PAPER

METABOLOMIC PROFILING, ANTIOXIDANT, ANTIPROLIFERATIVE AND ANTIMICROBIAL ACTIVITY OF MEDEMIA ARGUN PALM

I.B. ABDEL-FARID*[1,2], G.A. TAHA[2], M.G. SHEDED[2], M. JAHANGIR[3] and U.A. MAHALEL[1,2]

[1]Biology Department, College of Science, Jouf University, Sakaka, Saudi Arabia

[2]Botany Department, Faculty of Science, Aswan University, Aswan 81528, Egypt

[3]Department of Food Science & Technology, University of Haripur, Haripur, Pakistan

*Corresponding author: bayoumi2013@aswu.edu.eg

ABSTRACT

Metabolomic profiling, antioxidant, anticancer and antimicrobial activity of three parts (leaves, male parts and fruits) of the *Medemia argun* palm were evaluated. Secondary metabolites content showed a significant difference among the evaluated parts. Multivariate data analysis (MVDA) classified the parts into three groups based on their metabolomic profiling and the total antioxidant capacity (TAC) of their extracts. The highest content of secondary metabolites, particularly in the leaves and fruits, was reflected in the DPPH radical scavenging activity and consequently in the IC_{50} of their extracts. The leaves and fruits extracts showed the lowest IC_{50}, followed by the male parts extract (62.8, 78.9 and 134.4 μg/ ml, respectively). Individual polyphenols were also determined by HPLC, which revealed the dominance of rutin, spigenin-7-glucoside, vanillic and rosmarinic acids, and kaempferol in the leaves. *p*-hydroxybenzoic, caffeic, syringic, sinapic, and cinnamic acids and chrysin were the dominant polyphenols in the male parts extract. Ferulic acid, luteolin and apigenin were the dominant polyphenols in the fruits extract. *Medemia argun* extracts showed very strong antiproliferative activity against hepatocellular carcinoma (HepG-2) and lung cancer cell lines (A549). Of the three parts that showed very strong antiproliferative activity, the male parts extract showed prominent antiproliferative activity against HepG-2, while the leaves extract showed more

prominent activity against A549 (IC_{50} was 0.587 and 1.038 μg/ml, respectively). The leaves and fruits extracts showed antimicrobial activity against *Bacillus subtilis* and *Staphylococcus aureus* (Gram-positive bacteria) and *Pseudomonas aeruginosa* (Gram-negative bacteria). The male parts showed moderate antibacterial activity only against *B. subtilis*. No extracts affected the growth of *Escherichia coli*, *Candida albicans* and *Aspergillus flavus*. The biological activity of the *M. argun* palm will be discussed in the light of secondary metabolites content in the plant. To the best of our knowledge, this is the first study concerning the biological activity of *M. argun*.

Keywords: antiproliferative activity, antimicrobial activity, DPPH, HPLC, *Medemia argun*, polyphenols

1. INTRODUCTION

Recently, the search for natural compounds as antioxidants from plant materials has become a topic of interest for scientists due to the safe nature and low cost of these sources (LINDENSCHMIDT *et al.*, 1986; GURIB-FAKIM, 2006); to avoid the utilisation of synthetic compounds which have negative side effects; and because of the very high costs of the available synthetic compounds (ODUJE *et al.*, 2016). Polyphenols is a group of natural plant compounds that showed very strong antioxidant activity against free radicals, which cause many oxidative processes (LANGLEY-EVANS, 2000; PANDEY and RIZVI, 2009). More than 2000 years ago, the consumption of food rich in antioxidant compounds such as polyphenols, anthocyanins, saponins and carotenoids was recommended in traditional Chinese medicine due to their health benefits (LANGLEY-EVANS, 2000; MOHAMED, 2009).

The *Medemia* palm tree is a rare and mysterious genus found in the south of Egypt and the north of Sudan. It has only one species (*M. argun*), which resembles *Hyphaene,* particularly in its leaf, flower and inflorescence morphology (IBRAHIM and BAKER, 2009).

There is a lack of information about the metabolomics and biological activity of *M. argun*. Very few studies have been conducted on *Medemia argun,* and most of these focused on the profiling of essential oils and the proanthocyanidin fraction of the fruits by targeted metabolomics analysis, which was unable to reveal the whole picture of the *Medemia* metabolome. The essential oils from different parts of the fruits (mesocarp and headspace of seeds) were profiled and the results indicated that there was a significant variation in essential oils among the evaluated parts of the fruits (HAMED *et al.*, 2012). Higher performance liquid chromatography and electrospray ionisation mass spectroscopy revealed that the *M. argun* nut is a rich source of proanthocyanidin (HAMED *et al.*, 2014). In another study, incubation of the proanthocyanidin fraction with blood platelets and plasma reduced the formation of 3-nitrotyrosine and diminished the oxidation of thiol groups, in addition to the reduction of the level of carbonyl groups in proteins caused by treatment with peroxynitrite (MOREL *et al.*, 2014). Masullo *et al.* (2016) investigated the butanol extract of *Medemia* fruits, revealing the presence of eight compounds.

Neither a complete picture of *Medemia* metabolome of different parts such as leaves, fruits and male parts, nor the biological activity of these parts is evaluated. The objective of this study is to evaluate different parts such as leaves, male parts and fruits for their metabolomic content using targeted and non-targeted analysis in combination with MVDA, and to assess their biological activity against different microorganisms and against human carcinogenic cell lines.

2. MATERIALS AND METHODS

2.1. Plant materials

Parts of the *M. argun* palm (leaves, male parts and fruits) were collected from Aswan University desert garden. They were dried, separately ground into powder and stored in closed containers until used.

2.1.1 Plant extraction

100 mg of dried plant materials was dissolved in 4 ml of methanol-water (80%). The mixture was vortexed for one min and then placed in a 60ºC water bath for 1 h. The mixtures were centrifuged at 800 rpm for 10 min and the supernatants were used for determination of secondary metabolites such as saponins, phenolics, flavonoids, flavonols and tannins.

2.2. Plant analysis

2.2.1 Determination of carbohydrates

Carbohydrates were determined where the absorbance was read at 620 nm with a spectrophotometer (Thermo Spectronic Genesys 5) (MORRIS, 1948). The concentrations of carbohydrates in different parts of *M. argun* were calculated and expressed as mg/ g DW.

2.2.2 Determination of anthocyanins

Plant samples were dissolved in acidified methanol in brown tubes or in well-closed tubes covered with aluminum foil and incubated at +4ºC for 24 h (PADMAVATI *et al.,* 1997). The absorbances of the supernatants after centrifugation were recorded at 530 nm and 657 nm. The anthocyanins content was calculated using the following equation:

$$\text{Anthocyanin concentration } (\mu\text{mol/ g}) = ([A530 - 0.33 \times A657]/31.6) \times (\text{volume [ml]}/\text{weight [g]}).$$

2.2.3 Determination of saponins

Saponins were determined using vanillin reagent and the absorbance of samples and standard was read at 473 nm. Total saponins content was expressed as mg saponins equivalent (mg SE/ g extract) (EBRAHIMZADEH and NIKNAM, 1998).

2.2.4 Determination of total phenolics content

Folin-Ciocalteu reagent was used to determine total phenolics content in different parts of *M. argun*. The absorbance of samples and standard (gallic acid) was read at 700 nm (SINGLETON *et al.,* 1999). Total phenolics content was expressed as mg gallic acid equivalent (mg GAE/ g extract).

2.2.5 Determination of flavonoids

Aluminium chloride was used to determine flavonoids content in different parts of *M. argun*. The absorbance of samples and standard (quercetin) was measured at 510 nm (ZHISHEN *et al.,* 1999). The content of flavonoids was expressed as mg quercetin equivalent (mg QE/ g extract).

2.2.6 Determination of flavonols

Flavonols content was determined spectrophotometrically using aluminium chloride and sodium acetate. The absorbance of samples and standard (quercetin) was read at 440 nm (KUMARAN and KARUNAKARAN, 2007). The content of flavonols was expressed as mg of quercetin equivalent (mg QE/ 100 g extract).

2.2.7 Determination of total tannins

Tannins content was determined using vanillin reagent. The absorbance of samples and standard (catechol) was read at 550 nm (JULKUNEN-TITTO, 1985). The amount of total tannins was expressed as mg of catechol equivalent (mg CE/ g extract).

2.2.8 Determination of total antioxidant capacity (TAC) and 2,2-Diphenyl-1-picryl-hydrazyl (DPPH) free radical scavenging activity assay

Total antioxidant capacity and DPPH radical scavenging activity were estimated based on PRIETO *et al.* (1999) and BLOIS (1958), respectively. The experimental details of TAC and DPPH radical scavenging activity were reported (ABDEL-FARID *et al.,* 2014). DPPH radical scavenging activity (%) of different concentrations of the plant crude extracts was calculated from the equation: %DPPH = (A_0-A_s) / A_0 X 100, where A_0 is the absorbance of the control and A_s is the absorbance of the evaluated sample. Then the % inhibitions were plotted against concentrations and IC_{50} was calculated from the graph. The experiment was performed in triplicate and average absorption was recorded for each concentration.

2.3. Profiling of polyphenols in different parts of *M. argun* using HPLC

HPLC analysis was performed using Agilent Technologies 1100 series liquid chromatography equipped with an auto sampler and a diode-array detector. The mobile phase was formed from acetonitrile (solvent A) and acetic acid in water (2% v/v) (solvent B). 0.45 μm Acrodisc syringe filters (Gelman Laboratory, MI) were used to filtrate samples before injection. 20 μl injection volume, 0.8 ml/ min flow rate and 60 min run time was used with the following gradient programme: 100% B to 85% B for 30 min, 85% B to 50% B for 20 min, 50% B to 0% B for 5 min and 0% B to 100% B for 5 min. Simultaneously, peaks were monitored at 280, 320 and 360 nm and identified by congruent retention times and UV spectra with comparison with the standards (KIM *et al.*, 2006; MANSOUR *et al.*, 2018).

2.4. Biological activity

2.4.1 Antiproliferative activity

2.4.1.1 Cancer cell lines, medium and in vitro antiproliferative activity

Human hepatocellular carcinoma HepG-2 and lung carcinoma cell lines (A549) were obtained from an American culture collection. PRMI-1640 medium supplemented with 10% foetal bovine serum (FBS), 2 ml glutamine containing 100 U/ml streptomycin and 100 U/ml penicillin at 37ºC/5% CO_2 were used for cell culturing. From each *M. argun* extract, several concentrations were prepared in 1% DMSO (0.049, 0.098, 0.195, 0.391, 0.781, 1.56, 3.13, 6.25, 12.5 and 25 μg/ ml).

2.4.1.2 Determination of inhibition concentration 50% (IC_{50}) for Medemia palm extracts using sulforhodamine B (SRB) colorimetric assay

The antiproliferative activity of *M. argun* extracts was assessed using SRB assay based on Vichai and Kirtikara (2006). The antiproliferative test procedures, from cell harvesting to measurement of colour intensity using a microplate reader and calculation of IC_{50} of each extract, were described in VICHAI and KIRTIKARA, 2006 and EL-NAGGAR *et al.*, 2015.

2.4.2 Antimicrobial activity

Antimicrobial activity of the evaluated extracts was determined using disc diffusion assay (BAUER, *et al.*, 1966). Discs were impregnated with the stock solutions of the evaluated parts of *M. argun*, where each disc received 500 µg from the *Medemia* extracts. Positive control was also prepared where each disc received 200 µg of ampicillin and amphotericin (antibacterial and antifungal agents), respectively. Mueller-Hinton agar plates were inoculated with Gram (+) bacteria (*Staphylococcus aureus* and *Bacillus subtilis*); Gram (-) bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) were incubated at 35-37ºC for 24-48 h. *Candida albicans* was incubated at 30ºC for 24-48 h and filamentous fungi as *Aspergillus flavus* was incubated at 25ºC for 48 h (BAUER *et al.*, 1966). The inhibition zone was measured in millimetres, each experiment was repeated three times, and the average of three readings of the diameters of the inhibition zones was calculated.

2.5. Statistical analysis

Spectrophotometer data was subjected to multivariate data analysis (MVDA) such as principal component analysis (PCA) and hierarchical clustering analysis (HCA) using SIMCA-P software (version 12.0). The statistical differences among the content of secondary metabolites in different parts of *M. argun* were evaluated using analysis of variance (ANOVA) from Minitab (version 12.21). The correlation between the determined metabolites in different parts and TAC was assessed using Pearson's correlation test. Data was presented as the average of three readings ± the standard deviation.

3. RESULTS

3.1. Phytochemical analysis and metabolomic profiling of different parts of *M. argun* (argun palm) using spectrophotometer and HPLC combined with multivariate data analysis

The metabolites content of different parts (leaves, male parts and fruits) of *M. argun* is shown in Table 1. The flavonols content differed significantly in the leaves and fruits ($P<0.05$). Carbohydrates showed no significant difference among the evaluated parts ($P>0.05$). Fruits were characterised by a higher content of total saponins, total phenolics, total flavonoids and total condensed tannins. Phenolics and flavonoids content showed significant differences among the evaluated parts, while for total saponins and tannins content the only significant difference was observed between the leaves and fruits and the male parts and fruits (Table 1). Male parts were characterised by higher anthocyanins content, which showed a significant difference among the evaluated parts ($P<0.05$).

Table 1. Phytochemical analysis, total antioxidant capacity, DPPH radical scavenging activity and IC_{50} of different parts of *Medemia argun* palm.

Metabolites	Leaves	Male parts	Fruits
Carbohydrates (mg/g DW)	124.5±0.2[a]	122.9±0.05[a]	122.2±0.41[a]
Saponins (mg saponins equivalent/g)	15.3±0.7[a]	16.2±0.7[a]	18.7±0.04[b]
Phenolics (mg/ gallic acid equivalent/ g)	13.3±0.9[a]	36.04±0.06[b]	72.3±3.9[c]
Flavonoids (mg quercetin equivalent/ g)	13.3±0.9[a]	27.6±3.6[b]	43.1±4.4[c]
Flavonols (mg quercetin equivalent/ 100 g)	76.12±1.2[a]	67.9±9.35[ab]	45.2±7.0[b]
Anthocyanin (μmole/ g)	0.34±0.04[a]	0.41±0.01[b]	0.26±0.02[c]
Tannins (mg catechols equivalent/ g)	2.28±0.45[a]	2.47±0.09[a]	5.53±0.5[b]
TAC (ascorbic acid equivalent μg/ g)	0.305±0.007[a]	0.285±0.032[a]	0.153±0.006[c]
DPPH (100 μg/ ml)	85.7±1.34[a]	37.5±4.9[b]	72.5±0.42[c]
IC_{50} (μg/ ml)	62.8±9.9[a]	134.4±3.5[b]	78.9±2.9[a]

Different letters in the same row means significant difference at $P<0.05$.

To reduce the dimensionality of the metabolomic data and to evaluate the similarity and dissimilarity between different parts of *M. argun* from the perspective of their phytochemical composition and metabolomic profiling, the data of the three parts of *M. argun* was subjected to PCA, followed by HCA. PCA separated the parts into three groups: leaves, fruits and male parts, as shown in the score scatter plot of PC1 vs. PC2 (Fig. 1A). The score scatter plot also showed that there is a similarity between the leaves and male parts resulting from the approximation of the two parts in the negative part of PC1 (left hand side of the ellipse) (Fig. 1A). The score loading plot (Fig. 1B) revealed the metabolites contributed to the separation obtained in the score scatter plot.

Leaves had higher content of carbohydrates, flavonols, TAC and DPPH radical scavenging activity. Fruits had higher content of saponins, tannins, flavonoids and phenolics. The male parts had higher content of anthocyanins. The score biplot confirmed the results of the PCA score scatter and score loading plots (Fig. 2A). HCA showed the classification of the three parts in three groups with a similarity between the leaves and male parts (Fig. 2B).

Metabolomic profiling of individual polyphenols in different parts of the *M. argun* palm was evaluated using HPLC. 24 standards were injected and their peaks are shown in Fig. 3. In the evaluated parts, 18 individual polyphenols were detected and their concentrations were determined (Table 2). Rutin, apigenin-7-glucoside, kaempferol, and rosmarinic and vanillic acids were the dominant polyphenols in the leaves extract of *M. argun*. Caffeic, *p*-hydroxybenzoic, sinapic, syringic, and cinnamic acids and chrysin were the dominant polyphenols detected in the male parts extract, where ferulic acid, apigenin and luteolin were the dominant polyphenols in the fruits (Table 2).

Metabolomic profiling of individual polyphenols in different parts of *M. argun* palm was evaluated using HPLC. 24 standards were injected and their peaks are shown in Fig. 3. In the evaluated parts, 18 individual polyphenols were detected and their concentrations were determined (Table 2). Rutin, apigenin-7-glucoside, kaempferol, rosmarinic and vanillic acids were the dominant polyphenols in leaves extract of *M. argun*. Caffeic, *p*-hydroxybenzoic, sinapic, syringic, cinnamic acids and chrysin were the dominant polyphenols detected in male parts extract, where ferulic acid, apigenin and luteolin were the dominant polyphenols in fruits (Table 2).

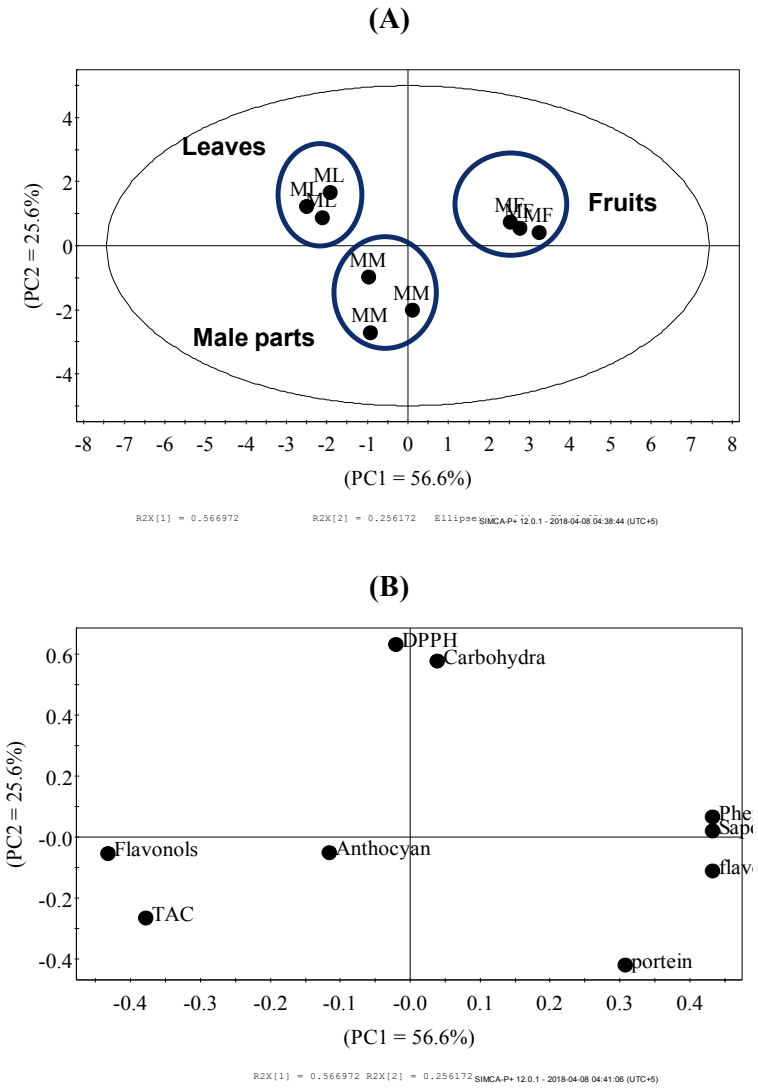

Figure 1. Score scatter plot of PC1 vs. PC2 (A), and the score loading plot of PC1 vs. PC2 of the metabolomic profiling of different parts of *Medemia argun* palm (B). ML = *Medemia argun* leaves, MM = *Medemia argun* male parts and MF = *Medemia argun* fruits.

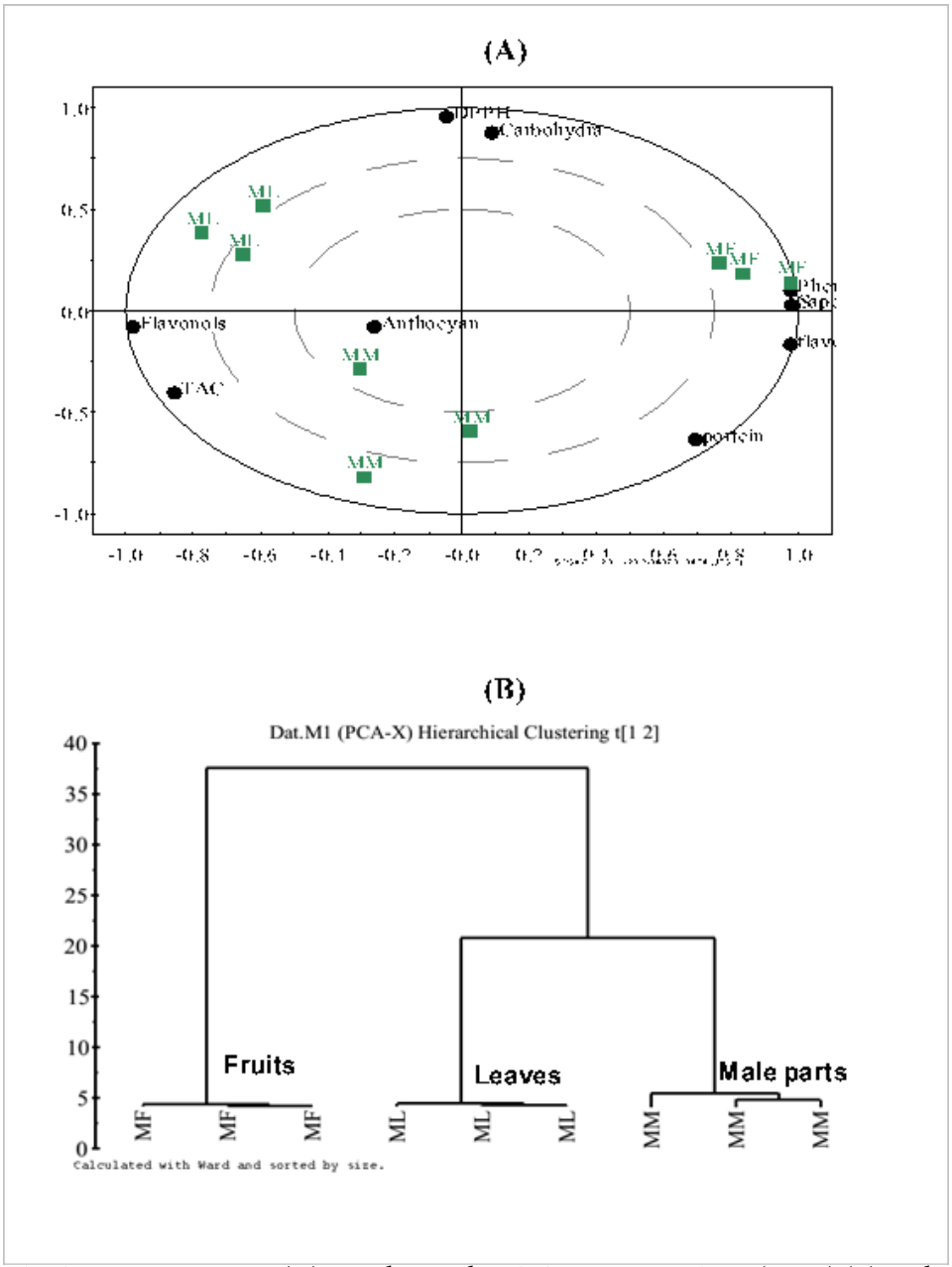

Figure 2. Score biplot of PC1 vs. PC2 (A), and hierarchical clustering analysis (HCA) (B) of the metabolomic profiling of different parts of *Medemia argun* palm. Labeling of each group is the same as in Fig. 1.

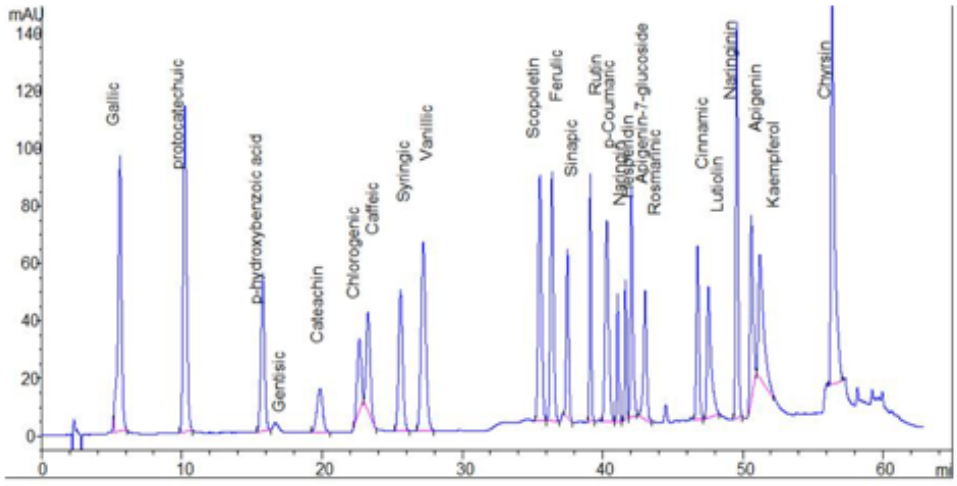

Figure 3. HPLC chromatogram of 24 polyphenols standards at 280 nm (TAHA *et al.,* 2020).

Table 2. Individual polyphenols (mg/ g) in different parts of *M. argun* palm.

	Leaves	Male parts	Fruits
Protocatechuic acid	0.167	0.152	ND
p-hydroxybenzoic acid	0.158	0.371	0.110
Catechins	0.316	0.295	ND
Chlorogenic acid	0.071	ND	ND
Caffeic acid	ND	0.206	ND
Syringic acid	0.112	0.267	0.081
Vanillic acid	0.066	ND	0.05
Ferulic acid	0.05	0.010	0.033
Sinapic acid	ND	0.179	0.169
Rutin	12.55	2.352	0.538
p-coumaric acid	0.821	0.722	0.70
Apigenin-7-glucoside	2.85	0.288	0.255
Rosmarinic acid	0.631	0.127	0.111
Cinnamic acid	0.012	0.088	0.017
Luteolin	ND	ND	0.033
Apigenin	ND	0.079	0.229
Kaempferol	0.211	0.131	0.073
Chrysin	ND	0.044	ND

ND = non-detectable.

3.2. Antioxidant, DPPH free radical scavenging activity and IC_{50} of *M. argun* palm

DPPH values differed significantly among the evaluated parts ($P<0.05$). TAC showed a significant difference between the leaves and fruits and the male parts and fruits (Table 1). The leaves and fruits had the highest values of DPPH radical scavenging activity (85.7 and 72.5%, respectively), whereas the male parts showed the lowest percentage (37.5%). The richness of leaves and fruits in bioactive metabolites was reflected in the DPPH radical scavenging activity and also in the IC_{50} of the extracts under evaluation. The leaves and fruits showed the lowest IC_{50} compared to the male parts (62.8, 79.9 and 134.4 μg/ ml, respectively) (Table 1).
The higher the free radical scavenging activity, the lower the IC_{50} and vice versa. This means that leaves and fruits are more active than male parts as antioxidant agents.
Pearson's correlation was performed to assess the relation between the estimated metabolites and TAC in the three evaluated parts of the *M. argun* palm. TAC positively correlated with the total flavonols and anthocyanins content ($P<0.05$), and r values: 0.799 and 0.913, respectively.

3.3. Antiproliferative activity of different parts of *M. argun*

The results of the IC_{50} of different parts of the *M. argun* palm are shown in Table 3. The three evaluated parts of *M. argun* showed very strong antiproliferative activity against hepatocellular carcinoma (HepG-2) and lung cancer cell lines (A549). The male parts of the *M. argun* palm showed the lowest IC_{50} against HepG-2 cell lines (0.587 μg/ ml), followed by the fruits and leaves extracts with IC_{50}: 1.247 and 1.476 μg/ ml, respectively. The leaves extract of *M. argun* was the strongest antiproliferative extract against lung carcinoma cell lines (A549) with the lowest IC_{50} (1.038 μg/ml), followed by the male parts and fruits extracts (IC_{50}: 2.369 and 3.551 μg/ ml, respectively) (Table 3). The dead cells in both cell lines incubated with *M. argun* were dose dependent as they increased with the concentrations used (Fig. 4). Although all extracts showed very strong antiproliferative activity against hepatocellular carcinoma and lung cancer cell lines, the effect of the male parts and fruits extracts was more pronounced than that of the leaves extract on HepG-2 cell lines, and the leaves and male parts extracts exerted stronger antiproliferative activity against lung cancer cell lines than the fruits extract (Table 3 and Fig. 4). Hepatocellular carcinoma (HepG-2) revealed more susceptibility to *M. argun* extracts than lung cancer cell lines (A549) (Fig. 4).

Table 3. IC_{50} (μg/ ml) of *M. argun* extracts against hepatocellular carcinoma (HepG-2) and lung cancer cell line (A549).

	HepG-2	A549
Leaves	1.476	1.038
Male parts	0.587	2.369
Fruits	1.247	3.551

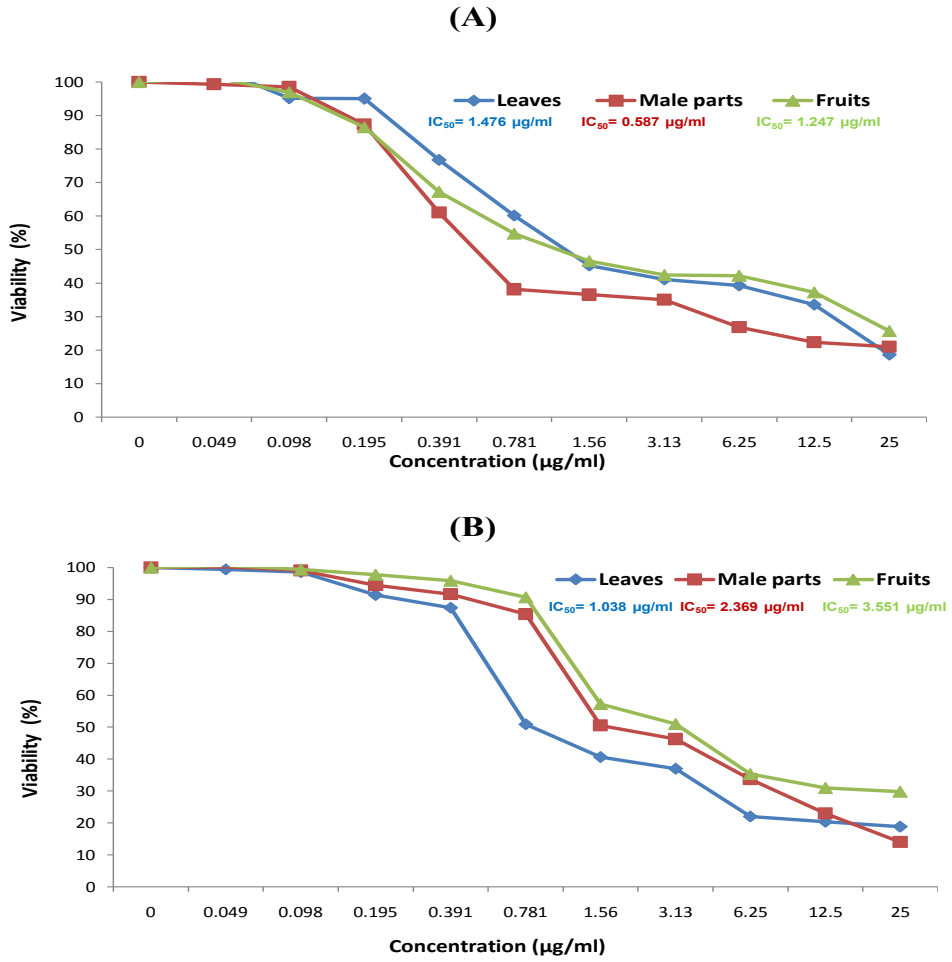

Figure 4. The viability percentage (%) of different parts of *Medemia* palm extracts against hepatocellular carcinoma HepG-2 (A) and lung cell line carcinoma A549 (B). Each experiment was repeated three times.

3.4. Antimicrobial activity of different parts of *M. argun*

The antimicrobial activity of different parts of an *M. argun* palm was evaluated using paper diffusion disc assay. Only the leaves and fruits extracts of *M. argun* exerted antimicrobial activity against *B. subtilis, S. aureus* and *P. aeruginosa* (Table 4). The effect of the fruits extract was more prominent than the leaves extract on Gram-positive bacteria. The leaves extract showed more effect than the fruits extract against *P. aeruginosa* (Table 4). Gram-positive bacteria showed more susceptibility than Gram-negative bacteria to the methanol extracts of the *M. argun* palm (Table 4). Only the male parts extract showed antibacterial activity against *B. subtilis* and no extract from the evaluated parts showed antimicrobial activity against *C. albicans* and *A. flavus*, meaning that fungal strains had more resistance than bacterial strains against *M. argun* palm extracts.

Table 4. Antimicrobial activity (in term of clear zones) of different parts of *M. argun*.

		Zone of inhibition (mm)			
		Control	**Leaves**	**Male parts**	**Fruits**
Gram +ve bacteria	*Bacillus subtilis*	28.0±2.0	11.3±2.5*	9.0±0.0*	12.7±0.06*
	Staphylococcus aureus	31.0±6.5	12.3±2.3*	0.0±0.0*	12.3±2.3*
Gram -ve bacteria	*Pseudomonas aeruginosa*	32.4±1.1	12.7±1.5*	0.0±0.0*	11.0±2.0*
	Escherichia coli	29.0±1.7	0.0±0.0*	0.0±0.0*	0.0±0.0*
Fungi	*Aspergillus flavus*	14.6±1.5	0.0±0.0*	0.0±0.0*	0.0±0.0*
	Candida albicans	16.3±1.1	0.0±0.0*	0.0±0.0*	0.0±0.0*

+ Control is ampicillin as antibacterial agent and amphotericin B as antifungal agent. Each disc was impregnated with 500 μg from each extract; whereas the disc of positive control was impregnated with 200 μg from each control. The data presented is a mean of 3 replicates with the standard deviation. *mean there a significant difference between the diameter of clear zones with the plant extracts and that of the positive control at $P<0.05$.

4. DISCUSSION

Under normal conditions, plants produce many bioactive water soluble secondary metabolites such as polyphenols, terpenoids, steroids, alkaloids, saponins, glucosinolates, isothiocyanates and tannins (KRUSE et al., 2000; DIXON, 2001). These metabolites are very important not only for a plant itself for its defense against pathogens and herbivores and tolerance for different biotic and abiotic stresses (PANDEY and RIZVI, 2009), but also for humans, as the metabolites are the main source of medicines as antioxidant and free radical scavenging activities for different oxidative stress linked diseases (OLAJUYIGBE and AFOLAYAN, 2011). Polyphenols are groups of secondary metabolites such as phenolic and cinnamic acids, flavonoids, anthocyanins, stilbene and lignans; they are considered as potentially health beneficial antioxidants. Consumption of a polyphenol-rich diet provides protection against a series of dangerous diseases such as cancers, diabetes, cardiovascular diseases, neurodegenerative disease and osteoporosis (PANDEY and RIZVI, 2009).

To assess the biological activities of plant extracts, the phytochemical and metabolomic profiling of plants is very important in attributing any biological activity to the plants' metabolites content. To reveal the whole picture of metabolomics of a given plant, many analytical techniques should be used in combination with MVDA. *M. argun* has received very little attention from researchers, and search engine queries for the plant found only five articles that focus only on the targeted phytochemical studies of *M. argun* (HAMED *et al.,* 2012, 2014, MOREL *et al.,* 2014; MASULLO *et al.,* 2016; SAID *et al.,* 2017). The significant variations in the bioactive secondary metabolites content in different parts of *M. argun* were in line with many previous reports, which showed clearly that each part of a given plant has its own phytoanticipins and metabolomic content (HAMED *et al.,* 2012; ABDEL-FARID *et al.,* 2014; TAHA *et al.,* 2020).

The highest percentages of DPPH free radical scavenging activity of the leaves and fruits of *M. argun* can probably be attributed to the richness of these parts in bioactive secondary metabolites such as polyphenols, saponins and tannins, as explored by spectrophotometer

and HPLC analysis. The positive correlation between TAC and DPPH free radical scavenging activity and secondary metabolites content, particularly polyphenols, was well documented in many previous studies (BASAR *et al.*, 2013; ABDEL-FARID *et al.*, 2014; DIACONEASA *et al.*, 2015; MANSOUR *et al.*, 2016).
The highest content of these previously mentioned secondary metabolites not only affected the DPPH radical scavenging activity and TAC, they also positively affected the antiproliferative activity of *M. argun* extracts. The cytotoxic activity of some Saudi and Egyptian desert plants against hepatocellular, breast and lung cancer cell lines was attributed to the highest content of these secondary metabolites in their extracts (ALENAD *et al.*, 2013; EL-NAGGAR *et al.*, 2015; TAHA *et al.*, 2020). The individual polyphenols in *M. argun* extracts such as rutin, catechin, kaempferol, rosmarinic, vanillic, rosmarinic, caffeic, *p*-hydroxybenzoic, protocatechuic, sinapic, syringic, ferulic, cinnamic acids, apigenin-7-glucoside, luteolin, apigenin and chrysin were well known as anticancer agents that worked individually or in combination (synergistic effect) (FUCHS and MILBRADT, 1993; VELIKA and KRON, 2012; FONSECA *et al.*, 2015; WILKINS *et al.*, 2017; CHANDRA and VISWANATHSWAMY, 2018; YAMAGATA *et al.*, 2018). The interaction of some of these active secondary metabolites with cancer-associated receptors seems to trigger specific mechanisms and consequently causes the death of cancer cells (WANG *et al.*, 2008).
The antimicrobial activity of *M. argun* was also attributed to its active metabolites content, particularly polyphenols. The suppression of proteases and/or inactivation of the microbial adhesions may be the mechanism of polyphenols toxicity against microorganisms (COWAN, 1999). The correlation between the content of secondary metabolites such as polyphenols and saponins and the antimicrobial potentiality was reported (CISOWSKA *et al.*, 2011; ALVES *et al.*, 2013; NAYAKA *et al.*, 2014; GULL *et al.*, 2015). The different responses of Gram-negative and -positive bacteria to *M. argun* extracts may be due to the nature of the cell wall, which varies between Gram-positive and -negative bacteria. Not only the type of bacteria but also the metabolites content of the plant part used and also the extraction method and the extraction solvent used may control the response of bacteria to the extract (GULL *et al.*, 2015). *M. argun* extracts showed no antifungal activity. Similar to these results, the proanthocyanidin fraction isolated from the *M. argun* nut has not affected the growth of *Cephalosporium gramineum* (MARTYNIUK *et al.*, 2017).

5. CONCLUSION

M. argun has high bioactive secondary metabolites content, such as polyphenols (with its different classes) and saponins, which were affected positively on potentiality against hepatocellular carcinoma and lung cancer cell lines. The highest content of these metabolites was reflected in the antiproliferative activity of *M. argun* against carcinogenic cell lines, and it also extended to its TAC and free radical scavenging activity. Moreover, the high secondary metabolites content, particularly polyphenols in the *M. argun* palm, was affected positively on potentiality as antibacterial activity. *M. argun* will be a promising plant in future for industrial and pharmaceutical medicines. Pharmacological and pharmaceutical studies are required in which separated individual polyphenols as well as plant extracts should be tested against different carcinogenic cell lines in vitro and in vivo in animals in amelioration experiments. The effect of *M. argun* extracts on diabetes in mice is also desirable.

ACKNOWLEDGMENTS

We would like to thank the Unit of Environmental Studies and Development (UESD) for providing the lab facilities to accomplish this work.

REFERENCES

Abdel-Farid I.B., Sheded M.G. and Mohamed, E.A. 2014. Metabolomic profiling and antioxidant activity of some *Acacia* species. Saudi j. Biol. Sci. 21:400-408. DOI: doi.org/10.1016/j.sjbs.2014.03.005

Alenad A.M., Al-Jaber N.A., Krishnaswamy S., Yakout S.M., Al-Daghri N.M. and Alokail, M.S. 2013. *Achillea fragrantissima* extract exerts its anticancer effect via induction of differentiation, cell cycle arrest and apoptosis in chronic myeloid leukemia (CML) cell line K562. J. Med. Plants Res. 7:1561-1567.

Alves M.J., Ferreira I.C. Froufe H.J., Abreu R.M.V., Martins, A. and Pintado M. 2013. Antimicrobial activity of phenolic compounds identified in wild mushrooms, SAR analysis and docking studies. J. Appl. Microbiol. 115:346-357. DOI: doi.org/10.1111/jam.12196

Basar M.H., Hossain S.J., Sadhu S.K. and Rahman M.H. 2013. A comparative study of antioxidant potential of commonly used antidiabetic plants in Bangladesh. Orient. Pharm. Exp. Med. 13:21-28.

Bauer A.W., Kirby W.M.M., Sherris J.C. and Turck M. 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 45:493-496.

Blois M.S. 1958. Antioxidant determinations by the use of a stable free radical. Nature 181:1199-1200.

Chandra Y.P. and Viswanathswamy A.H.M. 2018. Chemo preventive effect of rutin against N-nitrosodiethylamine-induced and phenobarbital-promoted hepatocellular carcinoma in wistar rats. Indian J. Pharm. Educ. Res. 52:78-86. DOI: doi.org/10.5530/ijper.52.1.9

Cisowska A., Wojnicz D. and Hendrich, A.B. 2011. Anthocyanins as antimicrobial agents of natural plant origin. Nat. Prod. Commun. 6:149-156.

Cowan M.M. 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12:564-582.

Diaconeasa Z., Leopold L., Rugină D., Ayvaz H. and Socaciu C. 2015. Antiproliferative and antioxidant properties of anthocyanin rich extracts from blueberry and blackcurrant juice. Int. J. Mol. Sci. 16:2352-2365. DOI: doi.org/10.3390/ijms16022352

Dixon R.A. 2001. Natural products and disease resistance. Nature 411:843-847.

Dosumu O.O., Nwosu F.O. and Nwogu, C.D. 2006. Antimicrobial studies and phytochemical screening of extracts of *Hyphaenethebaica* (Linn) Mart fruits. Int. J. Trop. Med. 1:186-189.

Ebrahimzadeh, H. and Niknam, V. 1998. A revised spectrophotometric method for determination of triterpenoid saponins. Indian Drugs 35:379-381.

El-Naggar S.A., Abdel-Farid I.B., Elgebaly,H.A. and Germoush, M.O. 2015. Metabolomic profiling, antioxidant capacity and *in vitro* anticancer activity of some compositae plants growing in Saudi Arabia. Afr. J. Pharm. Pharmacol. 9:764-774. DOI: doi.org/10.4172/1948-5956.C1.075

Fonseca S.F., Lima D.B., Alves D., Jacob R.G., Perin G., Lenardao E.J. and Savegnago, L. 2015. Synthesis, characterization and antioxidant activity of organoselenium and organotellurium compound derivatives of chrysin. New J. Chem. 39:3043-3050.

Fuchs J. and Milbradt, R. 1993. Skin anti-inflammatory activity of apigenin-7-glucoside in rats. Arzneim.-Forsch**.** 43:370-372.

Gull T., Sultana B., Bhatti I.A. and Jamil A. 2015. Antibacterial potential of *Capparis spinosa* and *Capparis decidua* extracts. Int. J. Agric. Biol. 17:727-733. DOI: doi.org/10.17957/IJAB/14.0007

Gurib-Fakim A. 2006. Medicinal plants: traditions of yesterday and drugs of tomorrow. Mol. Aspects Med. 27:1-93. DOI: doi.org/10.1016/j.mam.2005.07.008

Hamed A.I., Al-ayed A.S., Moldoch J., Piacente S., Oleszek W. and Stochmal A. 2014. Profiles analysis of proanthocyanidins in the argun nut (Medemia argun-an ancient Egyptian palm) by LC–ESI–MS/MS. J. Mass Spectro. 49:306-315. DOI: doi.org/10.1002/jms.3344

Hamed A.I., Lleonardi M., Stochmal A., Oleszek W. and Pistelli A. 2012. GC-MS analysis of aroma of *Medemia argun* (mama-n-khanen or mama-n-xanin), an ancient Egyptian fruit palm. Nat. Prod. Commun. 7:633-636.

Ibrahim H. and Baker W.J. 2009. *Medemia argun* - Past, Present Future. Palms 53:9-19.

Julkunen-Titto R. 1985. Phenolics constituents in the leaves of northern Willows. Methods for the analysis of certain phenolics. J. Agric. Food Chem. 33:213-217. Doi: doi.org/10.1021/jf00062a013

Kim K.H., Tsao R., Yang R. and Cui S.W. 2006. Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions. Food Chem. 95: 466-473. DOI: doi.org/10.1016/j.foodchem.2005.01.032

Kruse M., Strandberg M. and Strandberg B. 2000. Ecological effects of allelopathic plants-a review. NERI Tech. Rep. 315:5-67.

Kumaran A. and Karunakaran R.J. 2007. *In vitro* antioxidant activities of methanol extracts of five *Phyllanthus* species from India. LWT-Food Sci. Technol. 40:344-352. DOI: doi.org/10.1016/j.lwt.2005.09.011

Langley-Evans S.C. 2000. Antioxidant potential of green and black tea determined using the ferric reducing power (FRAP) assay. Int. J. Food Sci. Nutr. 51: 181-188.

Lindenschmidt, R.C., Tryka, A.F., Goad, M.E., Witschi, H.P., 1986. The effects of dietary butylated hydroxy toluene on liver and colon tumor development in mice. Toxicology 38:151-160. DOI: doi.org/10.1016/0300-483x(86)90116-2

Mansour R.B., Jilani I.B.H., Bouaziz M., Gargouri B., Elloumi N., Attia H., Ghrabi-Gammar Z. and Lassoued S. 2016. Phenolic contents and antioxidant activity of ethanolic extract of *Capparis spinosa*. Cytotechnology 68:135-142. DOI: doi.org/10.1007/s10616-014-9764-6

Martyniuk S., Hamed A.I., Gebala B. and Stochmalm A. 2017. Effect of the proanthocyanidin fraction from *Medemia argun* on the *in vitro* growth and activity of selected soil microorganisms. J. Element 2:143-150. DOI: doi.org/10.5601/jelem.2016.21.2.1089

Mohamed A.H., Hegazy M.F., Moustafa M.F., El-Sayed M.A., Abdel-Farid I.B., Esmail A.M., Abdel-Razik M.H., Mohamed N.S., Nenaaf G., Mohamed T.A., Shahat A.A., Karchesy J., Matsuda H. and Pare P.W. 2012. *Euphorbia helioscopia*: chemical constituents and biological activities. Int. J. Phytopharmacol. 3(1):78-90.

Mohamed A.N.E. 2009. Antioxidant and anticancer activities of doum fruit extract (*Hyphaene thebaica*). Afric. J. Pure Appl. Chem. 3:197-201.

Morel A., Hamd A.I., Oleszek W., Stochmal A., Glowacki R. and Olas B. 2014. Protective action of proanthocyanidin fraction from *Medemia argun* nuts against oxidative/nitrative damages of blood platelet and plasma components. Platelets 25:75-80. DOI: doi.org/10.3109/09537104.2013.769511

Msullo M., Hamed A.I., Mahalel U.A., Pizza C. and Piacente S. 2016. Phenolic compounds from the fruits of *Medemia argun*, a food and medicinal plant of ancient Egypt. Nat. Prod. Commun. 11:279-282.

Morris D.L. 1948. Quantitative determination of carbohydrates with Dreywood's anthrone reagent. Science 107:254-255.

Nayaka H.B., Londonkar R.L., Umesh M.K. and Tukappa A. 2014. Antibacterial attributes of apigenin, isolated from *Portulaca oleracea* L. Int. J. Bacteriol. 2014:1-9. DOI: doi.org/10.1155/2014/175851

Oduje A.A., Rapheal O.S.and John A.C. 2016. Assessment of the antioxidative properties of *Hyphaene thebaica* fruit and its comparative inhibitory activities with butylhydroxyanisole on A-amylase and A-glucosidase enzymes. Int. J. Complement. Altern. Med. 4:1-6.

Olajuyigbe O.O. and Afolayan A.J. 2011. Phytochemical assessment and antioxidant activities of alcoholic and aqueous extracts of *Acacia mearnsii* De Wild. Int. J. Pharmacol. 7:856-861. DOI: doi.org/10.3923/ijp.2011.856.861

Padmavati M., Sakthivel N., Thara K.V. and Reddy A.R. 1997. Differential sensitivity of rice pathogens to growth inhibition by flavonoids. Phytochemistry 46:499-502. DOI: doi.org/10.1016/S0031-9422(97)00325-7

Pandey K.B. and and Rizvi K.I. 2009. Plant polyphenols as dietary antioxidants in human health and disease. Oxid. Med. Cell. Longev. 2:270-278.

Prieto P., Pineda M. and Aguilar M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal. Biochem. 269:337-341. DOI: doi.org/10.1006/abio.1999.4019

Said R.B., Hamed A.I., Essalah K., Al-ayed S., Boughdiri S., Tangour B., Kowalcyzk M., Moldoch J., Mahalel U.A. Oleszek W. and Stochmal A. 2017. Fast characterization of C-glycoside acetophenones in *Medemia argun* male racemes (an Ancient Egyptian palm) using LC-MS analyses and computational study with their antioxidant effect. J. Mol. Struct. 1145:230-239. DOI: doi.org/10.1016/j.molstruc.2017.05.105

Singleton V.L., Orthofer R. and Lamuela-Raventós R.M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-ciocalteu reagent. Methods Enzymol. 299:152-178. DOI: doi.org/10.1016/S0076-6879(99)99017-1

Taha G.A., Abdel-Farid I.B., Elgebaly H.A., Mahalel U.A., Sheded M.G., Bin-Jumah M. and Mahmoud A.M. 2020. Metabolomic profiling and antioxidant, anticancer and antimicrobial activities of *Hyphaene thebaica*. Processes 8(3):1-13. DOI: doi.org/10.3390/pr8030266

Velika B. and Kron I. 2012. Antioxidant properties of benzoic acid derivatives against superoxide radical. Free Radicals Antioxid. 2:62-67. DOI: doi.org/10.5530/ax.2012.4.11

Vichai V. and Kirtikara K. 2006. Sulforhodamine B colorimetric assay for cytotoxicity screening. Nat. protoc. 1:1112-1116. DOI: doi.org/10.1038/nprot.2006.179

Wang H., Chiu L.C., Ooi V.E. and Ang, P.O. 2008. Seaweed polysaccharides with anticancer potential. Bot. Mar. 51:313-319. DOI: doi.org/10.1515/BOT.2008.041

Wilkins L.R., Brautigan D.L., Wu H., Yarmohammadi H., Kubicka E., Serbulea V., Leitinger N., Liu W. and Haaga J.R. 2017. Cinnamic acid derivatives enhance the efficacy of transarterial embolization in a rat model of hepatocellular carcinoma. Cardiovasc. Interventional Radiol. 40:430-437. DOI: doi.org/10.1007/s00270-016-1515-y

Yamagata K., Izawa Y., Onodera D. and Tagami M. 2018. Chlorogenic acid regulates apoptosis and stem cell marker-related gene expression in A549 human lung cancer cells. Mol. Cell. Biochem. 441:9-19. DOI: doi.org/10.1007/s11010-017-3171-1.

Zhishen J., Mengcheng T. and Jianming W. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 64:555-559. DOI: doi.org/10.1016/S0308-8146(98)00102-2

Paper Received May 3, 2020 Accepted June 16, 2020