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Comparison of ultrasound and maceration methods on antioxidant and antimicrobial efficacy of phenolic compounds extracted from *Cynodon dactylon* L. of Algeria

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Abstract. An investigation was carried out to extract the polyphenols from *Cynodon dactylon* L. by ultrasound-assisted extraction (UAE) and maceration assisted extraction (MAE), and to assess the antioxidant and antimicrobial activities, as well as the evaluation of the cytotoxic effect of ethanolic extracts. The yields of crude extracts were 9.40 % for the MAE extract and 12.52 % for the UAE extract. The results showed that the extract obtained by MAE contains a high level of polyphenols and flavonoids estimated by 42.14 ± 0.75 mg EAG/gE and 23.57 ± 0.78 mg EQ/gE. In contrast, the content of condensed tannins in the extract of UAE (19.34 ± 0.48 mg EC/gE) is higher. The evaluation of the antioxidant activity revealed a considerable antioxidant response, the MAE extract represents the most active extract, with an IC_{50} =7.52 ± 0.037 mg/mL for the DPPH test, and 15.83 ± 0.37 mg EAA/gE for the FRAP test. The results of antimicrobial activity showed that all the strains targeted have high susceptibility to the two ethanolic extracts of *Cynodon dactylon* L. The evaluation of cytotoxicity against RBCs was carried, the results shown the non-toxic effect of *C. dactylon* extracts and hence support its ethnomedicinal application.

***Keywords*:** *Cynodon dactylon*; phenolic compounds; antioxidant activity; antimicrobial activity; cytotoxicity.

1. Introduction

While globalization has generated many benefits for society, it has also raised new challenges, particularly with regard to health. In recent years, there have been notable increases in the occurrence of many diseases which affected the human health around the world [1]. One of the health challenges are infectious diseases. There are many pathogenic microorganisms who cause infections which treatment with the usual antibiotics is not effective. The pathogenic bacteria have developed resistance to the antibiotic drugs [2]. Antibiotic-resistant bacteria are expected to cause 10 million fatalities each year, according to a report submitted to the United Nations in 2019 [3]. In the present scenario, this multiple drug resistance necessitates a search for new antimicrobial substances from natural sources [4].

Plants are widely used as a potent source for isolation of several drugs and formulations in treatment of many diseases. There arises a need to screen medicinal plants for bioactive compounds as a basis for further pharmacological studies [5]. Besides, plant extracts have been shown to be clinically effective and relatively less toxic than existing drugs [6]. Characterization and proper quality confirmation are imperative steps in guaranteeing the quality of home-grown medication, which helps in rationalizing its security and adequacy [7].

Cynodon dactylon L. Pers is a perennial herb with some traditional properties such as anti-inflammatory, diuretic, antiemetic, antidiabetic and blood purifying agent and other diseases [8], because of the presence of important phytoconstituents - flavonoids, carotenoids, phenolics, phytosterols, glycosides, and saponins [9]. It used also as a folk remedy for cancer, convulsions, cystitis, diarrhea, hemorrhage, hypertension [10].

Extraction is the first and most important step in isolating bioactive chemicals. The extraction method has been shown to have a substantial impact on the polyphenol content as well as the antioxidant potential of plant extracts [11]. Ultrasound is used to the cell disruption and particle size reduction that helps in improved penetration of the solvent into the cell and enhances mass transfer to be extracted as compared with conventional methods [12]. As a result, the phytochemical characteristics and activities of *C. dactylon* were studied using both conventional and ultrasonic techniques.

The main objective of this study was designed to evaluate this medicinal herb and to figure out the biological activities of the aerial part of *C. dactylon* extracts. Moreover, this study evaluates the effect of the extraction methods: Maceration (MAE) and Ultrasound (UAE) on the plant compounds, the antioxidant, antimicrobial activity and cytotoxicity of ethanolic extracts of *C. dactylon*.

2. Experimental

2.1. Materials

Chemicals used for this work (2,2-diphenyl-1-picrylhydrazyl (DPPH), $AlCl_3$, NH_4OH, ascorbic acid, catechin, dimethyl sulfoxide (DMSO), Dragendorff, EtOH, Folin-Ciocalteu, gallic acid, HCl, $FeCl_3$, $MgCl_2$,

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MeOH, $K_3[Fe(CN)_6]$, quercetin, Na_2CO_3, NaCl, sodium dodecyl sulfate (SDS), NaOH, sodium phosphate buffer (SPB), H_2SO_4, trichloroacetic acid (TCA), vanillin) were purchased from Sigma-Aldrich and used as received.

2.2. Methods

2.2.1. Phytochemical tests. The solution used for the chemical group detection tests was conducted on the aqueous extracts of the vegetable powders obtained by decoction [13]. 5 g of dried powder of *C. dactylon* were boiled with 100 ml of distilled water for 30 min in a water bath. Then the extracts are filtered using Whatman N°01 paper. This preparation has been preferred to be as close as possible to the conditions of use of *C. dactylon* in traditional medicine. We used the analytical techniques published in literature [14-17].

2.2.2. Extraction of polyphenols. Maceration and ultrasound-assisted extraction procedures were employed for polyphenols extraction from *C. dactyl*on powder.

2.2.2.1. Maceration assisted extraction (MAE). The extraction is carried out according to the published methods [18, 19], with some modification. 20 g of *C. dactylon* were extracted with 400 ml of 80% ethanol in an Erlenmeyer flask at room temperature for 24 h. Erlenmeyer flasks were completely covered with aluminum foil to prevent degradation of molecules photosensitive. This maceration is repeated 3 times with solvent renewal. The hydro-alcoholic extract is recovered after filtration using an N°01 filter paper, the ethanol is removed from the filtrate by evaporation under reduced pressure in a rotary evaporator (Büchi R-210) and then oven-dried for at least 48 hours at temperature does not exceed 40°C, collected in amber glass bottles and stored at refrigeration temperature.

2.2.2.2. Ultrasound-assisted extraction (UAE). Indeed, the extraction can be carried out in a very simple manner using an ultrasonic bath [20]. The ratio of vegetable powders to ethanol for assisted ultrasonic extraction is the same as for maceration, to facilitate comparison between the two methods. 20 g of *C. dactylon* were placed here in a 500 ml Erlenmeyer flask, itself placed in an ultrasonic bath (Power-Sonic, China) thermostatically controlled at 30 °C to compensate for the temperature increases caused by the high molecular agitation generated by the ultrasound for 30 min. The resulting mixture was subsequently treated in the same way as the mixture obtained by maceration [21].

2.2.3. Quantitative estimation of phenolic compounds

2.2.3.1. Total polyphenols estimation. The total polyphenol content of *C. dactylon* was measured by the Folin-Ciocalteau method [23]. Briefly, 200 µl of each extract dissolved in distilled or point of range water is added to 1 ml of Folin-Ciocalteu reagent (diluted 10 times in distilled water). After 4 min of incubation at room temperature, 800 µl of Na_2CO_3 (7.5%) also diluted in distilled water, are added to the mixture. The whole, previously shaken, is incubated in the dark for 2 hours. The absorbance is then read at 765 mm by a UV / visible spectrophotometer (SpectraMax PC 340). The concentration of total polyphenols for each sample is calculated from the regression equation for a calibration range established with gallic acid as a control under the same operating conditions as the extracts. The absorbance obtained is for 1 mg of dry extract calculated from the regression equation ($y = 3.9602x + 0.0341$; $R^2 = 0.9974$) for a calibration range in aqueous medium (0 to 0.2 mg/ml), The results were expressed in mg of gallic acid equivalent per g of dry vegetable material extracted (mg EAG/g E).

2.2.3.2. Flavonoid estimation. The method [24] used to determine the flavonoid contents of our samples is using $AlCl_3$ as reagent. Briefly, a 1 ml intake of extract or standard (prepared in 80% methanol) is added to 1 ml of a freshly prepared solution of $AlCl_3$ (2% in methanol). After 10 min of reaction, the absorbance is read with a spectrophotometer (SpectraMax PC 340) at 430 nm. The absorbance obtained is for 1 mg of dry extract calculated from the regression equation ($y = 11.198x - 0.0189$; $R^2 = 0.9932$) for a calibration range in aqueous medium established with quercetin (0-0.2 mg/ml). The results expressed in mg of quercetin equivalent per g of dry vegetable material extracted (mg EQ/g E).

2.2.3.3. Condensed tannins estimation. The determination of condensed tannins in extracts of *C. dactylon* is carried out according the published methods [25]. Briefly, to 400 µl of each sample or standard, 3 ml of a solution of vanillin (4% in methanol) and 1.5 ml of concentrated HCl are added. The mixture is incubated for 15 min, and the absorbance is read at 500 nm. The absorbance obtained is for 1 mg of dry extract calculated from the regression equation ($y = 2.3905x - 0.0059$; $R^2 = 0.9974$) for a calibration range in aqueous medium established with catechin (0-0.5 mg/ml). The results expressed in mg of catechin equivalent per g of extract (mg EC/g E).

2.2.4. Evaluation of biological activity. The biological activities to be carried out are the antibacterial, the antioxidant and the hemolytic activity of the crude polyphenol extracts (MAE and UAE) of *Cynodon dactylon*.

2.2.4.1. Antioxidant Activity

a. DPPH radical trapping test. This spectrophotometric assay uses the stable radical, DPPH as a reagent [26]. The test was performed by mixing 50 µl of extract or standard with 1.95 ml of DPPH dissolved in methanol (4 %). After shaking, the reaction was placed safe from light during 30 min and the absorbance was read at 517 nm. Inhibition of free radical DPPH in percent (I %) was calculated in following way:

$$I\,\% = \left(A_{blank} - A_{sample} / A_{blank}\right) \times 100 \qquad (1)$$

Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration. Tests were carried out in triplicate [27].

b. Ferric reducing antioxidant power (FRAP). Reducing power of extracts of *C. dactylon* was measured by the methods published in literature [28]. This method is based on the reduction of Fe^{3+} in $[Fe(CN)_6]^{3-}$ to Fe^{2+}. For this purpose, 1 mg of each extract was diluted with 1 ml of distilled water. 0.5 ml of diluted extract was mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5

ml of 1% $K_3[Fe(CN)_6]$ solution, followed by incubating in a water bath at 50 °C for 30 min. At the end of the incubation, 2.5 ml of 10% TCA were added to the mixture and centrifuged at 3000 rpm for 10 min. The supernatant (2.5 ml) was mixed with 2.5 ml of deionised water and 0.5 ml of 0.1% $FeCl_3$ freshly prepared was added, absorbance of these mixtures was measured at 700 nm using a UV spectrophotometer. A calibration curve with different concentrations (0 to 0.5 mg/ml) is carried out using ascorbic acid with the same experimental procedure. The results are expressed in mg equivalent of ascorbic acid per g of extract (mg EAA/g E)

2.2.4.2. Antimicrobial activity. Susceptibility of the microbial strains to the polyphenolics compounds and ethanol extract were investigated by using the agar diffusion method described in literature [29]. This method used to research the antimicrobial activity of *C. dactylon* extracts with discs of 6 mm in diameter which impregnated with 10 µl of each extract of *C. dactylon* with different concentrations (2.5 mg/ml, 5 mg/ml, 7.5 mg/ml, 10 mg/ml) dissolved in DMSO, are deposited using forceps on the surface of the agar medium. After the plates were incubated at 37 °C during 24 h for bacteria and at 25 °C during 48 h for fungi, the diameters of the distinctly clear zones were measured in millimeters. All the tests were performed in triplicate [30]. Negative controls (disks impregnated with DMSO) and positive controls (antibiotics) were tested. All tests are repeated three time. Gentamicin (GEN^{10}) and Penicillin (P^{10}) are the antibiotic chosen because of their broad spectrum of action.

2.2.4.3. Cytotoxicity against hRBCs. The hemolytic effect test of the plant studied is carried out according to published methods [31-33]. Freshly drawn blood on a heparinized tube is centrifuged at 4000 rpm for 5 min. After removal of the supernatant, the pellet is washed twice with 10 mM PBS, pH 7.4 containing 150 mM NaCl, then suspended again in this same buffer [32].

Different concentrations of plant extracts (20 mg/ml, 10 mg/ml, 5 mg/ml, 1 mg/ml and 0.1 mg/ml) were dissolved in the PBS and SDS with the same concentrations [31]. hRBCs are suspended in the 10 mM PBS buffer pH 7.4 (0.5 ml are brought into contact with 9.5 ml of PBS 10 mM, pH 7.4) [32]. The samples were added 1:1 (v/v) to the RBC suspension. The tubes are mixed gently, and the erythrocyte suspension is incubated at 37 °C for 60 min. As soon as the extract, which corresponds to the reaction time zero, samples of 500 µl from the reaction solution are taken at regular intervals (each 15 min), to which we have added 2 ml of an ice-cold washing solution (NaCl 150 mM, $MgCl_2$ 2 mM). After centrifugation at 4000 rpm for 5 min, we recovered the supernatant on which we measured the hemoglobin leakage by reading the optical density at a wavelength of 548 nm [32].

A negative control tube is prepared in the same experimental procedures without extract. A tube of total hemolysis contains the erythrocyte suspension and 2% aqueous SDS solution [31]. The hemolysis rate of the different extracts is calculated as a percentage (%) relative to the total hemolysis according to the following formula [33]:

$$Hemolysis\ rate\ (\%) = \left[\left(A_{Extact} - A_{Negative\ Control}\right)/A_{Positve\ Control}\right] \times 100 \quad (2)$$

2.3. Statistical analysis

The obtained results are expressed as an average ± standard deviation. Data was statistically analyzed by applying analysis of variance (ANOVA) technique to determine significance level. XLSTA software was used for conducting statistical analysis of data.

3. Results and discussion

3.1. Phytochemical screening

The results obtained by the phytochemical tests carried out on the aqueous extract of the *C. dactylon* plant are shown in Table 1.

Table 1. Results of phytochemical tests of the aerial part of *Cynodon dactylon*

Test	Reagent	Results
Polyphenols	$FeCl_3$	+
Flavonoids	Mg^{2+}	+
Alkaloids	Mayer	+
	Dragendorff	-
Terpenoids	H_2SO_4	+
Saponins	Foam test	+
Free quinones	NaOH	+
Anthraquinone	NH_4OH	-
Cardiac glycosides	H_2SO_4	+
Reducing sugars	Fehling	+

(+): Presence; (-): Absence

The phytochemical tests realized have highlighted various secondary metabolites in the aerial part of the plant including free quinones, saponosides, terpenes, alkaloids, reducing sugars, cardiac glycosides, flavonoids and polyphenols. Thus, the absence of certain compounds such as anthraquinones.

3.2. Extraction yield

The extraction yields obtained show that there is a slight difference between the yields of the two extractions and are respectively 9.40% for the maceration extract and 12.52% for the ultrasound extract.

3.3. Total polyphenol, flavonoids and condensed tannins content

The results of the estimation of the phenolic compounds (Table 2) showed that the extract obtained by MAE contains a high level of total polyphenols and flavonoids (42.14 ± 0.75 mg EAG/gE and 23.57 ± 0.78 mg EQ/gE), compared to the extract obtained by the UAE (29.93 ± 0.14 mg EAG/gE and 13.53 ± 0.33 mg EQ/gE) respectively. The total polyphenols and flavonoids content are very highly significantly different ($P < 0.05$) between the two extraction methods. From an explicative point of view, the low polyphenols and flavonoids content may be due to the cavitation effects induced by the ultrasonic microenvironment, which could cause changes in the molecular structure and conformation of compounds [34]. Also, the long extraction time increases the possibility of the oxidation of phenolic compounds [35-39].

In contrast, the content of condensed tannins (Table 2) in the extract of UAE (19.34 ± 0.48 mg EC/g E) is higher than the extract of MAE (15.99 ± 0.63 mg EC/g

E). The condensed tannins content is very highly significantly different ($P < 0.05$) between the MAE and UAE. Numerous studies have observed that the extended exposure time to sound radiations increased the temperature of the water bath [40]. Moreover, the increment of the temperature results in the improvement of the solute solubility and diffusion coefficient and decreases of solvent viscosity, which promotes the extraction [41]. These can explain the higher content of condensed tannins in the UAE extract.

Table 2. Total polyphenol, flavonoid and condensed tannins contents of ethanolic extracts of the tested plants

Extract	Total polyphenols	Flavonoids	Condensed tannins
MAE	29.93 ± 0.14^a	23.57 ± 0.78^a	15.99 ± 0.48^b
UAE	24.04 ± 0.63^b	13.53 ± 0.33^b	19.34 ± 0.63^a

All values were significant at $P < 0.05$. Different letters represent values that are significantly different between UAE and MAE methods at 5%.

3.4. Antioxidant activity

3.4.1. DPPH test. The DPPH • radical is generally one of the most widely used compounds for the rapid and direct evaluation of antioxidant activity due to its stability in radical form and the simplicity of the analysis [42]. The results obtained are expressed as a percentage of 50 % inhibition of the free radical DPPH• and compared to the reference molecules (ascorbic and gallic acid).

The scavenging activities of *C. dactylon* extracts on DPPH ($y = 3.4907x + 0.0235$; $R^2 = 0.9935$). According to the results (Table 3), MAE extract had high antioxidant activity ($IC_{50} = 7.52 \pm 0.037$ mg/ml) then UAE extract (IC_{50} 8.83 ± 0.032 mg/ml) and the difference between the two methods was significant with $P < 0.0001$. This could be explained by the extraction methods which had different levels of total polyphenols and flavonoids [43].

3.4.2. FRAP test. Ferric reducing power is linked with the antioxidant potential of a compound so it may serve as a good indicator of antioxidant activity. In this assay, reducing power assay is based on the reduction of Fe^{3+} to Fe^{2+} by antioxidant compounds [44].

The FRAP results (Table 3) indicate that the extract obtained by *MAE* had the highest antioxidant activity, reaching a value of 15.83 ± 0.37 (mg EAA/g). However, no significant difference between the two methods used were detected. The reducing power property of plant extracts indicates that the antioxidant compounds are electron donors [45, 46]. This can be interpreted by the richness of these extracts in phenolic compounds, it can therefore be deduced from this test that the polyphenols in particular the flavonoids play a very important role in the chelation of the transition metals [47].

Table 3. Antioxidant activities (FRAP and DPPH scavenging assays) of ethanolic extracts of *C. dactylon*.

Sample	DPPH (mg/mL)	FRAP (mg EAA/g)
MAE	7.52 ± 0.037^a	15.83 ± 0.37^a
UAE	8.83 ± 0.032^b	15.38 ± 0.12^a

All values were significant at $P < 0.05$. Different letters represent values that are significantly different between UAE and MAE methods at 5%.

3.5. Antimicrobial activity

The antibacterial activity of ethanolic extracts which we obtained by two methods (EAM, UAE) of *Cynodon dactylon* L were screened in this study against bacteria and fungi by the technique of diffusion on disc [48]. The antibacterial activity was determined by measuring the diameter of zone of inhibition recorded (Table 4).

Table 4. Antimicrobial activity of extracts ultrasound assisted extract and maceration assisted extract extracts from *C. dactylon*

Extracts	Dose (mg/ml)	Inhibition diameters (mm)					
		P. aeruginosa	*S. typhimurium*	*S. aureus*	*B. cereus*	*A. carbonarius*	*A. parasiticus*
MAE	**2.5**	11.66±0.57	08.66±1.15	12.33±1.15	10.00±0.00	07.33±0.57	06.00±0.00
	5	11.66±0.57	09.00±0.00	13.33±0.57	11.00±1.15	08.00±0.57	06.00±0.00
	7.5	12.00±0.57	09.33±0.57	13.33±0.57	10.00±0.00	12.16±0.28	07.00±0.00
	10	13.00±0.57	10.00±0.57	14.00±1,00	10.00±0.57	10.00±1.15	13.16±1.04
UAE	**2.5**	07.66±1.15	07.66±1.52	09.00±1,00	07.00±1.73	06.00±0.57	06.00±0.00
	5	10.00±0.00	08.00±1.73	09.66±0.57	08.00±1.73	06.50±0.50	06.00±0.00
	7.5	09.00±1,00	09.00±1.52	10.66±0.57	09.00±1.00	09.66±0.57	06.00±0.00
	10	10.00±1.52	09.00±1.52	11.33±0.57	10.00±1.15	09.00±0.57	06.00±0.00

The results obtained show that all the extracts of *Cynodon dactylon* L have a significant inhibitory activity against bacterial and fungal growth with a different degree linked to the content of the extracts on polyphenols. These confirm the broad spectrum of antimicrobial activity of *C. dactylon* extracts.

In the present study, the two ethanolic extracts of *C. dactylon* were found to be active against the tested bacterial strains of Gram-positive *Staphylococcus aureus* and *Bacillus cereus*, which shows significant antibacterial activity with an inhibition zone diameter of 12.33 ± 1.15 and 10.00 ± 0.00 mm respectively for MAE extract, also 9.00 ± 1.00 and 7.00 ± 1.73 mm respectively for UAE extract at a minimum concentration 2.5 mg/ml.

The two extracts of *C. dactylon* were found to be active also against the tested bacterial strains of Gram-negative *Pseudomonas aeruginosa* and *Salmonella typhimurium*, which showed significant antibacterial activity with an inhibition zone diameter of 11.66±0.57 and 08.66±1.15 mm respectively for MAE extract at a minimum concentration 2.5 mg/ml. These results increased with the increase of concentration. So, in the high concentration 10 mg/ml, we obtained 13.00±0.57

and 10.00±0.57 mm for *P. aeruginosa* and *S. typhimurium*. For the UAE extract the antibacterial activity are less important than the MAE extract, with 10.00±1.52 and 09.00±1.52 mm respectively for *P. aeruginosa* and *S. typhimurium* strains. This study revealed that there is no significance between gram-negative and gram-positive bacteria in term of susceptibility to the ethanolic extracts of our plant.

Fungi are ubiquitous in the environment, and infection due to fungal pathogens has become more common. The aim of this work was to evaluate in vitro the potential antifungal activity of *Cynodon dactylon* extracts which obtained with MAE and UAE against *Aspergillus carbonarius* and *Aspergillus parasiticus*. The two fungal strains, *Aspergillus carbonarius* and *Aspergillus parasiticus*, are evaluated in relation to our extracts (MAE and UAE), the results show that the extract of MAE has a high sensitivity for the strain of *A. parasiticus* by an inhibition zone of 13.16 ± 1.04 mm. On the other hand, this strain is completely resistant to the extract of UAE. Contrariwise, the *Aspergillus carbonarius* strain showed an inhibition zone of 10.00 ± 1.15 and 09.00 ± 0.57 mm respectively for MAE and UAE.

3.6. Cytotoxicity against hRBCs

This test can help more to understand and to develop antimicrobial and antioxidant compounds. A good antibiotic must be low toxic to human or animals [49]. Considering the use of this compound in medicine, the study of their hemolytic property must be carried out, considering the sensitivity of human beings to the hemolysis induced by certain substances [50].

The results (Figure 1) show the evolution of the hemolytic effect as a function of time, hemolysis rate (%), for 60 min, in a PBS buffer medium (pH = 7.4) containing an erythrocyte suspension, incubated at 37 °C, and in the presence of various concentrations of *C. dactylon* extracts.

In the present study, the results show slight difference between the two extracts, where the percentages of hemolysis of the UAE (Figure 1.c) concentrations tested are less than the hemolysis of the MAE (Figure 1.b) concentrations, with maximum hemolysis of 12.78% and 11.33% for MAE and UAE respectively. As we discuss the previous results, this is referring to the extraction method used in our study and content of phenolic compounds.

The results obtained show that the percentages of hemolytic effect are directly proportional to the increase in concentrations and ethanolic extracts (MAE and UAE) and in contact time with human red blood cells. After 60 min, the percentages of hemolysis of the UAE extract tested are less than the hemolysis of the MAE extract. Therefore, at a concentration of 20 mg/ml, after 60 min of contact with human erythrocytes (Figure 2), maximum hemolysis (12.78%) is obtained with the MAE extract followed by the UAE extract (11.33%) of *C. dactylon*.

However, when these two results are compared to the cytotoxicity of SDS, it becomes clear to us that SDS is toxic more than eight times the toxicity of the extracts.

Therefore, we can say that these two extracts may be slightly hemolytic at high concentrations and after one hour of contact with hRBCs.

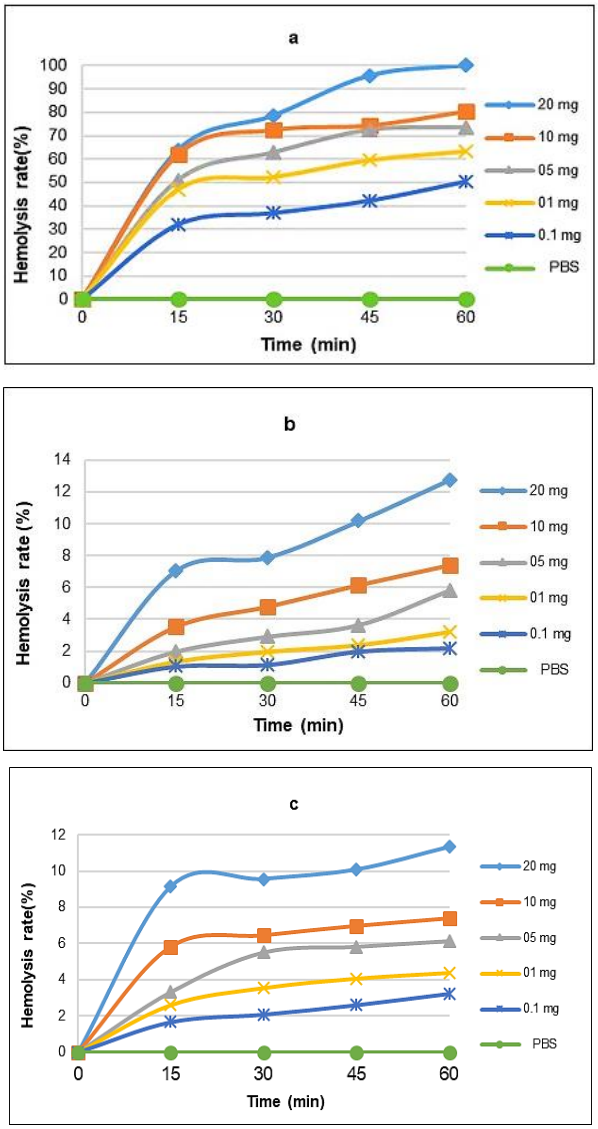

Figure 1. a. Effects of SDS on the leakage of intracellular hemoglobin in *hRBCs*; b. Effects of MAE extract solubilized in PBS on the leakage of intracellular hemoglobin in *hRBCs*; c. Effects of UAE extract solubilized in PBS on the leakage of intracellular hemoglobin in *hRBCs*

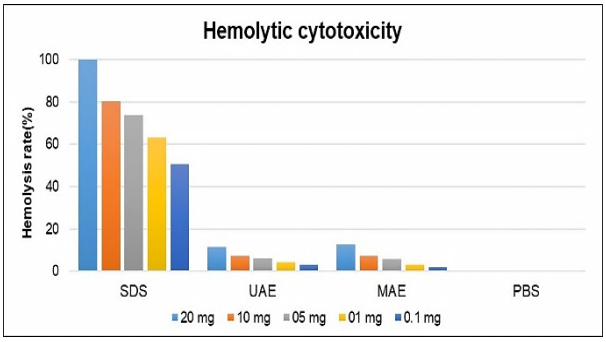

Figure 2. Comparison of the hemolytic cytotoxicity of the different ethanolic extracts on the *hRBCs* tested at different doses after 60 min of incubation.

The data on the patterns of hemolysis of polyphenols are scarce, and they are not evaluated on *Cynodon dactylon*. Therefore, this study provides new knowledge

about total effect of ethanolic crude extract of *C. dactylon*. Therefore, and based on [51], which mentioned that polyphenols are well tolerated by the human body, our results suggested the non-toxic effect of the extract thus making it suitable for the preparation of drugs involved in the treatment of various diseases.

4. Conclusions

The ethanolic extracts of *C. dactylon* L suggests that this plant represents a promising source of natural compounds. *C. dactylon* compounds have essential biological activities, which vary from one extract to another depending on the extraction procedure that has an impact on the type of the compounds present in the extracts as well as the effectiveness of their biological activities. Moreover, this preliminary study indicates a satisfactory effect of the compounds of *C. dactylon* extracts which making it suitable for the preparation of drugs involved in the treatment of various diseases.

Conflict of interest

Authors declare no conflict of interest.

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