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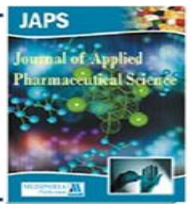

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Effect of *Conyza aegyptiaca* on the frog semi isolated heart

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ABSTRACT

Conyza aegyptiaca (L.) Asteraceae is currently used in traditional medicine for many purposes including cardiovascular diseases. For this purpose, the present study was understood to investigate crude extract and different fractions of the plants on the frog heart activity and furthermore its antioxidant was investigated. Results indicated that the crude extract and fractions reduce all heart activity both amplitude and frequency. In contrast, crude extract and fractions do not have antioxidant properties. This investigation reveals that utilization of ethanolic extract of *Conyza aegyptiaca* to alleviate cardiovascular diseases may be passed through direct effect on the heart. This result confirms the use of this plant in traditional medicine.

***Key words*:** *Conyza aegyptiaca*, Crude extract, Fractions, Cardiovascular disorders.

INTRODUCTION

Communities in developing countries both in rural and urban areas rely to traditional medicine which concerned more than 80% of these populations (Authors). Based on the utilization of knowledge related to plants and others natural resources, African Traditional medicine can be subdivided in three categories: popular knowledge, medicinal plants sold in the markets and finally system of traditional healers who maintain jealously their secrecies and transmit them from generation to generation within the families. The use of medicinal plants varies according to these three categories. *Conyza aegyptiaca* is medicinal plant used throughout those three categories. Traditional popular knowledge within Ewe land (South of Benin, Togo and Ghana) indicate that, *Conyza aegyptiaca* (L.), Asteraceae is used mainly to treat female sterility and several diseases. In Togo, *Conyza aegyptiaca* leads mainly the mountain area of the South-West where it is known under the local name of «*Ahlomè*» or «egbe veve » (that means bitter herb). Indeed, *C. aegyptiaca* is very bitter plant used traditionally as decoction or infusion. Traditional healers used also the areal part of the plants to obesity, diabetes and cardiovascular diseases and many close species as anti-malarial remedies (Clarkson et al. 2004; Pillay et al., 2008).

Previous studies indicate that this plant has fungicides and fungi statics effects on different mycosis such as *Microsporum canis*, *Trychophyton mentagrophytes*, *Candida zeylanoides* (unpublished data). The areal part of the plant contains [2,4-dihydroxy-6-(beta-D-glucopyranosyloxy)phenyl]-butan-1-one, roseoside, and kaempferol-3-O-beta-D-glucopyranoside (Mahmoud et al., 2009). These authors have shown that [2,4-dihydroxy-6-(beta-D-glucopyranosyloxy)phenyl]-butan-1-one has antioxidant effect (Mahmoud et al., 2009).

Indeed the prevalence of cardiovascular diseases is increasing in our communities due to the westernization of the style of the life in many tropical countries. Cardiovascular diseases could

result from vascular perturbation through atherosclerosis, heart dysfunction and over production of reactive oxygen species in the human body.

The present study aims to investigation the effect of *C. aegyptiaca* on the activity of the semi-isolated heart and on its antioxidant capacity.

MATERIALS AND METHODS

Plant Material

Arial part of *Conyza aegyptiaca* collected from Danyi in the mountain area of Togo, 200 km northwest of Lomé. Voucher specimen (N° 2541) was identified by the Department of Botany at Faculty of Sciences (Université de Lomé -Togo, West Africa). This plant material was washed, cut into the small pieces and dried under air conditioner and reduced to a coarse powder by grinding with a mortar and pestle.

Animals

Animals used in this experiment are *Buffo regularis* (collected on the campus of Universite de Lome and acclimated for two to three weeks) and Wistar rat either sex (provided by the Laboratory of Physiology/Pharmacology of natural substances, Faculty of Sciences, Université de Lomé.

Chemicals

Ethanol, methanol, n-butanol, dichloromethane, potassium chloride, l'acetylcholine malondialdehyde barbituric acid Chlorure de potassium, sodium chloride. All those chemicals are purchased Sigma-Aldrich or DBH. Solvent used are analytical grade.

Extraction and Purification

The dried powder of the aerial part (50 g) of *Conyza aegyptiaca* was extracted, during a period of 72 hours, with ethanol (95%) at room temperature (50 ml for 5 g plant product). At the end of the extraction period, the mixture was filtered using a funnel fitted with cotton and moreover with filter paper Whatmann N1. The extract was concentrated under vacuum using a rotary evaporator to afford a residue (11.8%). Thin layer chromatography (TLC) was performed on the crude extract with the following solvent system: methanol/dichloromethane ($MeOH/CH_2Cl_2$: 5/95). This preliminary screening has shown that the crude extract contains several compounds comprising polar and non-polar substances. The crude residue was consequently loaded on a silica gel column and eluted with $MeOH/CH_2Cl_2$ using a gradient from 1/99 to 10/90. Several fractions, indicated as follow: F1, F2, F3, F4, F5, F6 and F7 were collected, and concentrated using the rotary evaporator. The proportions of F1, F2 and F5 are not significant and then are not tested.

Heart Perfusion

The heart of *Buffo regularis* was dissected in situ and the vena cava was connected to the transducer through polyethylene tubing, PE 240 (7451) (OD: .095''; ID: .066'') for recording heart activity. Preparation was kept for 10 minutes for equilibration during which the organ was perfused with Ringer at room temperature. At the end of equilibration period, 100 µl of extract or fractions solutions at 10 mg/ml were injected in the vena cava through the catheter and the changes in the heart activity was monitored through amplitude and frequency of the heart. Acetylcholine (10^{-7} g/ml) and adrenaline (10^{-3} g/ml) were used as reference substances.

Antioxidant assays

Antioxidant capacity of the crude extract and fractions were evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) reducing assay as described (McCune and Johns, 2004; Kpegba et al., 2010) and inhibition of malondialdehyde (MDA) formation test (Agbonon et al., 2009). For 2,2-diphenyl-1-picrylhydrazyl assay, 0.25 mL of crude extract or fractions at different concentrations a methanol solution of extract at different concentrations (100 ; 200 ; 400 ; 800 et 1600 µg/ml) was mixed with 1.5 mL of DPPH at 100 µmol/L. After 10 min, the change in the absorbance was determined at 517 nm and quercetin was used as a positive control. For the inhibition of malondialdehyde assay, the liver of rat was homogenized in 10 mL KCl solution (1.5%). The homogenate was centrifuged at 1107 g for 10 minutes. Malondialdehyde formation (lipid peroxidation) was induced in the supernatant via the addition of hydrogen peroxide and iron sulfate ($FeSO_4$) (Agbonon et al., 2009). Crude extract, fractions solutions at 80 µg/mL (final concentration), quercetin (32 µg/mL) or distilled water were individually (10µL) added to tubes containing 1 mL of the liver homogenate. This was followed by the addition of 111 µL $FeSO_4$ (1mM) and hydrogen peroxide (1mM) into each tube, respectively. In the other test tubes containing the basal concentration of MDA, 1 mL of homogenate was added to 232 µL of distilled water. The tubes were incubated at room temperature for 4 hours under light conditions. All assays were performed in triplicates.

Lipid peroxidation was determined by measuring malondialdehyde (MDA) concentration in the tissue. The supernatant (200 µL) of liver homogenate, treated as mentioned above, was exposed to 0.6 mL of 1 % phosphoric acid and 1 mL of 1 % thiobarbituric acids and the mixture was heated to 100 °C for 50 min. At the end of the incubation period, the mixture was cooled in ice for 10 min and 2 mL of 1-butanol was added and the mixture was centrifuged and the supernatant was removed. The absorbance was read at 535 nm using a plate reader (Molecular Devices, Sunnyvale, CA).

RESULTS AND DISCUSSION

The crude extract and fractions of *C. aegyptiaca* act with different intensities on the amplitude and frequency of the contraction of the frog heart. All the substances tested lead to decrease the cardiac activity. As shown in figure 1, the crude extract decrease the amplitude without affected the heart frequency. The fractions which have the most significant effect on

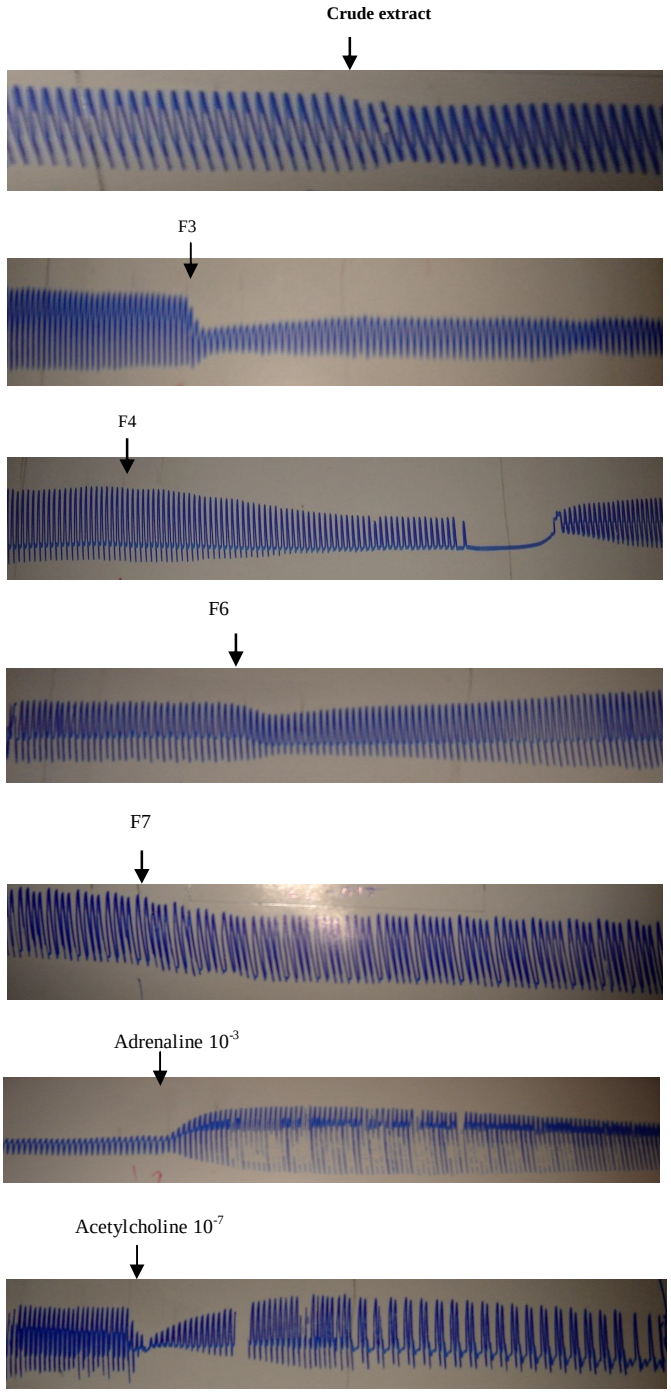

Fig 1. Effect of crude extract and fractions of *Conyza aegyptiaca* on the frog heart activity in situ. *Buffo regularis* heart was connected to the transducer and 100 µl of extract or fractions solutions at 10 mg/ml were injected in the vena cava through the catheter and the changes in the heart activity was monitored through amplitude and frequency of the heart.

the heart activity are F3, F4 and F6. These fractions decrease significantly the amplitude of the heart contraction. The effect on the heart amplitude is peculiarly significant two fractions F3 and F4. The decrease resulted from these two fractions are too pronounced that their presence in any extract of *Conyza aegyptiaca* may justify its cardio-moderator effect. Indeed, *C. aegyptiaca* is used traditionally to alleviate cardiovascular disorders. It is well

Table 1. Effect of crude extract and fractions of *Conyza aegyptiaca* on the amplitude and frequency of frog heart. Extract or fractions solutions were injected in the vena cava of *Buffo regularis*. Means are ± SD (n = 4).

Crude extract/ Fractions	% of variation of frequency	% of variation of the amplitude
Crude extract	-3.12 ± 3.12	-15.22 ± 14.65
F3	3.33 ± 3.33	-39. 44 ± 15.82
F4	5.55 ± 5.55	6.25 ± 3.6
F6	3.33 ± 3.33	-20.42 ± 8.26
F7	-8.32 ± 8.32	8.38 ± 2.13
Acetylcholine 10^{-7}	5.55 ± 5.55	-82.95 ± 7.95
Adrénaline 10^{-3}	12.5	166.6

Table2. Free Radical–Scavenging activity of crude extract and fractions of *Conyza aegyptiaca* using DPPH method; Means are ± SD (n = 3).

Substances	CI50 (µg/ml)
Crude extract	1243.3 ± 16.2
F3	> 1600
F4	> 1600
F6	> 1600
F7	> 1600
Quercetin	21.26 ± 2.3

Table 3. Effect of crude extract and fractions of *Conyza aegyptiaca* on the malondialdehyde (MDA) formation induced by hydrogen peroxide in rat liver. MDA formation was induced in vitro on the rat liver homogenate by hydrogen peroxide. Means are ± SD (n = 3).

Crude extract/Fractions	Concentration of MDA (nM/ml
Crude extract	83.90 ± 7.76
F3	84.10 ± 6.32
F4	74.71 ± 8.78
F6	77.15 ± 7.87
F7	68.08 ± 13.51
Control	78.95 ± 10.46
Basal MDA	3.33 ± 0.75
Quercetin	19.42 ± 0.177

established that cardiovascular diseases have several causes that may involve the dysfunction of the heart (Mao et al., 2010) and or the failure in normal activity of the blood vessels in systemic circulation (Klein et al., 2007; Burke et al., 2008). Blood vessels obstruction leading to cardiovascular disorder, result mainly from reactive oxygen species and oxidative stress, and known as atherosclerosis.

Antihypertensive substances may have central effect on the heart or peripheral effect on blood vessels by inhibiting atherosclerosis. Compounds that have antioxidant effect are particularly therapeutics importance in atherosclerosis pathway of cardiovascular diseases because reactive oxygen species (ROS) are involved in the physiopathology of atherosclerosis (Vogiatzi et al., 2009). For those purposes, antioxidant effect of the crude extract and different fractions are investigated. The results showed in tables 1 and 2 indicated that the crude extract and fractions have no significant effect on DPPH reduction and on the MDA formation induced by hydrogen peroxide in rat liver. Those results indicated that the ethanol extract and different fraction of *Conyza aegyptiaca* isolated in the present study have no antioxidant and then may not inhibit atherosclerosis. However, previous investigations were demonstrated antioxidant effects of compounds isolated from the arial part of this plant (Mahmoud et al., 2009).

The close specie *Conyza dioscordis* also contains antioxidant substances such as quercetin, quercetin 4'-glucoside (Sayed et al. 1991; El-Hamouly and Ibrahim, 2003; Al-Jaber et al., 2011). Furthermore, it is reported that Conyza sumatrensis is used traditionally in Uganda to treatment tuberculoses (Tabuti et al., 2010). Those data indicated that Conyza species have many bioactive compounds that could be beneficial for cardiovascular diseases but also as antibiotics.

Among fractions, the effect of F7 is not significant and when compare this effect to the result of the crude extract, it is possible to speculate that the proportion of F7 in the crude extract would more important than the three others fractions (F3, F4 and F6).

The main finding of this study is that *C aegyptiaca*, used traditionally for a long time in the treatment of hypertension, would act directly on the heart by cardio moderation mechanism. Furthers investigations are needed to evaluate the effect of compounds derived from *C. aegyptiaca* on adrenaline receptors on the heart and if possible isolate the compounds in the different fractions.

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