ACTA SCIENTIARUM POLONORUM
Acta Sci. Pol., Technol. Aliment. 11(3) 2012, 249-257
pISSN 1644-0730 eISSN 1889-9594 www.food.actapol.net/

ANTIOXIDANT ACTIVITY OF DIFFERENT PARTS FROM *ANNONA SQUAMOSA*, AND *CATUNAREGAM NILOTICA* METHANOLIC EXTRACT

Abdalbasit Adam Mariod[1]✉, Siddig Ibrahim Abdelwahab[2], Sara Elkheir[1], Yousif Mohamed Ahmed[1], Putri Narrima Mohd Fauzi[3], Cheah Shiau Chuen[3]

[1]Food Science and Technology Department, College of Agricultural Studies, Sudan University of Science and Technology P.O. Box 71 Khartoum North, **Sudan**
[2]UPM-MAKNA Cancer Research Laboratory, Institute of Bioscience, University Putra Malaysia Serdang, 43400, Selangor, **Malaysia**
[3]Centre of Natural Products and Drug Discovery (CENAR), Department of Pharmacology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, **Malaysia**

ABSTRACT

Background. We extracted phenolic compounds from *Annona squamosa* (leaves, bark, roots and seedcake), and *Catunaregam nilotica* (leaves, bark and seedcake) using methanol and their antioxidant activity was evaluated employing various established in vitro systems.
Material and methods. *Annona squamosa* (leaves, bark, roots and seedcake), and *Catunaregam nilotica* (leaves, bark and seedcake) were used in the study. Antioxidant activity was estimated using oxygen radical absorbance capacity, MTT assay and DPPH assays, and polyphenols profile was determined by HPLC method.
Results. The total phenolic content was determined by Folin-Ciocalteu method and the highest amounts were 171.5, 170.4, 169.5, and 167.9 g/kg plant extract as GAE for *A. squamosa* roots, *C. nilotica bark*, *C. nilotica* leaves, and *A. squamosa bark,* respectively. The leaves extracts of the two trees showed high flavoniod content. The results showed that *C. nilotica* and *A. squamosa* extracts displayed antioxidant activities, with IC_{50} values ranging from 7.81 to 62.5 and from 7.81 to 125.0 µg/ml, respectively using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. The different parts extracts from two trees showed good antioxidant activity evaluated by oxygen radical absorbance capacity and MTT assay systems.
Conclusion. These results suggested that *Annona squamosa* and *Catunaregam nilotica* phenolic compounds could be utilized as a natural antioxidant.

Key words: *Annona squamosa*, antioxidant activity, *Catunaregam nilotica*, 1,1-diphenyl-2-picrylhydrazyl (DPPH), oxygen radical absorbance capacity (ORAC), cytotoxic activity, MTT assay

INTRODUCTION

Catunaregam nilotica is a wild fruit belong to the family Rubiaceae and known locally in Sudan as kerkir. It is widespread in Central and East Africa, as well as in Cameroon and Nigeria [Steentoft 1988]. In Sudan it is found in south and north Kordofan states. It grows as a medium height shrub (usually less than 3 m) with grey drupes, stiff spines, and deciduous leaves clustered below the spines. It has

✉basitmariod@yahoo.com

© Copyright by Wydawnictwo Uniwersytetu Przyrodniczego w Poznaniu

a broad range of applications in the indigenous medical system [Farid et al. 2002]. *Catunaregam nilotica* still grows as a wild plant in different areas in western Sudan states. The seed kernels of *C. nilotica* collected in Sudan have a high potential from an economic aspect because of their high protein and oil content [Mariod et al. 2010]. Two new saponins were isolated from the fruits of *Catunaregam nilotica* [Lemmich 1995]. Mariod et al. [2010], extracted *C. nilotica* oil using two different methods they reported very high oil content (40.0%) with linoleic, oleic, palmitic and stearic as the major fatty acids, and high amount of tocopherol (110.5 mg/100 g oil).

Annona squamosa L. (Annonaceae), commonly known as custard apple is a native of west Indies, it is widely grown throughout the tropics in India and popularly cultivated in the north eastern parts of Thailand, mainly for its edible fruit. Its seed is well known for killing head lice but has no report about the active component [Intaranongpai et al. 2006]. The plant is deciduous and small; reaching a maximum of 6 m in height with many lateral branches grows well in regions of medium humidity. Its seeds comprise 30% of its fruits weight, which is edible [Cardeiro et al. 2005]. The major fatty acids of *A. squamosa* oil were oleic, linoleic, palmitic, and stearic. The tocopherol content of *A. squamosa* was 16.6 mg/100 g oil, with delta-tocopherol as the predominant tocopherol [Mariod et al. 2010]. Petroleum ether extract of *Annona squamosa* exhibited significant wound healing activity in excision, incision, burn and dead space wounds [Shenoy et al. 2009]. *A. squamosa* seed oil was reported to be used in soap and plasticizer industry, as well as in alkyd manufacturing, the seeds are acrid and poisonous. Bark, leaves and seeds contain the alkaloid anonaine [Morton 1987]. The plant is attributed with medicinal properties which include antifertility, anti-tumor and antimalarial activities [Pillaya et al. 2008]. The young leaves of *Annona squamosa* are used extensively for their antidiabetic activity [Shirwaikar et al. 2004]. Annonaceous acetogenins were isolated from *Annonaceae* plants which exhibited a broad range of biological bioactivities such as cytotoxic, antitumoral, antiparasitic, pesticidal and immunosuppresive activities [Yang et al. 2009].

There was a noticeable correlation between total polyphenols and free-radical DPPH scavenging activity [Roesler et al. 2007]. The role of antioxidants in preventing oxygen radical and hydrogen peroxide induced cytotoxicity and tissue damage in various human diseases is becoming increasingly recognized [Roesler et al. 2007], the antioxidants constituents of plant materials are very important for the maintenance of health and protection against many diseases (e.g. cancer) and raising interest among scientists, food manufacturers and consumers as the trend for the future is toward functional food with specific health effects [Loliger 1991]. Typical compounds that possess antioxidant activity include phenols, phenolic acids and their derivatives, flavonoids, tocols, phospholipids, amino acids and peptides, phytic acid, ascorbic acid, pigments, and sterols. Phenolic antioxidants are primary antioxidants which act as free-radical terminators [White and Xing 1996]. High performance liquid chromatography (HPLC) with diode array detection (DAD) is an indispensable tool for the provisional identification of the main phenolic structures present in foods [Chirinos et al. 2009]. Little information is available concerning the chemical composition and antioxidant activities of *Annona squamosa* and *Catunaregam nilotica*. In particular, phenolic compounds in the two plants have not been well characterised. In the present study, we investigated the antioxidant activity of *Annona squamosa* and *Catunaregam nilotica* methanolic extract. The aims of this study were to (1) determine total phenolic of the two plants methanolic extract; (2) measure the antioxidant activity of in the two plants by various assay methods such as 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, ORAC antioxidant activity, and cytotoxic activity (3) identify the main phenolic compounds present in methanolic extract by HPLC-DAD.

MATERIAL AND METHODS

Materials

All solvents used were of HPLC grade. Methanol, ethyl acetate, hexane, chloroform, butylated hydroxyanisole (BHA), and Folin-Ciocalteau reagent as were obtained from Merck (Merck, Darmstadt, Germany). Trolox (6-hydroxy-2, 5,7,8-tetramethylchroman-2-carboxylic acid), fluorescein (3,6'-dihydroxy-spiro [isobenzofuran-1 [*3H*], 9'[*9H*]-xanthen]-3-one), AAPH (2,2'-azobi(2-amidinopropane) dihydrocloride), ferulic

acid, chlorogenic acid p-coumaric acid, 3,4-dihydroxy-benzoic acid, (-)-epicatechin and (+)-catechin, and syringic acid were obtained from Sigma-Aldrich, (Germany), gallic acid and vanillin were obtained from Fluka Chemie AG (Switzerland). The pure flavonoid compound quercetin was obtained from Lyon, Nord-Genay (France).

Methods

Extraction of phenolic compounds

Twenty grams of the dried ground *Annona squamosa* leaves (ASL), bark (ASB), root (ASR), cake (ASC) and *Catunaregam nilotica* leaves (CNL), bark (CNB), and cake (CNC) were extracted with 80% methanol by sonication (Hwasin Technology, Seoul, Korea) to obtained methanolic extract with solid to solvent ratio of 1:10 ($w \cdot v^{-1}$) at room temperature for 1 hour. Each extraction process involved homogenization of ME and solvent at 13 000 rpm for 15 minutes followed by sonication (Hwasin Technology, Seoul, Korea) at constant temperature of 30°C for 1 hour. The methanolic extracts were filtered through filter paper Whatman no 1. Then solvents were removed by using rotary evaporator (Buchi, Flawil, Switzerland). The yield of each extract was measured before kept in –80°C for further analysis.

Total phenol content

The total phenol content (TPC) extracts of Annona *squamosa* leaves (ASL), bark (ASB), root (ASR), cake (ASC) and *Catunaregam nilotica* leaves (CNL), bark (CNB), and cake (CNC) was determined by the Folin-Ciocalteau spectrophotometric method at 765 nm (Shimazu Co. Ltd., Kyoto, Japan), the absorbance was measured after 2 hrs at 765 nm, the result was calculated as galic acid equivalent (mg/kg oil) [Taga et al. 1984]. The measurements were repeated three times. For the replicated samples, relative standard deviation (RSD) was reported.

Total flavoniod

The total flavonoid content of Annona *squamosa* leaves (ASL), bark (ASB), root (ASR), cake (ASC) and *Catunaregam nilotica* leaves (CNL), bark (CNB), and cake (CNC) was determined following [Arvouet-Grand et al. 1994]. Briefly, 5 mL of aluminum trichloride ($AlCl_3$) (Labosi, Paris, France) in methanol (Fluka Chemie, Switerland) was mixed with the same volume of methanol extract (0.01 or 0.02 $mg \cdot mL^{-1}$). Absorption readings at 415 nm spectrophotometer (Shimadzu Corp. Kyoto, Japan) were taken after 10 min against a blank sample consisting of a 5 mL extract solution with 5 mL methanol without $AlCl_3$. The total flavonoid content was determined using a standard curve with Rutin (Sigma-Aldrich Chemie, Steinheim, Germany) (0-50 $mg \cdot L^{-1}$) as the standard. The mean of three readings was used and expressed as mg of rutin equivalents (RE) 100^{-1} g of extract.

Antioxidant activities (AOA) measurement

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity test. The antioxidant activity of phenolic extracts from *Annona squamosa* leaves (ASL), bark (ASB), root (ASR), cake (ASC) and *Catunaregam nilotica* leaves (CNL), bark (CNB), and cake (CNC) was measured following the method of Gordon et al. [2001] using the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH). A methanolic solution (100 µL) of the phenolic compounds extracted from Annona *squamosa* leaves (ASL), bark (ASB), root (ASR), cake (ASC) and *Catunaregam nilotica* leaves (CNL), bark (CNB), and cake (CNC) was placed in a cuvette and 0.5 mL of a methanolic solution of DPPH (50 mg DPPH 100 mL^{-1} MeOH) was added. After 30 min incubation in darkness and at ambient temperature (23°C), the resultant absorbance was recorded at 515 nm. The decrease in absorbance at 515 nm was determined using a spectrophotometer (Shimadzu Co., Ltd., Kyoto, Japan). The absorbance of the DPPH radical without antioxidant, i.e. the control was measured. The data is commonly reported as IC_{50}, which is the concentration of antioxidant required for 50% scavenging of DPPH radicals in the specified time period. All determinations were performed in triplicate.

ORAC antioxidant activity assay. The oxygen radical absorbance capacity (ORAC) is based largely on the the procedure described by Pérez-Jiménez and Saura-Calixto [2006] slightly modified: 175 µL of the sample/blank were dissolved with PBS at concentrations of 160 $\mu g \cdot ml^{-1}$, pH 7.4, 75 mM and serial dilutions for the Trolox standards were prepared accordingly. ORAC assay was performed in a 96-well black microplate with 25 µl of samples/standard/positive control and 150 µl of fluorescence sodium

salt solution, followed by 25 μl of 2,20-azobis (2-amidinopropane) dihydrochloride (AAPH) solution after 45 min incubation at 37°C (200 μl total well volume). Fluorescence was recorded until it reached zero (excitation wavelength 485 nm, emission wavelength 535 nm) in a fluorescence spectrophotometer Perkin--Elmer LS 55, equipped with an automatic thermostatic autocell-holder at 37°C. The positive control was Quercetin and the negative control was blank solvent/PBS. Data were collected every 2 min for a duration of 2 hr. Results are calculated using the differences of areas under the fluorescein decay curve between the blank and the sample and are expressed as Trolox equivalents.

HPLC-DAD system for analysis of phenolic compounds. HPLC analysis was performed using Agilent G1310A pumps (Agilent, Stevens Creek Blvd Santa Clara, USA), with diode array detector and chromatographic separations were performed on a LUNA C-18 column (5 μm, 250 × 4.6 mm; Phenomenex, Torrance, California, USA). The composition of solvents and used gradient elution conditions were described previously by [Chirinos et al. 2009] with some modifications. The mobile phase was composed of solvent (A) water-acetic acid (94:6, v/v, pH 2.27) and solvent (B) acetonitrile. The solvent gradient was as follows: 0 to 15% B in 40 min, 15 to 45% B in 40 min, and 45 to 100% B in 10 min. A flow rate of 0.5 ml/min was used and 20 μl of sample were injected. Samples and mobile phases were filtered through a 0.22 μm Millipore filter, type GV (Millipore, Bedford, MA) prior to HPLC injection. Each fraction was analyzed in duplicate. Phenolic compounds were identified and quantified by comparing their retention time and UV-Vis spectral data to known previously injected standards as a lack of flavonoid standards they were not identified [Chirinos et al. 2009].

Cell culture. All the cells used in this study were obtained from American Type Cell Collection (ATCC) and maintained in a 37°C incubator with 5% CO_2 saturation. MCF-7 human breast carcinoma cells, HepG2 human hepatocellular carcinoma cells, HT--29 human colon adenocarcinoma cells and WRL-68 normal hepatic cells were maintained in Dulbecco's modified Eagle's medium (DMEM). Whereas A549 non-small cell lung cancer cells, WI-38 normal lung fibroblast cells and PC3 prostate adenocarcinoma cells were maintained in RPMI medium. Both medium were supplemented with 10% fetus calf serum (FCS), 100 units·ml^{-1} penicillin, and 0.1 mg·ml^{-1} streptomycin.

MTT assay. Cellular viability – Different cell types from above were used to determine the inhibitory effect of ASR, ASB, ASS, CNB, and CNS on cell growth using the MTT [3-(4,5-dimethylthiazole-2-yl) 2,5-diphenyltetrazolium bromide] assay. This colorimetric assay is based on the conversion of the yellow tetrazolium bromide (MTT) to the purple formazan derivatives by mitochondrial succinate dehydrogenase in viable cells. For measurement of cell viability, cells were seeded at a density of 1×10^5 cells·ml^{-1} in a 96-well plate and incubated for 24 hours at 37°C, 5% CO_2. Next day, cells were treated with the test agents and incubated for another 24 hours. After 24 hours, MTT solution at 2 mg·ml^{-1} was added for 1 hour. Absorbance at 570 nm were measured and recorded. Results were expressed as a percentage of control giving percentage cell viability after 24 hours exposure to test agent. The potency of cell growth inhibition for each test agent was expressed as an EC_{50} value, defined as the concentration that caused a 50% loss of cell growth. Viability was defined as the ratio (expressed as a percentage) of absorbance of treated cells to untreated cells.

Statistical analysis

Each value is a mean of three replications. Values of different parameters were expressed as the mean ±standard deviation (mean ±SD). The discussion is based on the one-way analysis of variance (ANOVA; $P < 0.05$). All statistical analyses were performed using the SPSS of the windows statistical package (Release 8.0).

RESULTS AND DISCUSSION

Total phenolic content

Many studies investigated natural sources of phenolic antioxidants, which could substitute synthetic additives, and also play an important role in preventing many diseases. Special attention is focused on the isolation of natural phenolic antioxidants from inexpensive sources or byproducts of agricultural and food industries, such as oilseeds and oilseed meals. They contain phenolic compounds of various chemical

natures, such as tocopherols, carotenoids, flavonoids, lignans, lignins, phenolic acids and tannins [Matthäus 2002].

Figure 1 shows the total phenol of methanolic extracts of *Annona squamosa*, and *Catunaregam nilotica* bark, leaves, root and seedcake, the results showed that the two plants extracts contain extremely high contents of total phenols. The highest amounts were 171.5, 170.4, 169.5, and 167.9 g/kg plant extract as GAE for ASR, CNB, CNL, ASB methanolic extracts, respectively. Overall, the lowest concentrations were found in the methanolic ASC, ASL and CNC extracts at 75.6, 93.6 and 112.5 g/kg plant extract as GAE, respectively. Considering the high total phenol content of *Annona squamosa*, and *Catunaregam nilotica* extracts compared to recent results of fruits extracts published in the literatures such as *Annona crassiflora* peel, *Ceratonia siliqua* L. (Carob pods), and guava [Roesler et al. 2007, Kumazawa et al. 2002, Jimenez et al. 2001].

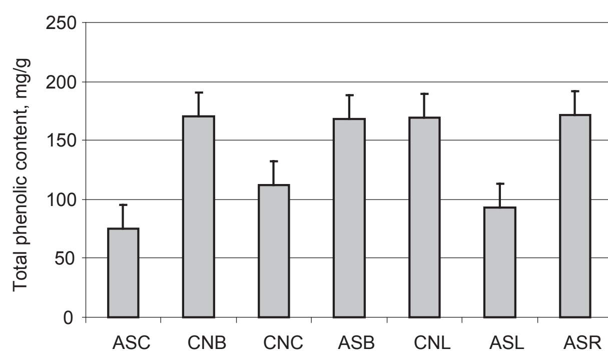

Fig. 1. Total phenol (results are mean ±SD (n = 3)) of methanolic extracts of *Annona squamosa*, and *Catunaregam nilotica* bark, leaves, root and seedcake

Total flavonoid

The most widespread and diverse phenolics are the flavonoids, which are built upon a C6-C3-C6 flavone skeleton in which the three-carbon bridge between the phenyl groups is commonly cyclised with oxygen. Several classes of flavonoids are differentiated on the degree of unsaturation and the degree of oxidation of the carbon segment [Robards et al. 1999].

Figure 2 shows the total flavonoid of methanolic extracts of *Annona squamosa*, and *Catunaregam nilotica* bark, leave, root and seedcake. Figure 1 shows the amount of total flavonoid of methanolic extracts of *Annona squamosa*, and *Catunaregam nilotica* bark, leave, root and seedcake, the results showed that the leaves extracts of the two plants showed high flavoniod content. CNL showed the highest amounts 298.8 followed by ASL 222.6 mg/g. The seedcake extracts of the two plants (ASC and CNC) recorded very low amount of flavonoid 27.6 and 23.1 expressed as mg of rutin equivalents (RE) 100 g of extract. CNB, ASB, and ASR showed medium concentrations as 123.6, 102.5 and 106.6 mg/100 g of extract, respectively.

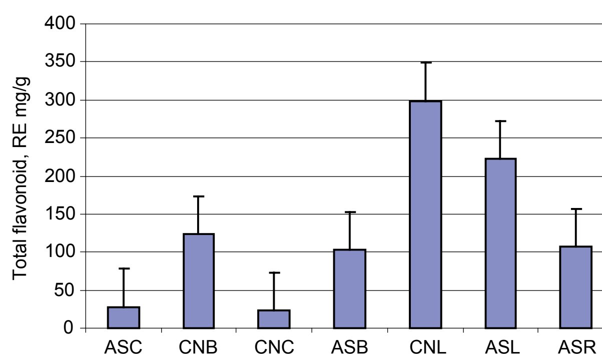

Fig. 2. Total flavonoid (results are mean ±SD (n = 3)) of methanolic extracts of *Annona squamosa*, and *Catunaregam nilotica* bark, leaves, root and seedcake

Plant flavonoids (mainly rutin) are partly responsible for the antioxidant characteristics of plant extracts [Molyneux 2004]. Among the tested samples, ASL and CNL methanolic extracts showed the most antioxidant activity (IC_{50} 7.81 µg/ml), which may be attributed to the antioxidant effects of flavonoids.

Identification of phenolic compounds using HPLC-DAD

HPLC-DAD was used to identify and know what is/are the responsible active ingredient(s) in the crude methanolic extracts of ASR, ASL, CNL, CNB, CNS, ASB, and ASS.

Table 3 shows that the crude methanolic extracts of ASR, ASL, CNL, CNB, CNS, ASB, and ASS contains

syringic, gallic acid, hydroxybenzoic, chlorogenic, vanillin, ferulic and p-cumeric acids. These compounds have been identified according to their retention time and the spectral characteristics of their peaks compared to those of standards, as well as by spiking the sample with standards. Chlorogenic acid was detected to be the major phenolic component in all the methanolic extracts, and it is the higher in CNL and CNC showing the levels of 1.027 and 1.226 mg/100 g dry weight contributing about 81.6 and 89.3% to the total amount, respectively. *Annona squamosa* different parts methanolic extracts ASR, ASL, ASB, and ASS showed less amount of chlorogenic acid in comparison to *C. nilotica* different extracts (Table 3). The levels of total phenolic compounds in CNL, CNB, CNC, ASB, ASC, ASL and ASR methanolic extracts determined by HPLC were, 1.150, 0.026, 1.503, 0.303, 0.076, 0.4701, 0.04 mg/g dry weight, respectively, and thus lesser than (169.5, 170.4, 112.5, 167.9, 75.6, 93.6 and 171.5 mg/g) the ones obtained by the Folin-Ciocalteu method. This result is predictable due to the weak selectivity of the Folin-Ciocalteu reagent, as it reacts positively with different antioxidant compounds (phenolic and non-phenolic substances). The above mentioned HPLC-DAD results indicate that such phenolic compounds from the two trees may show higher antioxidant activity. Isolation and characterisation of such phenolic compounds may be useful in developing natural antioxidants.

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The extracts of *Annona squamosa*, and *Catunaregam nilotica* were assayed over a range of dilutions to establish the concentration of each extract required to scavenge 50% of the DPPH radical present in the assay medium, referred to as the IC_{50} defined as the concentration of the antioxidant needed to scavenge 50% of DPPH present in the test solution. Lower IC_{50} value reflects better DPPH radical scavenging activity [Ali et al. 2008]. The antioxidant activity of *Annona squamosa*, and *Catunaregam nilotica* bark, leaves, root and seedcake extracts was determined (Table 1). Free-radical scavenging potentials of the two plants extracts at different concentrations were tested by the DPPH method and *Annona squamosa* leaves, root, seedcake and bark methanolic extracts presented IC_{50} of 7.81, 7.81, 15.63 and 125.0 μg/ml, respectively, which seem to be more effective (higher antioxidant activity) than *Catunaregam nilotica* leaves, seedcake and bark extracts that presented IC_{50} of 7.81, 31.25 and 62.5 μg/ml, respectively.

Table 1. Results of DPPH (IC_{50}) and ORAC (μM of Trolox) antioxidant activities of *Annona squamosa*, and *Catunaregam nilotica* methanolic extracts

Extract	IC_{50}, μg·ml^{-1}	ORAC, μM of Trolox
CNB	62.5 ±0.5^a	47.08 ±0.23^a
CNC	31.3 ±0.3^b	44.94 ±0.34^b
CNL	7.81 ±0.1^c	72.72 ±0.89^c
ASC	15.6 ±0.2^d	87.95 ±0.96^d
ASB	125.0 ±0.4^e	84.62 ±0.31^d
ASL	7.81 ±0.1^c	29.60 ±0.17^e
ASR	7.81 ±0.1^c	65.20 ±0.51^f
Ascorbic acid	3.13 ±0.1^f	0.00
Quercetin	0.00	58.97 ±0.02^g

Values are mean of triplicates ±standard deviation. The same letters in columns denote the lack of statistically significant differences at $p < 0.05$.

Table 2. Effect CNL and ASL on different cells type expressed as EC_{50} values in 24 hours MTT assay

MTT assay Test agent	EC_{50} ±SD, μg/ml	
	CNL	ASL
A549	42.54 ±2.32^a	20.14 ±1.18^b
PC-3	34.42 ±2.97^a	36.91 ±2.57^b
MCF-7	22.75 ±1.98^a	19.42 ±2.37^b
HepG2	28.40 ±3.54^a	15.01 ±1.05^b
HT-29	53.20 ±4.24^a	17.84 ±1.99^b
WRL-68	44.47 ±1.27^a	17.64 ±1.94^b
WI-38	64.74 ±2.92^a	41.63 ±2.34^b

Results are mean ±SD (n = 3), results are given in mg/g extract. The same letters in rows denote the lack of statistically significant differences at $p < 0.05$.

Table 3. Phenolic compound content mg/100 g dry weight in *Annona squamosa*, and *Catunaregam nilotica* methanolic extracts

Compounds	CNL	CNB	CNC	ASB	ASC	ASL	ASR
Syringic acid	0.018 ±0.11	0.007 ±0.35	0.021 ±0.22	0.012 ±0.32	0.006 ±0.21	0.012 ±0.12	0.00
Hydroxybenzoic acid	0.03 ±0.21	0.00	0.052 ±0.12	0.00	0.00	0.058 ±0.02	0.005 ±0.01
Gallic acid	0.014 ±0.11	0.004 ±0.18	0.061 ±0.22	0.035 ±0.31	0.007 ±0.14	0.025 ±0.11	0.00
Chlorogenic acid	1.027 ±0.67	0.00	1.226 ±0.56	0.214 ±0.43	0.05 ±0.16	0.351 ±0.35	0.022 ±0.01
Vanillin	0.026 ±0.34	0.015 ±0.11	0.050 ±0.21	0.028 ±0.32	0.012 ±0.25	0.023 ±0.01	0.002 ±0.01
p-coumaric	0.005 ±0.01	0.00	0.031 ±0.23	0.014 ±0.16	0.001 ±0.21	0.0011 ±0.01	0.00
Ferulic acid	0.030 ±0.13	0.00	0.062 ±0.18	0.00	0.00	0.014 ±0.13	0.011 ±0.21
Total	1.150	0.026	1.503	0.303	0.076	0.4701	0.04

Values are means ±SD (n = 3), and they are given as mg/100 g dry weight of investigated *Annona squamosa* leaves (ASL), bark (ASB), root (ASR), cake (ASC) and *Catunaregam nilotica* leaves (CNL), bark (CNB), and cake (CNC) extracts.

ORAC antioxidant activity assay

The oxygen radical absorbance capacity (ORAC) assay is the only method that takes free radical action to completion and uses an area-under-curve (AUC) technique for quantitation and thus, combines both inhibition percentage and the length of inhibition time of the free radical action by antioxidants into a single quantity [Ali et al. 2008]. The assay has been widely used in many recent studies related to plant [Almeida et al. 2008].

To evaluate the antioxidant capacity of *Annona squamosa*, and *Catunaregam nilotica* methanolic extract, seven samples CNL, ASL, ASR, CNB, CNS, ASB, and ASS, were used and their potencies were compared with the positive control; Quercetin. The area under the curve (AUC) was calculated for the sample, standard and the positive control. ORAC results are shown in Table 1. ASC, ASB, CNL and ASR each display a higher level of antioxidant activity (87.95, 84.62, 72.72 and 65.20 µM of Trolox, respectively) than Quercetin at 5 µg/ml (58.97 ±0.02 µM of Trolox). CNB, CNC, and ASL showed lower level of antioxidant (47.08, 44.94, and 29.60 µM of Trolox, respectively) than Quercetin at 5 µg/ml (58.97 ±0.02 µM of Trolox). As general saying *Annona squamosa* methanolic extracts showed high antioxidant activity than *Catunaregam nilotica* methanolic extracts.

Cytotoxic activity

MTT assay was used to evaluate the cytotoxic activity of extracts from different parts of *A. squamosa*, and *C. nilotica*. MTT is reduced to an insoluble purple formazan by mitochondrial dehydrogenase. Cell viability was measured by comparison of the purple colour formation. Dead cells, on the other hand, did not form the purple formazan due to their lack of the enzyme. The effect of CNL and ASL on the cell viability in vitro is shown in Figure 3. Cells were cultured for 24 h with several different levels of CNL and ASL extracts. The curves showed that the growth of melanoma cancer cells was inhibited in a dose-dependent manner.

Crude extracts of ASR, ASL, CNL, CNB, CNS, ASB, and ASS were tested with a series of different doses on A549, PC-3, MCF-7, HepG2, HT-29, WRL--68 and WI-38, respectively, and After 24 hours, cell viability was determined by the MTT assay, only CNL and ASL (Fig. 3) were found effective. Test agents induced cell cytotoxicity in a concentration dependent manner. These dose titration curves allowed determining of EC_{50} for the test agents towards different cell lines (Table 2). These results indicated that cell lines differ in their sensitivity to the same test agent, which may be determined by multiple cell type-specific signaling cascades and transcription factor activities. Thus, at the same time no significant cytotoxic effects

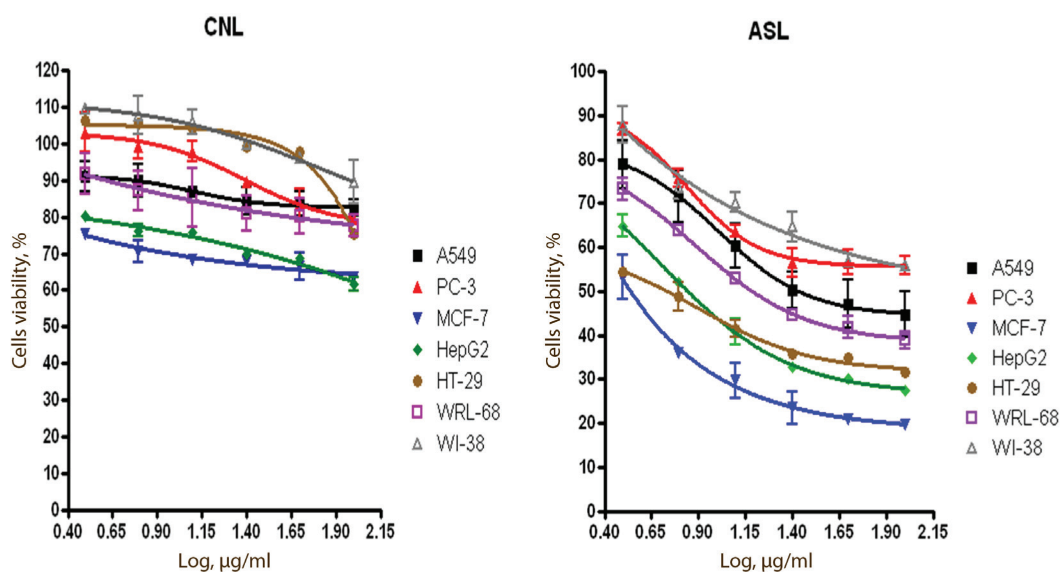

Fig. 3. Dose response curves (using GraphPad Prism) of extract (CNL, ASL) and fractions tested in the MTT assays

towards normal hepatic cells and normal lung fibroblast compared with cancer cells.

REFERENCES

Ali S.S., Kasoju N., Luthra A., Singh A., Sharanabasava H., Sahu A., Bora U., 2008. Indian medicinal herbs as sources of antioxidants. Food Res. Inter. 41, 1-15.

Almeida I.F., Fernandes E., Lima J.L.F.C., Costa P.C., Bahia F.M., 2008. Walnut (*Juglans regia*) leaf extracts are strong scavengers of pro-oxidant reactive species. Food Chem. 106, 1014-1020.

Arvouet-Grand A., Vennat B., Pourrat A., Legret P., 1994. Standarisation d'un extrait de propolis et identification des pricupaux constituants [Standarization of a propolis extract and identification of the main constituents]. J. Pharm. Belgique, 49, 6, 462-468.

Cardeiro M.C.R., de Andrade S.R.M., Ferreira F.R., Filqueiras H.A., Alres R.E., Kinpara D.I., 2005. *Annona species*. Univ. Southan Pton, Southan Pton, UK.

Chirinos R., Betalleluz-Pallardel I., HuamᎫn A., Arbizu C., Pedreschi R., Campos D., 2009. HPLC-DAD characterisation of phenolic compounds from Andean oca (*Oxalis tuberosa* Mol.) tubers and their contribution to the antioxidant capacity. Food Chem. 113, 1243-1251.

Farid H.A.R., Kunert O., Haslinger E., Seger C., 2002. Isolation and structure elucidation of iridoide and coumarin derivatives from *Xeromphis nilotica* (Rubiaceae). Monatsh. Chem./Chem. Monthly 133, 1453-1458.

Gordon M.H., Paiva-Martins F., Almeida M., 2001. Antioxidant activity of hydroxytyrosol acetate compared with that of other olive oil polyphenols. J. Agric. Food Chem. 49, 2480-2485.

Gramza-Michałowska A., Człapka-Matyasik M., 2011. Evaluation of the antiradical potential of fruit and vegetable snacks. Acta Sci. Pol., Technol. Aliment. 10 (1), 61-72.

Intaranongpai J., Chavasiri W., Gritsanapan W., 2006. Anti--head lice effect of Annona squamosa seeds. Southeast Asian J. Trop. Med. Public Health 37, 532.

Jimenez E.A., Rincon M., Pulido R., Fulgencio S.C., 2001. Guava fruit (*Psidium guajava* L.) as a new source of antioxidant dietary fiber. J. Agric. Food Chem. 49, 5489-5493.

Kumazawa S., Taniguchi M., Suzuki Y., Shimura M., Kwon M., Nakayama T., 2002. Antioxidant activity of polyphenols in carob pods. J. Agric. Food Chem. 50, 373-377.

Lemmich E., Cornett C., Furu P., Jŕstian C.L., Knudsen A.D., Olsen C.E., Salih A., Thiilborg S.T., 1995. Molluscicidal saponins from *Catunaregam nilotica*. Phytochem. 39, 63-68.

Loliger J., 1991. The use of antioxidants in foods. Free Rad. Food Add., 121-145.

Mariod A.A., Elkheir S., Ahmed Y.M., Matthaus B., 2010. *Annona squamosa* and *Catunaregam nilotica* seeds, the

effect of the extraction method on the oil composition. J. Am. Oil Chem. Soc. 87, 763-769.

Matthäus B., 2002. Antioxidant activity of extracts obtained from residues of different oilseeds. J. Agric. Food Chem. 50, 3444-3452.

Molyneux P., 2004. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songkl. J. Sci. Technol. 26, 211-219.

Morton J.F., 1987. Fruits of warm climates.

Pérez-Jiménez J., Saura-Calixto F., 2006. Effect of solvent and certain food constituents on different antioxidant capacity assays. Food Res. Inter. 39, 791-800.

Pillay P., Maharaj V.J., Smith P.J., 2008. Investigating South African plants as a source of new antimalarial drugs. J. Ethnopharm. 119, 438-454.

Robards K., Prenzler P.D., Tucker G., Swatsitang P., Glovera W., 1999. Phenolic compounds and their role in oxidative processes in fruits. Food Chem. 66, 401-436.

Roesler R., Catharino R.R., Malta L.G., Eberlin M.N., Pastore G., 2007. Antioxidant activity of Annona crassiflora: Characterization of major components by electrospray ionization mass spectrometry. Food Chem. 104, 1048-1054.

Roman G.P., Neagu E., Radu G.L., 2009. Antiradical activities of *Salvia officinalis* and *Viscum album* L. extracts concentrated by ultrafiltration process. Acta Sci. Pol., Technol. Aliment. 8 (3), 47-58.

Shenoy C., Patil M.B., Kumar R., 2009. Antibacterial and wound healing activity of the leaves of *Annona squamosa* Linn. (Annonaceae). Phytochem. 1, 44-50.

Shirwaikar A., Rajendran K., Dinesh Kumar C., Bodla R., 2004. Antidiabetic activity of aqueous leaf extract of *Annona squamosa* in streptozotocin-nicotinamide type 2 diabetic rats. J. Ethnopharm. 91, 171-175.

Steentoft M., 1998. Flowering plants in West Africa. Cambridge Univ. Pr., Cambridge.

Taga M.S., Miller E.E., Pratt D.E., 1984. Chia seeds as a source of natural lipid antioxidants. J. Am. Oil Chem. Soc. 61, 928-931.

White P.J., Xing Y., 1997. Antioxidants from cereals and legumes. Natural antioxidants: chemistry, health effects, and applications. 224-244.

Yang H., Li X., Tang Y., Zhang N., Chen J., Cai B., 2009. Supercritical fluid CO_2 extraction and simultaneous determination of eight annonaceous acetogenins in Annona genus plant seeds by HPLC-DAD method. J. Pharmaceut. Biomed. Anal. 49, 140-144.

Received – Przyjęto: 6.12.2011

Accepted for print – Zaakceptowano do druku: 6.03.2012

For citation – Do cytowania

Mariod A.A., Abdelwahab S.I., Elkheir S., Ahmed J.M., Fauzi P.N.M., Chuen Ch.S., 2012. Antioxidant activity of different parts from *Annona squamosa*, and *Catunaregam nilotica* methanolic extract. Acta Sci. Pol., Technol. Aliment. 11(3), 249-257.