



Original Research Article

Phytochemical studies of *Cynodon dactylon* (L.) and isolation and characterization of bis(2-ethylheptyl) phthalate from the plant

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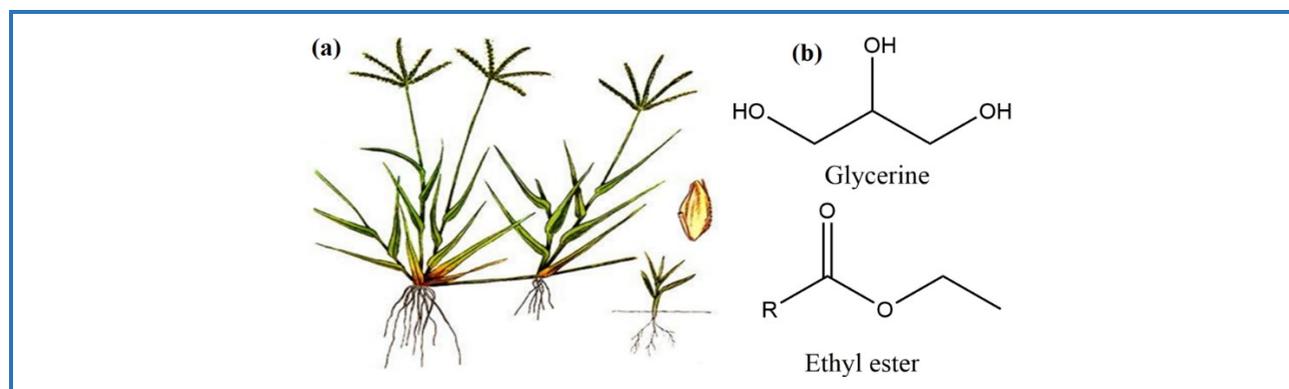
Fatty acid

ABSTRACT

In this study, the phylum *Cynodon dactylon* was assessed phyto-chemically. The results revealed that the plant had a rich source of phytochemicals as the root of this plant contains 830.27 mg/kg of carbohydrate. Also, 2.5 mg/g phenolic compounds were found in the ethanolic extract of the plant. Fatty acid composition of the plant as also investigated, wherein palmitic acid and linoleic acid had the highest contents in aerial parts of *C. dactylon* with 37.63% and 30.45%, respectively. In addition, high antiradical activity was found for the stem extract of the plant. Finally, bis(2-ethylheptyl) phthalate as an anti-oxidant and anticancer compound was extracted from the *C. dactylon* plant stem extract and fully identified and characterized using the FT-IR, CHN, ¹H NMR, ¹³C NMR analysis along with various 2D NMR techniques.

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Graphical Abstract



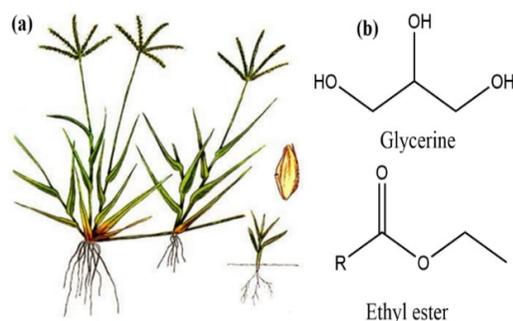
Introduction

Cynodon dactylon from the family of Graminae/Poacea, is a perennial plant with 1 to 30 cm long stems in the form of ascending stems or underground stems with short divisions [1, 2]. Its leaves are 2.5-10 cm long and are greenish-blue or dark green and have stellate-like inflorescences and have hyacinths without a peduncle in two rows on one side of the hyacinth [3]. Proliferation of the plant is mostly done by rhizomes, seeds or stolon and its habitat is mostly in the tropics. *Cynodon dactylon* has many medicinal properties for treating various diseases such as diarrhea, diabetes, hypertension, caries, wounds, and fever [4, 5]. It also has anti-microbial, anti-coagulant, anti-inflammatory, anti-malaria, anti-pain, and anti-virus effects. Extract of this plant can produce various types of phytochemicals such as alkaloids, saponins, phenols, flavonoids, tannins, sterols, glycosides, proteins, amino acids, and carbohydrates [1–6].

Phytochemical analysis showed that *Cynodon dactylon* contain flavonoids, alkaloids, glycosides, terpenoids, triterpenoids, steroids, saponins, tannins, resins, phytosterols, reducing sugars, carbohydrates, proteins, and volatiles and non-volatile oils, and also contain nutrients such as fat, fiber, iron, calcium, phosphorus, potassium and beta-carotene [3]. According to studies done on this plant, the highest content of glycosides is 12.2%, followed by tannins, resins and alkaloids, respectively [5–7]. Two examples of compounds isolated from this plant are shown in Figure 1.

Secondary metabolites are often small organic molecules produced by organisms, while organisms do not need them for growth, development and proliferation [8, 9]. Secondary metabolites can be classified according to the way they are synthesized and divided into three main groups, including: terpenoids, phenolic compounds, and highly diverse alkaloids [10]. Every year, several secondary metabolites are extracted from the plants and used as new sources for the treatment of various cancers; however, some of these natural compounds have unique anticancer effects and not directly used for treatment because of their physical and chemical or toxic properties. On the other hand, secondary plant metabolites can often lead to drug development, thus modifying the chemical structure of these compounds to increase their anticancer activities and reduce side effects [11].

Figure 1. a) *Cynodon dactylon* (L.) Pers.
b) structure of compounds extracted from *Cynodon dactylon* leaves



Phenols are the largest group of secondary metabolites used as anti-allergy, anti-inflammatory, anti-microbial, anti-bacterial, antiviral and antioxidant. Phenols are also used for treating the cardiovascular disease [4]. Although most of the properties are related to the removal of free radicals and antioxidant activity, phenolic compounds are beneficial to human health. Flavonoids also have significant effects on the cellular function of mammals with their biochemical and medicinal functions. One of the pharmacological effects of these natural compounds is their relaxant effect on the intestine, vessels and smooth muscle [2]. The antibacterial, antiviral, anti-inflammatory, anti-cancer and anti-allergic effects have been proven along with many other flavonoids bioactive activities.

Many studies have shown the antioxidant activity and health benefits of anthocyanins in various fruits and vegetables. It was reported that, the anthocyanins can reduce the risk of various types of cancer due to their antioxidant, anti-tumor and anti-inflammatory effects on the body. Studies have shown that anthocyanins have acquired their natural ability to fight cancer by blocking cell proliferation and inhibiting tumor formation and interfering with carcinogenesis [7].

Fatty acids are aliphatic monocarboxylic acids found in animals and plants. These compounds play an important role in cellular synthesis and energy metabolism and are involved in the treatment of a variety of diseases including, diabetes and sudden infant death syndrome [10–12]. Deficiency of these compounds in the body can lead to kidney damage and severe growth retardation and may lead to miscarriage in pregnant women [6]. Deficiency of these acids in the body causes problems such as dry skin, hair loss, reduced body resistance to radio waves, dry mouth and eyes as well as cancer [1]. This group of fatty acids includes omega-3 fatty acids (linolenic acid), omega-6 (linoleic acid) and some extent omega-9 (oleic acid).

Phthalates (also called phthalic acid diesters) are a group of chemicals commonly used in the plastics industry as lubricant [13, 14]. Lubricants are usually added to other materials, especially polyvinyl chloride and other polymers such as rubber and styrene, to make them flexible and elastic. Phthalates can be used in some food packaging materials, adhesive used for paper, boards and plastics, cellulose resuscitated (cellophane), paper sheets, aluminum foil and sealants for bottles [15].

Due to the importance of this genus, the phytochemical properties and secondary metabolites of *Cynodon dactylon* and the possibility of extracting the active compounds in the extract are studied. In continuation of previous researches on the phytochemical studies, herein, phenolic, flavonol, flavonoid, carbohydrate, ash, fat, and anthocyanin contents were measured from various extracts. Furthermore, fatty acid composition of the plant was also investigated. Finally, bis(2-ethylheptyl) phthalate as a biologically active compound was successfully extracted from the plant extract, characterized, and identified.

Experimental

Instrumentation and materials

All the chemicals were purchased from the Sigma and Merck or Fluka chemical companies and used as received without further purification. All the solvents were distilled under the N₂ atmosphere and dried before use. Progress of the reactions and purity of the products were accomplished by thin layer chromatography (TLC). FT-IR spectra were obtained using a JASCO FT/IR 4600 spectrophotometer using KBr pellet. Composition of the fatty acid was investigated by the gas chromatography (GC) method using a Shimadzu-14B gas chromatography equipped with HP-1 capillary column and N₂ as a carrier gas. Anisole was used as internal standard. The ¹H NMR (300 MHz) and ¹³C NMR (75 MHz) analysis were performed using a Bruker AVANCE3 3-300 MHz instrument in CDCl₃ and DMSO-*d*₆ as a solvent and TMS as an internal standard. Atomic absorption analysis was performed using a Shimadzu AA-6300 instrument.

Collection of plant materials

Healthy leaves of *C. dactylon* were collected in May 2017 from near and surrounding places of Birjand-Shokat Abad. The collected fresh leaves were washed 2-3 times with running water followed by drying up to 10 days under sterile conditions.

Measurement of water soluble carbohydrates

The amount of soluble carbohydrates in plant tissues was measured using antron reagent by spectrophotometric method [16]. In this method, the standard glucose plot at 625 nm was used. Preparation of extract: 0.1 g of herbal sample was added to 100 mL of distilled water and the mixture as stirred for 1 h. The extract was filtered by a whatman filter paper and the residue was stored for determination of soluble carbohydrates at 4 °C. Preparation of antron reagent: 76 mL of sulfuric acid was diluted and, and 0.1 g of thiourea and 0.1 g of antron was added to the acid solution. The reagent was kept at 4 °C for further testing. Preparation of stoke glucose solution: 0.01 g of glucose dissolved in 10 mL distilled water and this solution is used immediately after preparation.

Measuring plant ash content

The powder of the plant was oxidized and burned using a graphite furnace and turned into ashes. The ash content was calculated using the weight of the fresh and residual ash.

Anthocyanin content

Table 1. Determination of effective constituents of *C. dactylon* (L.) plant (aerial parts) in different solvents by spectrophotometric method

Compound (mg/ g)	Organ	Solvent used for extraction			
		EtOH 80%	Ethyl acetate	Chloroform	<i>n</i> -Butanol
Total phenolic	Stem	2.5	0.264	0.328	1.462
(mg gallic acid/g)	Root	0.04	0.021	0.001	0.017
Total flavonoid	Stem	6.716	0.969	0.650	2.456
(mg rutin/g)	Root	0.0345	0.102	0.073	0.048
Total flavonol	Stem	8.132	1.185	0.995	3.946
(mg rutin/g)	Root	0.147	0.210	0.164	0.121

Table 2. The amount of phenolic and flavonoid compounds of *Leptochloa uniflora* [23]

Solvent	Phenol (mg/g)	Flavonoid (mg/g)
Petroleum ether	36.03	634.34
Chloroform	22.38	616.67
Acetone	79.36	79.36
Methanol	148.0	148.06

Table 3. The amounts of carbohydrates, ash, and raw fat in stem and root of *Cynodon dactylon* (L.)

Component	Organ	
	Stem	Root
Carbohydrate concentration (g/kg) ^a	89.53	830.27
Ash (%)	13.22	5.22
Fat (%)	3.2	5.2

^a Gram of carbohydrate per 1 kg of the plant

The anthocyanin content of the samples was measured by the method reported by Wagner [17]. Briefly, 0.1 g of the dry plant was placed in a mortar and rubbed and sanded with 10 mL of acidic methanol (MeOH: HCl (conc.) 1:99). Then, the extract was poured into the test tubes and incubated in the dark for 24 h at 25 °C. It was then centrifuged at 4000 rpm for 10 min and the absorbance was recorded at 550 nm. Concentration of anthocyanin in $\mu\text{mol/g}^{-1}$ of the plant was calculated using the following equation: $A = \epsilon bc$, where $\epsilon = 33000 \text{ M}^{-1}/\text{cm}^{-1}$.

Antioxidant activity

Table 4. The amount of heavy metals in the plant in stem and root of *Cynodon dactylon* (L.)

Heavy metal	Stem (mg/kg)	Root (mg/kg)
Fe	42835.1	46541.8
Cu	303850	10054.85
Zn	589.35	271.65
Ca	23980	33865
Mg	38755	91365
Mn	2758	1439.1
K	141370	346180
Na	142745	66833

Table 5. Fatty acid composition of stem oil of the *Cynodon dactylon* (L.)

Entry	Fatty acid	Type	Content (%)
1	Myristic acid (C14:0) ^a	Saturated	3.07
2	Palmitic acid (C16:0)	Saturated	37.63
3	Margaric acid (C17:0)	Saturated	2.7
4	Stearic acid (C18:0)	Saturated	3.62
5	Arachidic acid (C20:0)	Saturated	1.95
6	Palmitoleic acid (C16:1 cis)	Unsaturated ($\omega - 7$)	1.77
7	Linolenic acid (C18:3)	Unsaturated ($\omega - 3$)	30.45
8	Oleic acid (sum of cis and trans isomers) (C18:1)	Unsaturated ($\omega - 9$)	5.32
9	Linoleic acid (sum of cis and trans isomers) (C18:2)	Unsaturated ($\omega - 6$)	13.47

^a Saturated: unsaturated bond ratio

Different concentrations of stem extract (6.25-25 mg/mL⁻¹) were prepared with MeOH 80% under the ultrasonic irradiation. The tubes were incubated in the dark for 15 min, then centrifuged and the resulting extracts were stored at 4 °C until conducting the experiments. To prepare the DPPH solution, 4.36 mg DPPH was dissolved in 100 mL of MeOH 80%. Subsequently, 75 μ L of the extract was mixed with 3.9 mL of DPPH and the absorption was recorded at 517 nm after 30 min of mixing. Controls consisted of DPPH solution and solvent. Inhibitory percentages were plotted in terms of concentration of extract in mg/mL and IC₅₀ was obtained from the equation of the line. The percent inhibition of DPPH free radicals was obtained by Equation 1.

$$\text{Inhibitio percentage} = \frac{A_b + A_s}{A_b} = 100 \quad (1)$$

Where A_b and A_s are the absorption of blank and sample (containing plant extract), respectively. The experiments were also repeated with butylated hydroxytoluene (BHT) as a strong standard antiradical and the results were compared with the plant extract.

Preparation of plant extract for extraction of bis(2-ethylheptyl) phthalate compound

40 g of the *C. dactylon* leaves powder were transferred to a conical flask (800 mL), and then 480 mL of the chloroform was added to the flask. The conical flask containing *C. dactylon* leaves were shaken well for 24 h. Then, the extract was filtered using a whatman filter paper No.1 and the filtrate used for further analysis.

Measurement of phenolic compounds

To prepare the standard solutions, 1000 ppm gallic acid and 0.01 g of gallic acid were dissolved in 10 mL of 50% EtOH and subsequently 0, 20, 40, 60, 80, 100, and 120 ppm gallic acid solutions were prepared. Phenolic compounds were measured by a method proposed by Škerget [18]. Briefly, 0.5 mL of diluted or standard sample, 2.5 mL of folin ciocalteu reagent (10%) and 2 mL of sodium carbonate solution (7.5%) were mixed in darkness at room temperature. After 1 h, the absorbance of the solution was read at 760 nm.

Measurement of total flavonoid compounds

To prepare standard solutions (routine solution 1000 ppm), 0.01 g of routine was dissolved in 10 mL of 50% EtOH and subsequently 10, 20, 40, 60, 80, 100, and 120 ppm solutions were prepared. The flavonoid content was measured using the colorimetric method, which is based on the formation of a flavonoid-aluminum complex and a maximum absorption at 415 nm. 1 mL of the standard or extract was added to 1 mL of $AlCl_3 \cdot 6H_2O$ solution. Then, 3 mL of 10% sodium acetate solution was added to the solution. After 40 min, absorbance of the solutions at 415 nm was recorded by a spectrophotometer.

Measurement of total flavonol in extract

Measurements of flavonols were performed with a slightly modified aluminum chloride colorimetric method [19]. Routine was used as a standard and the absorption was recorded after 2.5 h by a spectrophotometer at 440 nm. Calibration curves were plotted using standard solutions of absorbance and measured based on the obtained equation of the concentration of flavonols in the extract.

Determination of raw fat and composition of fatty acid present in stem of C. dactylon

The percentage of raw fat in the powder stem and flower of *C. dactylon* was determined using the soxhlet in hexane for 5 h [20]. Extraction of triglycerides from the plant (aerial parts) was also performed in *n*-hexane for 24 h at room temperature. Transformation of triglyceride to the corresponding methyl ester was accomplished according to a procedure described elsewhere [21]. Then, the fatty acid composition of the oils was determined using the gas chromatography.

Results and Discussion

Phenol, flavonoid and flavonol

The flavonoid content was determined using the plotted calibration curve of standard solutions. For this purpose, extraction was performed with solvents of different polarity. Total phenol results were expressed in mg of gallic acid per gram of the plant, and total flavonoid and flavonol content in mg of rutin per gram of the plant. The values of the active compounds extracted are demonstrated in [Table 1](#). The highest extraction of phenolic compounds was related to 80% ethanol extract of plant stem (2.5 mg/g) and the lowest amount was in root chloroform extract (1.04×10^{-3}). The highest and lowest flavonoid content in 80% ethanol extract were shoot and root, respectively ([Table 1](#)). As the results show, the highest extraction of phenolic compounds in the extract with EtOH 80% (2.5 mg/g) was related to aerial parts of the plant, and the least phenolic compounds were related to the root chloroform extract of the plant that was 1.04×10^{-3} mg/g. It was reported that, high phenolic compounds are the main reason for the high antioxidant activity of some extracts, including polar extracts such as ethanol [14–17]. Because, based on the available evidences, there is a direct relationship between the amount of phenolic compounds and the antioxidant power of plants. Also, flavonoid and flavonol content was the highest in the stem extract of the plant with 80% ethanol solvent.

Based on the studies on *Leptochloa uniflora*, from Graminae/Poacea family, the amount of phenolic and flavonoid compounds has been measured by different solvents and the results were summarized in [Table 2](#). According to these results, this plant has high phenolic compounds and high flavonoid content. The presence of these compounds in a plant increases its antioxidant properties, which enhances the antioxidant properties of the plant, which is rich in phenolic and flavonoid content compared to the Graminae/Poacea family [22]. In another study, the amount of phenolic compounds for methanol and aqueous extracts of the *Oryza sativa* from the family of Graminae/Poacea, was 80.7 and 88 mg/g, respectively, which is acceptable [23].

Carbohydrates

According to the results, aerial parts and especially root of this plant have a rich source of carbohydrates with 89.53 and 830.27 mg/kg, respectively. High levels of water soluble sugars in *C. dactylon* can be an adaptive mechanism to increase the drought resistance. In stress conditions (in the plant), to maintain the osmotic balance and the ability to absorb more water from the root, enhancing the compounds such as carbohydrates that are involved in cell structure and promoting the plant growth to improve the osmotic regulation [16, 12, 22]. Significant carbohydrate levels have also been reported from studies of other Graminae/Poacea family. For example, the water-soluble carbohydrate content of *Bambusa vulgaris* was 62.48 g/kg [24]. Water soluble carbohydrates in the leaves of the *Napier grass*, from the Graminae/Poacea family, were also calculated as 174 g/kg [25]. According to these studies, these plants usually grow in arid and low-water areas due to their high carbohydrate content [26].

Ash content

The content of ash for stem and root of *C. dactylon* was determined as 13.22% and 5.22 % (Table 3). The higher the ash content of the plant reflects the higher mineral content in the plant. As the plant extracts minerals from the soil, they are stored in the tissue of the stem, leaf, and root. Therefore, the higher the mineral content in the soil, the higher its mineral content and, consequently, the higher the mineral content of the plant ash. According to studies on a number of different genes of Graminae/Poacea family, the % plant ash has been calculated. For example, % ash for a plant from the Graminae/Poacea family *Bambusa vulgaris* was 12.53% [27]. Also, *Phragmites australis* from Graminae/Poacea family has 20.3% ash [28]. In general, the % ash in Graminae/Poacea family is acceptable, and therefore the amount of metal elements extracted from them has desirable values [12].

In addition, the amount of heavy metal ions in the ash was measured using atomic absorption method. Table 4 represents the amount of Fe, Cu, Zn, Ca, Mg, Mn, K, and Na in the plant.

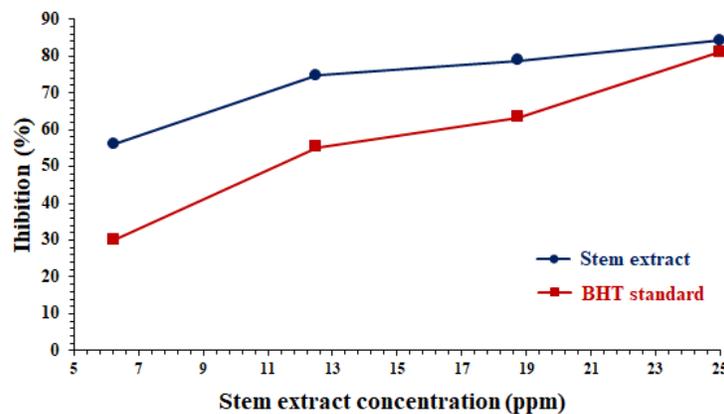


Figure 2. Inhibition percentage of stem extract of *C. dactylon* and BHT at various concentrations

Anthocyanin content

Using $A = \epsilon bc$, the concentration of anthocyanin in the aerial parts of the plant was found to be 1.3 $\mu\text{mol/g}$. According to a study on anthocyanin content in *Leptochloa uniflora* [29], the anthocyanin from the Graminae/Poacea family was 1.8 $\mu\text{mol/g}$, which is higher than other plants. Generally, plants in this family have acceptable levels of anthocyanin compounds.

Raw fat and fatty acid contents

The percentage of crude fat in shoots and flowers of chickpea was 3.2 and 2.5, respectively, which was not high compared to other Graminae/Poacea family [30]. The analysis of plant stem oil revealed 9 fatty acids (Table 5) that make up 100% of the total fatty acids in the oil. According to Table 5, palmitic acid with 37.63% was the highest amount. Then 30.45% for linolenic acid and 13.47% for cis and trans-linoleic isomers was observed respectively. Also, the results showed that 51.01% of stem oil is unsaturated fatty acids and cis fatty acids and 48.97% is saturated fatty acids. 37.63% of stem oil is palmitic acid and 30.45% is linolenic acid, which is a component of saturated fatty acids and omega-3 fatty acids, respectively. According to studies on different Graminae/Poacea family, different amounts of fatty acids have been extracted from them. For example, in the plant *Cymbopogon citratus*, 6 fatty acid compounds were extracted, the most of which being linolenic acid (36.63%), which is higher than that of *C. dactylon*. The amount of unsaturated fatty acids was 36.48% and 48.56%, respectively [31]. Also, in another study on the oil and fatty acid content of the plant *Oryza sativa*, from the Graminae/Poacea family, 17 fatty acid compounds were extracted from the plant, 88.99% of which were identified. In this plant, the highest percentage of fatty acid related to meristic acid was reported to be 42.8%, which is high compared to *C. dactylon* [32].

Antioxidant activity

After calculating the concentration of the methanol extract of plant water and BHT standard, IC_{50} (ability to eliminate 50% of DPPH free radicals) was obtained from the percent inhibition diagram in various concentrations of the stem extract (Figure 2). According to the diagram in Figure 2, the IC_{50} for the stem of the plant was 24.36 $\mu\text{g/mL}$. DPPH test was also performed on BHT standard antioxidant and IC_{50} value for this compound was 16.13 $\mu\text{g/mL}$, as shown in Figure 2. This indicates that, a lower concentration of the plant extract is needed to eliminate the DPPH free radicals. From the amount of IC_{50} obtained from the MeOH extract, it could be concluded that DPPH free radical inhibitor compounds in the plant are more potent than BHT in a same concentration.

Characterization of bis(2-ethylheptyl) phthalate

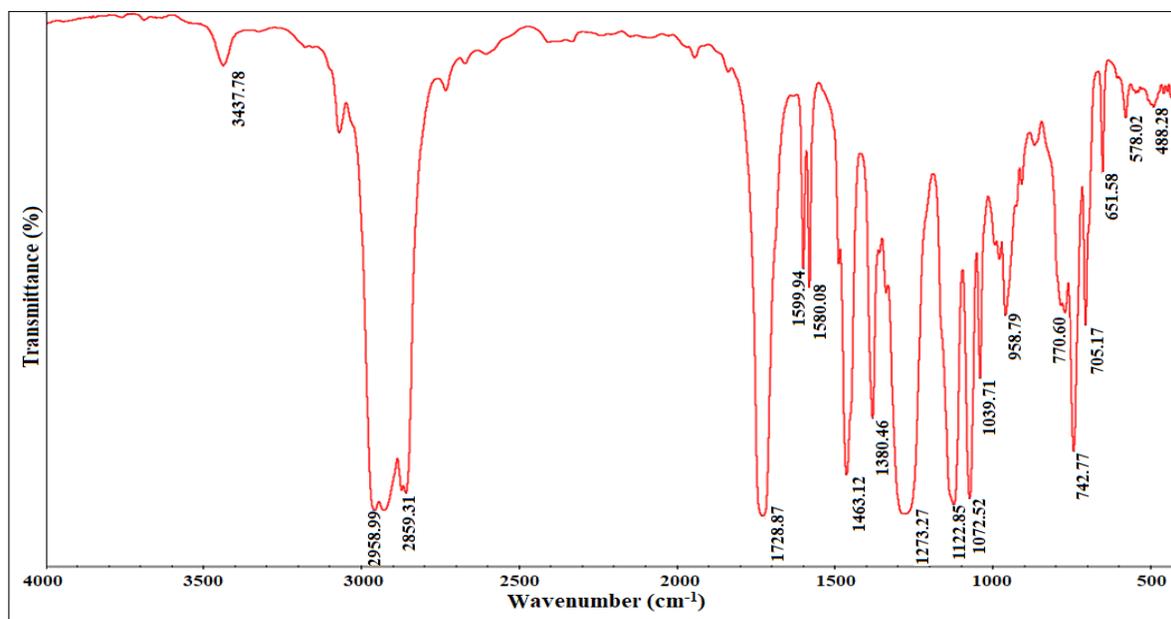
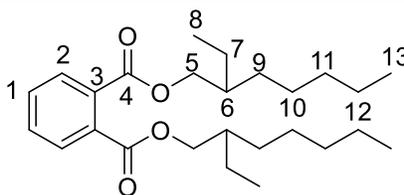


Figure 3. FT-IR spectrum of bis(2-ethylheptyl) phthalate

Table 6. ^1H and ^{13}C NMR data of bis (2-ethylheptyl) phthalate in CDCl_3



Position	δ_{C}	δ_{H}	#H, split (J in Hz)
1	130.88	7.53	2H, dd (8.8)
2	128.80	7.70	2H, dd (8.8)
3	132.47	-	-
4	167.74	-	-
5	68.13	4.21	4H, t (6.2)
6	38.75	1.65	4H, m
7	23.76	1.40	4H, m
8	10.96	0.90	6H, m
9	30.3	1.25	4H, m
10	28.9	1.29	8H, m
11	29.71	1.29	8H, m
12	22.99	1.31	4H, m
13	14.05	0.89	6H, m

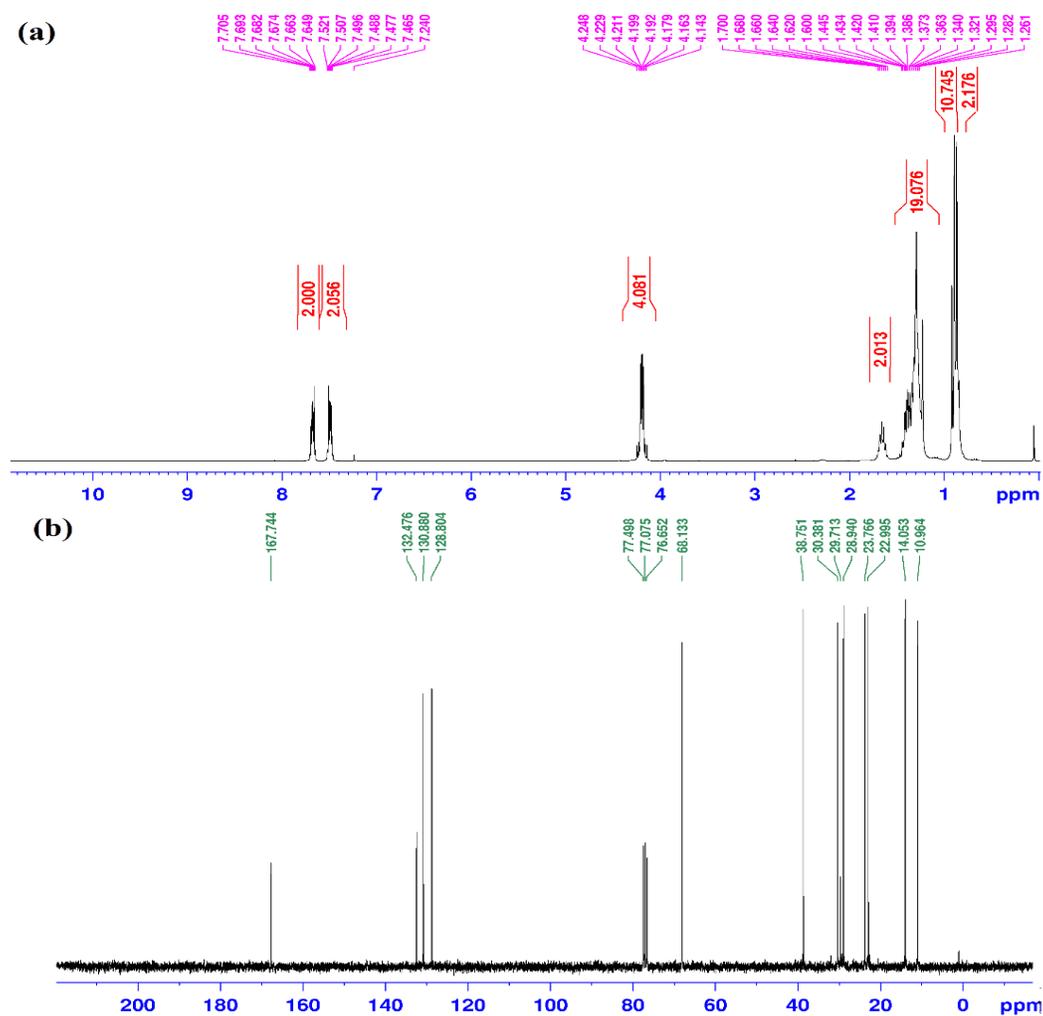


Figure 4. ^1H NMR and ^{13}C NMR of bis(2-ethylheptyl) phthalate in CDCl_3

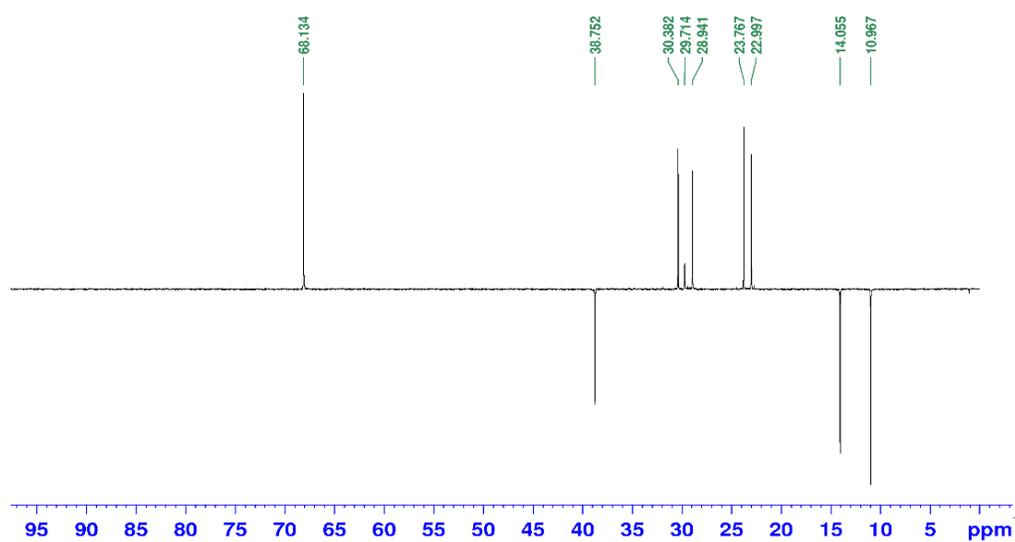


Figure 5. DEPT spectrum of bis(2-ethylheptyl) phthalate (75 MHz, CDCl_3)

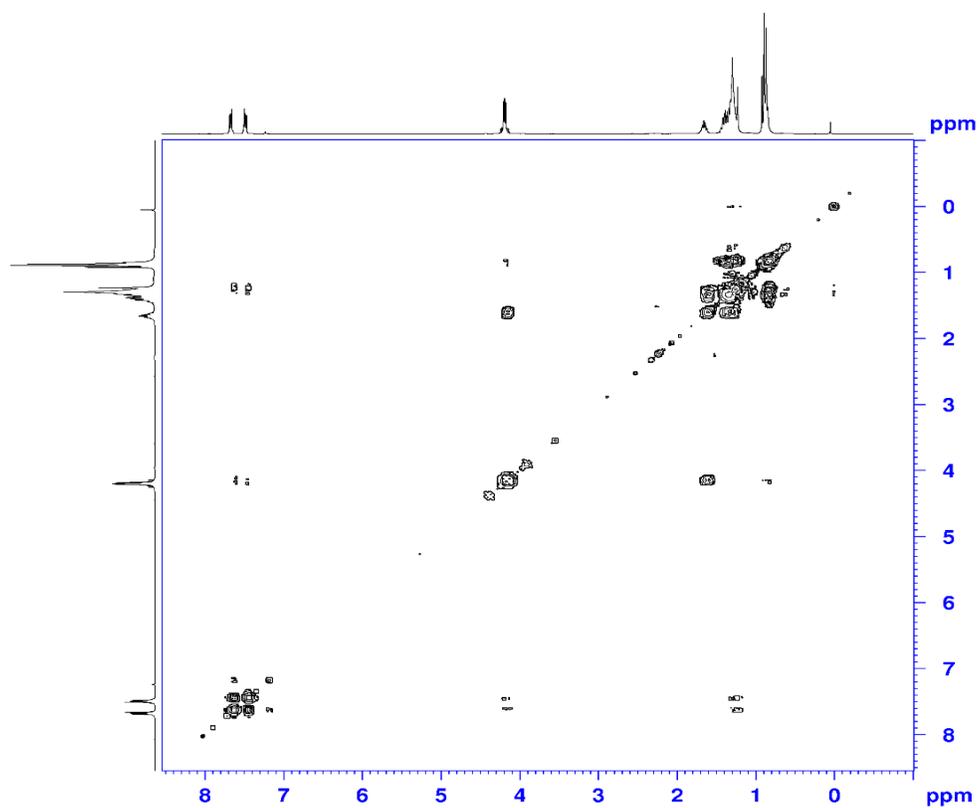


Figure 6. H-H COSY spectrum of bis(2-ethylheptyl) phthalate (300 MHz, CDCl₃)

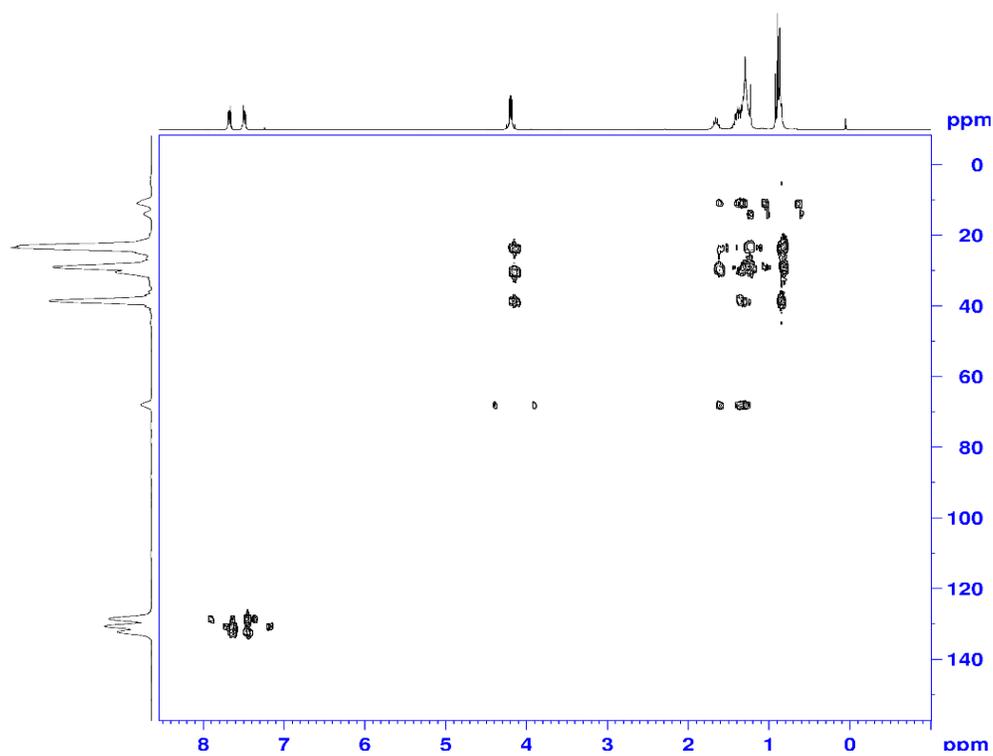


Figure 7. HMBC spectrum of bis(2-ethylheptyl) phthalate (CDCl₃)

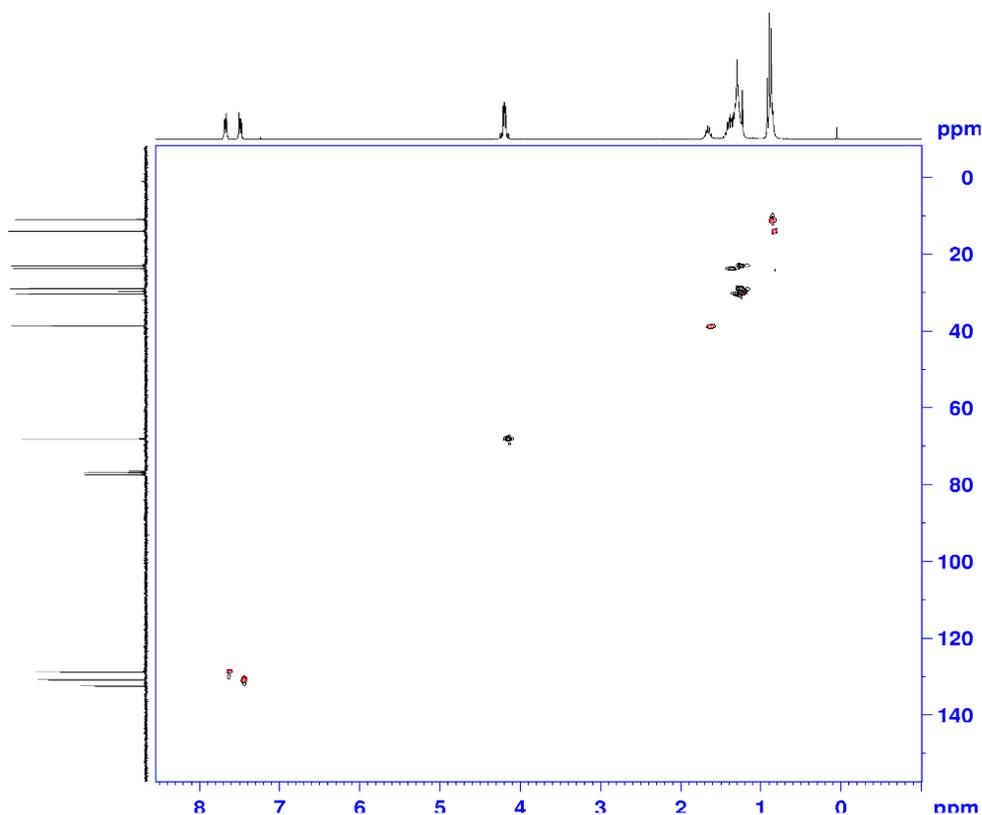


Figure 8. HSQC spectrum of bis(2-ethylheptyl) phthalate (CDCl_3)

The extracted bis(2-ethylheptyl) phthalate was fully characterized and identified by FT-IR, CHN, ^1H NMR, ^{13}C NMR, DEPT and various 2D NMR techniques such as HMQC, and HMBC. Elemental analysis of bis(2-ethylheptyl) phthalate (**1**) confirmed the chemical formula $\text{C}_{26}\text{H}_{42}\text{O}_4$ with 74.50% C, 10.33% H, and 15.15% O, very close to their theoretical values (74.60% C, 10.11% H, and 15.29% O).

A characteristic peak at 1728 cm^{-1} represent the stretching vibration of carbonyl groups (Figure 3). Also, the stretching vibrations related to aliphatic and aromatic C-H were appeared at 2859 cm^{-1} and 2958 cm^{-1} respectively in agreement with literature [14, 33].

Table 6 demonstrates the NMR data related to compound **1**. The ^1H NMR spectrum represents a typical AA'BB' system at $\delta = 7.70$ and 7.53 ppm (Figure 4a). Based on the chemical shift, coupling constants and how splitting, it was concluded that the compound is an ortho-disubstituted benzene ring involve the same substituent in both positions.

The ^{13}C , HMQC, and DEPT, NMR signals (Figure 5, 6, 7 and 8) confirmed the presence of two alkyl methane, two carbonyl carbons, four aromatic methine, four methyl, and two *O*-bearing methyleneand groups. Based on DEPT-90 (Figure 5), there are 6 methylene groups (negative signals) at 68.1, 23.7, 30.3, 28.9, 29.7, and 22.9 ppm, respectively for C5, C7, C9, C10, C11, and C12 [14]. The

methyl groups were appeared at 14.0 and 10.9 ppm for C13 and C8, respectively. In addition, three peaks at 130.8, 128.8, and 38.7 ppm were assigned to C1, C2, and C6 respectively (Figure 3b).

Conclusions

Cynodon dactylon is a rich source of carbohydrate (830.27 g/kg in root) and phenolic compounds (2.5 mg/g in root). The results showed that the main constituents of stem oil of *C. dactylon* were oleic acid, palmitic acid, and linoleic acid. It was also found that, 51.01% of stem oil is unsaturated fatty acids and cis fatty acids and 48.97% is saturated fatty acids. 37.63% of stem oil is palmitic acid and 30.45% is linolenic acid, which is a component of saturated fatty acids and omega-3 fatty acids, respectively. The IC₅₀ for the stem of the plant was found to be 24.36 µg/mL, making the plant extract as a promising candidate for antioxidant activity. In this study, the biologically active, bis(2-ethylheptyl) phthalate was extracted from the *Cynodon dactylon* plant stem extract. Its structure was identified and proved by FTIR, CHN, 1D (¹H NMR, ¹³C NMR, DEPT), and 2D (H-H COSY, HSQC, HMBC) NMR methods.

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Disclosure Statement

No potential conflict of interest was reported by the authors.

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