart rate	25	0.20 cm
Adrenalin (1 mg/mL)	22	0.45 cm
0.5 mg/mL	22	0.83 cm
1 mg/mL	16	1.20 cm

Table 3 Result of isolated heart perfusion technique on compound 2

Doses	Frequency (Heart rate)	Average amplitude (forces height)
Normal heart rate	29	0.40 cm
Adrenalin (1 mg/mL)	15	1.25 cm
0.5 mg/mL	14	1.47 cm
1 mg/mL	12	2.25 cm

Table 4 Result of isolated heart perfusion technique on compound 3

Doses	Frequency (Heart rate)	Average amplitude (forces height)
Normal heart rate	22	1.05 cm
Adrenalin (1 mg/mL)	20	2.00 cm
0.5 mg/mL	19	1.40 cm
1 mg/mL	18	2.70 cm

Table 5 Result of isolated heart perfusion technique on compound 4

Doses	Frequency (Heart rate)	Average amplitude (forces height)
Normal heart rate	12	0.93 cm
Adrenalin (1 mg/mL)	10	1.90 cm
0.5 mg/mL	15	1.86 cm
1 mg/mL	17	2.80 cm

4. Discussion

4.1. Cardiotonic Activities of *A. esculentus* Fruit Extract and its Isolated Compounds

In this study, the electrocardiographs of frog heart that received the extract, isolated compounds and controls (adrenaline) were evaluated using kymograph. Electrocardiographs were used as index of cardiac function. In this study, a pronounced depression of the cardiac functions was observed in rat when diethyl ether was used as the anaesthetic agent. This corroborated earlier studies on the effect of anaesthetic agent on the heart ([11], [12]). This observation therefore necessitated the use of isolated *R. temporaria* heart tissues for the study as the use of anesthetic agent was avoided. In compliance with the animal right conventions, being an explorative study, it was comfortable to try the extracts and isolated compounds in amphibian pharmacology before progressing to mammalian subjects. The limitation of this model is that there is a wide difference between the anatomy of the heart of the amphibian with its three chambers and that of mammal with four chambers. Despite the limitation of the model, a proof of concept was established as to the effect of the extract on the cardiovascular system. The crude extract with dose 1mg/mL showed a positive inotropic (increase in force of contraction of the heart) and negative chronotropic (decrease in frequency or the rate of the heart beat) compared with the positive control (adrenaline of dose 1mg/mL) in the amphibian model. Compound 1 and 2, (quercetin analogues) showed a negative chronotropic effect and positive inotropic effect. Thus quercetin glycoside has been reported to serve a vital role in reducing cardiovascular diseases [13]. In vivo study in mice has also showed that, quercetin glycoside has the ability to inhibit the process of abnormal aortic aneurism of the heart due to its anti-inflammatory effect [14].The triterpene (compound 3) showed a negative chronotropic effect and a positive inotropic effect. The potential effect of ursolic acid analogues on the heart had been demonstrated by several investigators. For instance, Somova*, et al*., (2003) showed ursolic acid to be able to lower the heart rate of genetically hypertensive rats by 32% and also ursolic acid had been shown to have protective effect on the cardiovascular systems ([16], [17]) as well as inhibiting the activity of angiotensin converting enzymes [18]. Esculentoside (compound 4), an imidazole pyridine analogue showed positive inotropic and positive chronotropic effect on the animal. Olprinone is a classical imidazole [1,5a] pyridine analogues that had been used in Japan as cardiac stimulant. [19]. The study showed that the two flavonoids has a good negative chronotropic effect at dose 1 mg/mL (quercetin glycoside (16 heartbeat per 15 seconds) and quercetin diglycoside (12 heartbeat per 15 seconds)) and also the triterpene and alkaloid showed the best positive inotropic effect (2.70 cm and 2.80 cm respectively) at 1 mg/mL. Most of the compounds isolated in this study showed positive inotropic and negative chronotropic effect. Positive inotropic agents are used in the treatment of low cardiac output such as cardiogenic shock, heart failure and patients with critical hypoperfusion [20] [21]. Positive inotropic agents are also used for patients with bradycardia [22] [23]. In addition, agents with positive inotropic effect are required during resuscitation after cardiac arrest and act on potassium channel in smooth muscles leading to vasodilation [24] [23] whereas negative chronotropic agents have been shown to have therapeutic effect on patients with paroxysmal supraventricular tachycardia (PSVT) by suppressing the rate of cardiac pacemaker, atrioventricular nodal conduction and vasodilates the coronary vasculature [25] ([26], [27]).

5. Conclusion

This study isolated three different classes of compounds from *A.esculentus* namely Flavonols (isoquercitrin glycosides), Triterpene (Ursolic glycoside) and pyridine-imidazole (esculentoside). It also demonstrated that quercetin glycoside, Ursolic glycoside and the crude extract from *A. esculentus* fruits had negative chronotropic effects and also possess positive inotropic effects on the heart of *R. temporaria*. Due to the cardiovascular activities obtained with the extract and compounds isolated from *A. esculentus* fruits, this study recommend and encourage the intake of *A. esculentus* fruits for people with cardiovascular disorder especially individuals with bradycardia and tachycardia in reference to the positive inotropic effect and negative chronotropic effect observed in the extract of *A. esculentus* and its isolated compounds.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors have no conflict of interest.

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