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Total phenolic contents, flavonoids, and antioxidant activity of the aerial parts extracts of (*Calotropis procera*) naturally growing in Samnu Oasis, southwest of Libya

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المحتوى الكلي للفينولات والفلافونويدات والنشاط المضاد للأكسدة لمستخلصات الأجزاء الهوائية لنبات (*Calotropis procera*) الذي ينمو طبيعياً في واحة سمنو جنوب غرب ليبيا.

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Abstract:
The present study highlights the importance of "*Calotropis. proceraa* (Aiton) W. T. Aiton" [5]. as an important source of many bioactive substances. Therefore, it can be used in pharmaceutical industries. The present study seeks to quantify the overall phenolic and flavonoid content in the aerial parts extracts of (*C. procera*), besides evaluating its antioxidant potential. This research is significant as it will contribute to the understanding regarding the therapeutic qualities of the plant (*C. procera*) and focus on one of the natural resources with antioxidant capacity. Results of phytochemical screening of different aerial parts extracts of *C. procera* presented and reveal that the flavonoids were present in extracts of leaves, flowers, and fruits (6.55, 4.59, and 4.67 mg/g dry weight) respectively, as well as the phenolic concentrations showed were present in extracts of leaves, flowers, and fruits (10.45, 7.22, and 6.55 mg/g dry weight), respectively.
Moreover, results of the anti-oxidant test showed that the highest anti-oxidant activity was present in methanolic extracts of leaves with an IC_{50} value of (0.17 mg/ml). While the lowest anti-oxidant activity was attained from the extract of flowers with an IC50 value of 0.24 mg/ml and presented in methanolic extracts of fruits with an IC50 value of 0.19 mg/ml. Based on in vitro tests, a relationship was observed among the outcomes from antioxidant tests and the levels of total phenolics and flavonoids. Besides, *C. procera* is considered promising, good candidates for natural resources of anti-oxidants for medical uses.

Keywords: *Calotrops procera*, Secondary metabolism, Anti-oxidant activity, Phytochemical.

الملخص
الدراسة الحالية تسلط الضوء على أهمية نبات العشار و أسمه العلمي (*Calotropis procera* (Aiton) W. T. Aiton ([5] . النبات يعتبر مصدر هام للعديد من المواد النشطة بيولوجيًا, لذلك يمكن استخدامه في الصناعات الدوائية و تهدف الدراسة الحالية إلى تحديد محتوى المواد الفينولية والفلافونويدات في مستخلصات الأجزاء الهوائية للنبات بالإضافة إلى تقييم إمكاناتها المضادة للأكسدة. هذا البحث مهم لأنه يساهم في فهم الخصائص الطبية لنبات (*C. procera*) والتركيز على أحد الموارد الطبيعية ذات القدرة المضادة للأكسدة وقد بينت نتائج التحليل الكيميائي في الدراسة الحالية لمستخلصات الأجزاء الهوائية المختلفة من النبات المدروس عن وجود الفلافونويدات في مستخلصات الأوراق والزهور والثمار بقيم (6.55، 4.59، 4.67 ملجم / جم وزن جاف) على التوالي، إضافة لذلك أظهرت تركيزات الفينول وجودها في مستخلصات الأوراق والأزهار والثمار بالقيم (10.45، 7.22، 6.55 ملجم / جم وزن جاف) على التوالي. ومن ناحية أخرى أظهرت نتائج اختبار مضادات الأكسدة وحساب قيمة (IC_{50}) ويقصد به تركيز المستخلص الذي يلزم لتثبيط 50% من الشارادات الحرة التي تكون ناتجة عن مادة (DPPH). إن أعلى نشاط مضاد للأكسدة كان في المستخلصات الميثانولية للأوراق بقيمة (0.17 ملجم/مل). بينما تم الحصول على أقل نشاط مضاد للأكسدة في مستخلصات الزهور بقيمة (0.24 ملجم/مل وبناءً

على الاختبارات المعملية، لوحظت علاقة مباشرة بين نتائج اختبارات مضادات الأكسدة ومستويات الفينولات الكلية والفلافونويدات. بالإضافة إلى ذلك يمكن اعتبار *C. procera* مرشحًا واعدًا وجيدًا كمصدر نباتي طبيعي لمضادات الأكسدة للاستخدامات الطبية.

الكلمات المفتاحية: نبات (*Calotrops procera*)، نواتج الايض الثانوية، مضادات الأكسدة، التحليل الكيميائي النباتي.

Introduction

Calotropis Procera is one of the common poisonous plants in Libya [1]. *C. procera* is recognized for its therapeutic characteristics; several parts of the plant have been employed in traditional medicine by Libyan locals for the treatment of various diseases, including the (Leaves uses To cure leucoderma , treat migraine, to treat fever, eczema, asthma, cough and rheumatism and flowers and roots uses to treat Skin infection) [2].

In Libya subfamily Asclepiadaceae comprises 8 genera, 8 species distributed throughout Libya according to Ying et al [3]. The plant species of subfamily Asclepiadaceae are annual or perennial herbs, shrubs, and and trees in infrequent instances. with copious milky latex [4]. *C. Procera* belongs to family Apocynaceae, subfamily Asclepiadaceae [5,6]. Members of the *Calotropis procera* are primarily widespread within the tropical and subtropical zones of Africa, Asia, and America. [7]. They have been extensively utilized in traditional medicinal methods across North Africa, the Middle East, South Asia, and Southeast Asia [8]. It is frequently referred to as milkweed due to its capacity to generate substantial amounts of latex [9,10]. Latex, a milky liquid composed of various physiologically active substances is produced in large quantities by the plant *C. procera* [11].

The recent studies focus on the plant secondary metabolites that exhibit many biological characteristics [12]. The Secondary metabolites are molecules that do not directly participate in normal growth, development, or reproduction, yet are essential for the plant's interaction with its environment. In recent years, the significance of plant secondary metabolites has escalated due to their prevalent applications as pharmaceuticals, insecticides, and food additives [13].
Most of the phytochemical's compounds are beneficial to human health and the ecosystem. Bioactive chemicals in plants are responsible about the biological impacts of plant species [14]. Phenolic chemicals are the principal antioxidant elements of botanical extracts.; they deactivate hydroxyl and peroxyl radicals. Scientific evidence suggests that antioxidants significantly diminish the likelihood of chronic ailments, such as cardiovascular disease and malignancies [15]. The current study seeks to measure the aggregate phenolic concentrations and flavonoid levels in the aerial parts of (*C. procera*), beside evaluating its antioxidant potential. This research is significant as it will contribute to the understanding of the therapeutic qualities of the (Calotropis *procera*) and focus on one of the natural resources with antioxidant capacity.

Material and methods: Discerption of plant.

The scientific name of the plant is "Calotropis procera (Aiton) W. T. Aiton" [5]. The plant is classified as a dicotyledon, an evergreen shrub, or a small-branched tree, with a height range of 2 to 5 meters. All parts of the plant contain a milky white sap. Stems are erect with many branches. The plant has simple, opposite, and sessile leaves, while its flowers are hermaphrodite, actinomorphic, white with purplish ends, five sepals, free, imbricate petals, joined, valvate, five stamens, epipetalous, superior ovary, and two locular. The fruits are subglobose, big, greyish-green follicles as shown in Plate (1). , with a tuft of long, white, silky hairs on the axile placenta and numerous brown, flattened seeds inside each fruit.

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Plate (1): Some views of aerial parts of Calotropis *Procera*.

Preparation of plant samples:
Samples of the *Calotropis procera* (leaves, flowers, and fruits) were collected in October 2023 from their natural habitat in the Samnu Oasis, southwestern Libya. The samples were preserved in plastic bags, and their botanical identification was confirmed using descriptions from the Libyan Flora, then dried and ground into a powder. Methyl alcohol was used to extract 100 g of dried plant powder, which was soaked overnight with frequent shackling. After filtering, the solution was dried off by evaporation. For later usage, the dried residue was stored after being dissolved in dimethyl sulfoxide.

Phytochemical Analysis:
Determination of Flavonoids and total Phenolics.
The flavonoids contained in the aerial parts extracts of *C. prosera* were determined using the spectrophotometric method [16]. whereas the estimation of total phenolics is done using the technique outlined by Singleton et al. [17]. It is based on the Ciocalteu-Folin reagent, which changes the phenolic compounds to a blue complex.

Determination of antioxidant activity
The activity of antioxidants was evaluated in a methanolic extract of desiccated plant (aerial parts of *C. prosera*) as published by Koseam et al. [18]. with tiny modifications, as follows: Approximately 20 g of powder samples were extracted with 200 ml of 50% methanol for one week at room temperature. DPPH: free radical scanning activity. This test is based on the ability of a plant extract or compound to inhibit free radicals. A compound's ability to donate a hydrogen atom was evaluated using the scavenging- activity of the stable 1, 1-diphenyl--2-picrylhydrazy--l (DPPH) radical, as described in [19]. Technique Two milliliters of 0.15 mM DPPH were mixed with 1 ml of diverse botanical extracts at varying Concentrations. A control was implemented. created by adding 2 ml of DPPH to 1 ml of solvent (methanol 50%). The contents of the tubes were combined and left to stand for 30 minutes before measuring the absorbance at 517 nanometers. The antioxidant activity was measured as follows: (DPPH %= [1-(A spp./A c)] x 100). A spp. denotes absorbance of plant sample, A c denotes absorbance of control at 517 nm. The (IC50), defined as the quantity (mg) of plant in 1 ml of solution necessary to reduce the initial concentration of DPPH radicals by 50%, was calculated. The antioxidant activity of catechol (ascorbic acid) was assessed for comparison.

Statistical analysis
All results from these studies were presented as mean (± SE) of the mean. Statistical analyses were performed using -SPSS-20.

Results and discussion
Phytochemical Analysis: Determination of Flavonoids and total Phenolics.

Table (1) The content of the flavonoids and phenolic components (mg/g dry weight) of the aerial parts of *Calotropis procera*.

parts of plant	Flavonoids (mg/g dry weight)	Phenolics (mg/g dry weight)
Leaves	6.55±1.20	10.45±1.45
Fruits	4.67±0.73	7.22 ±1.25
Flowers	4.59 ±0.61	6.55 ±1.08

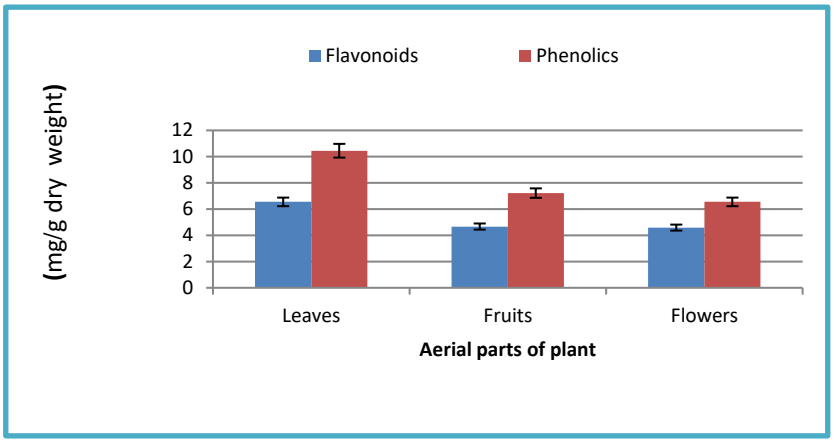

Figure (1) Flavonoids and Phenolics contents of the aerial parts of (*Calotropis procer*).

The results of phytochemical screening indicate that, the phytochemicals have the capacity to improve human health and produce new drugs against various diseases [20,21]. I n the other hand Scientific evidence suggests that, the importance of plants lies in certain chemicals in their cells, such as tannins, saponins, alkaloids, flavonoids and phenolic compounds [22,23]. Also, Habeeb et al indcated Calotropis *procera* has biological activities and the therapeutic potentials, this could be ascribed to the secondary metabolites such as (alkaloids, phenols and tannins) naturally found in plant [24]. Attained. The presence of these phytoconstitents in *calotropis prosera* were agreed with those mentioned by [25] Results of phytochemical (flavonoids and total phenolics) creening of current scientific paper of different Aerial parts extracts of *Calotropis procera* (Table 1) is presented revealed that :

Flavonoids are 0ne of classes of bioactive secondary metabolism with significant anti-oxidant [26]. The concentrations of flavonoid of the aerial parts presented a wide range of variations (figure 1). Leaves extract attained the highest value (6.55 mg/g dry weight) while flowers extract attained the lowest value (4.59 mg/g dry weight) Fruits extract attained (4.67, mg/g dry weight). it agrees more or less with Kanmegne and Omokolo reported analogous results.[27]. Also were agreed with reborted by Oraibi and Hamad [28]. Flavonoids are important compounds possess anti-oxidant [29].
Phenolics concentrations showed slightly wide range of variations (figure 2). in the aerial parts extracts of *Calotropis Procera* attained the highest value in leaves extract (10.45 mg.g-1 dry weight) Followed by fruits extract of with (7.22mg.g-1 dry weigh) while flowers extract attained the values of lowest phenolics content with (6.55 mg.g-1 dry weight). It agrees with that reported by Duan, Wong [30,31]. Flavonoids and phenols were higher values in all parts of *Calotropis procera* [32].
Phenolic components are very important botanical constituents which their (scavenging ability) on free radicals due to their hydroxyl groups. thus, highest concentration of phenols in extract of leaves (Table 1, Figure 2) have the highest antioxidant activity of (Table 2, Figure 2). This agreed with the report by Tosun et al [33].

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Antioxidant Activity: **DPPH free radical scavenging activity**.

Table (2) Percentage of (DPPH) radical- scavenging activity "(mean value ± standard error)" and (IC_{50} values) of methanolic extracts of the aerial parts of *Calotropis procera* and Catechol.

Aerial Parts extracts of C. *procera*	Concentration (ppm)	Scavenging activity (%)	IC_{50} mg/ml
Leaves	500	77.20±1.71	0.17
	400	65.12±1.41	
	300	51.55±1.20	
	200	42.27±0.92	
	100	29.13±0.63	
Fruits	500	70.56±1.54	0.19
	400	59.53±1.29	
	300	45.21±1.02	
	200	34.41±0.75	
	100	28.55±0.62	
Flowers	500	55.81±1.21	0.24
	400	42.55±0.92	
	300	30.34±0.66	
	200	21.25±0.46	
	100	5.90±0.13	
Catechol		0.15	

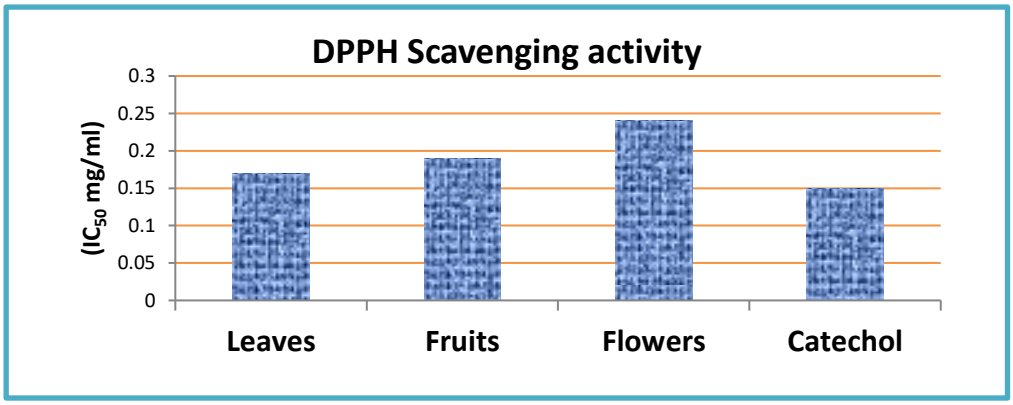

Figure (2) IC_{50} values (mg/ml) of the aerial parts of *Calotropis procera* and natural anti-oxidant catechol.

The DPPH assay is one of the important techniques for measuring antioxidant activity by using the stable purple free radical (DPPH). Anti-oxidants neutralize these radicals by donating hydrogen, resulting in a color change to yellow. The (IC50 value) is calculated to determine the concentration of anti-oxidant needed to inhibit 50% of the radicals, with lower values indicating higher effectiveness.

Table 2 Figure (2) represent the amount (mg/ml) of the methanolic crude extracts for the studied *Calotropis procera*. parts. and the IC50 of each part. The IC50 values ranged from 0.17 to 0.24 mg/ml. where the extract of leaves showed the highest anti-oxidant activity with IC50 value of 0.17 mg/ml. While the lowest anti-oxidant activity was attained from the extract of flowers with IC50 value of 0.24 mg/ml. The extracts of fruits with IC50 value of 0.19 mg/ml. The extracts of natural antioxidant catechol were attained the IC_{50} value of 0.15 mg/ml. The results are consistent with those of Wong et al., who reported that the highest total antioxidant activity was in the leaves of *Calotropis procera* it could be ascribed to the phenolic compounds in plants [30, 33]. In general, total phenolic compounds of plants are important constituents imparting it anti-oxidant potentially [31] Flavonoids are important compounds possess anti-oxidant [29,34].

Conclusion

The results revealed that the leaves have the highest flavonoid and phenolic content and show higher antioxidant activity than the flowers and fruits. *Calotropis procera* is a promising source of bioactive compounds, which can be used in the development and production of several drugs.

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