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EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
BOTANY

ISSN 2090-3812

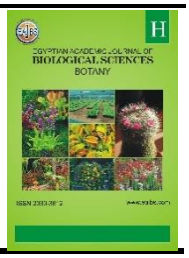
www.eajbs.com

Vol. 12 No.2 (2021)

Citation: Egypt. *Acad. J. Biolog. Sci.* (H. Botany) *Vol.12(2) pp191-205(2021)*
DOI: 10.21608/EAJBSH.2021.298891

Egypt. Acad. Journal Biolog. Sci., 12(2): 191-205 (2021)

Egyptian Academic Journal of Biological Sciences
H. Botany
ISSN 2090-3812
www.eajbsh.journals.ekb.eg.



Physiological Approaches to Evaluate the Antioxidant and Antimicrobial Activities of Two Wild Euphorbiaceous Species of the Egyptian Flora

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ARTICLE INFO
Article History
Received:30/09/2021
Accepted:27/12/2021

***Keywords*:**
Euphorbaceae, Medicinal plants, secondary metabolites, Antioxidant and Antimicrobial agents.

ABSTRACT

Although plant-based medicine is a traditional approach since the ancient eras, remarkable progress was observed last decades in using different conventions in introducing, analyzing and comparing medicinal plants on scientific bases. Thus, we established this work with the goal in mind to compare the morphological traits, essential bioactive secondary metabolites and the total antioxidant capacities of two *Euphorbaceae* members; *Euphorbia maculata* and *Euphorbia peplus*. Moreover, we aimed also to evaluate the antimicrobial activity of these two Euphorbes as biological resources against some microbial strains *viz*., *Staphylococcus aureus*, *Bacillus subtili*, *Escherichia coli*, *Salmonella typhimurium*, *Saccharomyces cerevisiae* and *Aspergillus flavus*. The obtained bio-growth markers referred to the stronger invading nature of *Euphorbia maculata* over *Euphorbia peplus*. This conclusion is additionally strengthened by the higher levels of antimicrobial activity of *E. maculata* versus *E. peplus.* In support, the total carotenoid content of *E.maculata* augmented also that of *E. peplus* by 49.4%. On the contrary, the total flavonoids and phenolics contents recorded 92.65% and 83.05 % increments for *E. peplus* over *E.maculata*. These contradictory results may give us a good interpretation of the convergent total antioxidant capacities; 5155.2 and 6685Ug/g F.Wt in *E. maculata* and *E. peplus* respectively. From the previously mentioned findings, the authors recommended, *Euphorbia maculata* and *Euphorbia peplus,* these invasive species, be employed as exceptional natural antimicrobial managers that could be adopted for the improvement of new medications dealing safely with infectious diseases. Besides, these biological resources consider more confident antioxidants in the food packaging industries.

INTRODUCTION

A tremendous number of plants possess a vast array of bioactive phytochemicals, which lend plants medicinal and therapeutic properties and allow plants to be utilized in numerous claims in the pharmaceutical industry for alleviating the ailments of humankind (Ncube *et al.*, 2008). Since the prehistoric era, people have used medicinal plants to treat infections as a common practice. Even so, as of now, plants are the only source of essential healthcare benefits for three-quarters of the world's population (Raji *et al*, 2019). Owing to their affordability and accessibility, medicinal herbs are indispensable modules of traditional medicine used all over the world (Natarajan *et al*., 2005).

The third-largest genus of flowering plants, Genus *Euphorbia*, is one of the prevalent genera and comprises a variety of plant types, including herbs, shrubs, and trees, either succulent or non-succulent. Its classification and chemistry are a rich scope for search as a result of the so-varied chemical composition of it's compounds which are frequently of significant value even being poisonous in many cases (Govaerts *et al*., 2000 and Mwine and Van Damme, 2011).

The euphorb plant distributes all over the world, especially in temperate and tropical regions of the Americas, Africa, as well as through the Caribbean. Because *Euphorbia* species have floristic diversity, they contribute to the economic significance of their localities (Bingtao *et al*., 2008).

The widespread distribution of Euphorbiaceae species them to a variety of circumstances, to which they must adapt, leading to the development of a wide range of secondary metabolites for survival/defense. Some of the modifications that help Euphorbs in colonisation and survival were determined as the succulence and CAM (crassulacean acid metabolism) pathway, which are characteristics of a respectable number of Euphorbiaceae (Mwine and Van Damme, 2011).

The therapeutic qualities of some Euphorbiaceae species may be a natural consequence of the stress factors found across the majority of the family's habitats. Diverse stress factors, such as temperature, salinity, and drought, among others, are thought to work in conjunction with genetic factors, such as gene expression and mutation loads, to cause the synthesis of a diverse collection of secondary metabolites, which lend the family its therapeutic properties (Mwine and Van Damme, 2011).

Different plant parts of the Euphorbs plant have been used in medicine. For instance, stem extracts have antipyretic, analgesic, sedative, anxiolytic, and inhibitory effects on platelet aggregation, anti-inflammatory behavior, antioxidant, and antimutagenic properties. These pharmaceutical properties are used in treating bronchitis, asthma, and various other lung conditions (Loh *et al*., 2009 and Basma *et al*., 2011).

Euphorbia maculata L. (Euphorbiaceae*)* is an annual herb, used extensively as folk medicine around the world, particularly in China, Japan, and Korea. (Asgarpour *et al*.2016). It is used frequently to treat hematuria, hemoptysis, diarrhoea, and sore swelling (Herbalism, 1999). Extracts of *E. maculata* have antiplatelet properties by preventing the production of thromboxane B2 (Kwon *et al*., 2015).

Euphorbia peplus L. (Euphorbiaceae), is an annual herb that is native to western Asia, North Africa, and Europe. Its sap is very irritating to the rapidly dividing human tissue and has long been used commonly as a traditional remedy for skin lesions (Noori *et al.,* 2009 and Nasrollahzadeh *et al*., 2011) as well as skin cancer especially non-melanoma skin cancer (NMSC). Diterpene esters were determined to be the sap effective compounds against skin diseases (Ramsay *et al*, 2011). These esters became the focus of continuing study worldwide since they are cytotoxic for a vast number of malignancies both in vivo and in vitro. Due to the toxicological hazards of synthetic antioxidants as food additives, their use has become controversial (Es-Safi *et al*., 2006). Furthermore, the overuse of synthetic antibacterial agents in human drugs caused the development of tolerant bacterial strains (Aouni *et al*., 2013). Additionally, there is a pressing need to find efficient medications that can replace or enhance those now in use due to the negative side effects and documented drug resistance (Amin *et al*., 2017). Thus, the need to introduce novel natural plant-based antibacterial and antioxidant agents becomes an urgent issue (Farah *et al*., 2014).

Qalyubia Governorate, Egypt has a diverse flora, which is rich with a great variety of important *Euphorbia* plants as invasive species. Even though, and to the best of our

knowledge, multiple of these Euphorbs have not yet undergone several biological analyses. This triggered our interest in comparing the antibacterial and antioxidant activities of two medicinal *Euphorbia*; *Euphorbia maculata* L. and *Euphorbia peplus* L. naturally in Qalyubia growing Governorate.

The objectives of this study could be outlined in some points which are (i) Comparing the morphological traits of *E. maculata*. *and E. peplus* (ii) Qualitative screening of some bioactive phytochemicals in both herbs (iii) evaluating the antioxidant and antimicrobial capacities of these Euphorbes methanolic (MeOH) extracts (iii) clarifying the probable correlations between carotenoids, phenolic compounds in terms to the antioxidant capacity.

MATERIALS AND METHODS

1. Plant Collection and Identification:

Healthy whole plants from *Euphorbia maculata and Euphorbia peplus* were collected in April 2021 from Benha University Botanical Garden, Qalyubia Governorate, Egypt. The plant specimens were picked up, and dipped in beakers filled with water to eliminate the adhering soil particles and the plants were blotted with tissue towel. Finally, the specimens were identified and the voucher was kept in the herbarium of the Botany Department, Faculty of Science, Benha University.

2. Measurements of Some Morphological Traits:

Lengths of fresh shoots and roots were measured with the help of a meter scale. The same specimens were weighed to record the fresh masses of shoots and roots and then placed in an oven until constant dry weights could be obtained.

3. Qualitative Analysis Of Phytochemical Components:

According to the methodology adopted by Savithramma *et. al.*, (2011) the methanolic (MeOH) extracts of *Euphorbia maculata* L. and *Euphorbia peplus* L. were screened for some bioactive phytochemicals as follows.

Terpenoids Check Test: By mixing 0.5 ml of the plant extract with 2 millilitres of concentrated sulphuric acid and chloroform V/V, the existence of terpenoids is verified by the emergence of a reddish-brown interface.

Steroids Check Test: the existence of steroids is proved by adding 2 mL chloroform and 1 mL sulphuric acid to 0.5 mL plant extract. The formation of reddish-brown interface rings confirmed that steroids are present.

Saponins Check Test: To identify saponins, 2 mL of plant extract and 2 mL of distilled water were stirred in a graduated cylinder for 15 minutes. Saponins were ascertained if 1-centimetre foam was formed.

Phenols Check Test: 1 mL of plant extract was mixed with 2 mL of distilled water and a few drops of 10% ferric chloride. Phenols are present if blue/ green colour development took place.

Flavonoids Check Test: To detect flavonoids, 2 mL of plant extract and 1 mL of 2N sodium hydroxide were combined. Flavonoids are represented by yellow coloration.

Tannins Check Test: 1 mL of 5% ferric chloride was added to 2 mL of the plant extract. Dark blue or greenish-black colouring is an indicator of tannins.

Coumarins Check Test: One milliliter of 10% sodium hydroxide was combined with 1 millilitre of plant extract to recognise the coumarins. The establishment of yellow color is an obvious sign of coumarins.

Quinones Check Test: To identify Quinone, 1 mL of concentrated sulphuric acid was combined with a known volume of the plant extract. Red coloration substantiates quinones.

Alkaloids Check Test: 2 mL of concentrated hydrochloric acid and 2 mL of plant extract were needed to which of Mayer's reagents was added. Alkaloids are present if white or green colour arises.

Glycosides Check Test: By mixing 3 mL of chloroform and 10% ammonium solution with 2 mL of plant extract. The development of pink colour is evidence that glycosides exist.

4. Estimation of Carotenoids Content (CC):

The photosynthetic pigments carotenoids were determined calorimetrically following the protocol of Lichtenthaler (1987). Then the fractions were calculated as µg g^{-1} fresh weight of leaves.

5. Quantitative Estimation of Certain Non-Enzymatic Secondary Metabolites and The Total Antioxidant Capacity:

5.1. Estimation of Total Phenolics Content (TPC):

Using the Folin-Ciocalteu reagent, total phenolic content was determined (Singleton and Rossi, 1965). The total phenolics were calculated as µg gallic acid equivalent to g^{-1} fresh weight of plant shoots.

5.2. Estimation of Total Flavonoids Content (TFC):

Following Zhuang *et al.*, (1992) protocol, total flavonoid content was assessed via the aluminum chloride calorimetric assay. The total flavonoids content was expressed as µg catechin equivalent to g^{-1} fresh weight of plant shoots.

5.3. Estimation of Total Antioxidant Capacity (TAC):

The phosphomolybdenum assay Prieto, *et al*. (1999) was adopted for calculating the total antioxidant capacity, which was expressed as the number of µg equivalent of ascorbic acid g^{-1} fresh weight of plant shoots.

6. Antimicrobial Activity Assay:

Agar-well diffusion technique (Heritage, *et al*., 1996) was used to evaluate the antimicrobial activity of MeOH extracts of the studied plants. In this study 6 microorganisms were used *viz*, the Gram +ve bacteria; *Staphylococcus aureus* and *Bacillus subtilis*, the Gram -ve bacteria; *Escherichia coli* and *Salmonella typhimurium*, as well as two fungal strains; the *Saccharomyces cerevisiae* and *Aspergillus flavus*. The tested microbial strains were obtained from the Botany and Microbiology Department, Faculty of Science, Benha University. The inoculated Petri dishes were kept in a refrigerator for 6 h, then incubated at 30 ± 2C° for 24 h, and finally, the clear inhibition zones were recorded in (cm).

7. Statistical Analysis:

The morphological traits were estimated by adopting five replicates for each parameter. Whereas, for the chemical determinations, each experiment was constructed three times, and the mean and ± standard deviation (SD) were calculated. SPSS software (IBM, v25) was utilized to run an ANOVA analysis of one-way analysis of variance. After that, post hoc Duncan's test was employed to determine the difference in growth, metabolic parameters and inhibition zones between the two examined plant extracts at a probability level (P) ≤ 0.05.

RESULTS AND DISCUSSION

1. Growth Biomarkers:

In this investigation, the studied plants have different habits; *E. maculata* is a prostrate Euphorbe (Fig. 1-A) grown naturally in Benha University Botanical Garden and even in cracks of the platform inside the garden. It creeps clandestinely underneath the cultivated species from the onset of spring until the beginning of the next winter season. On the other hand, *E. peplus* exhibited an erect form habit (Fig. 1-B), grown in Benha University Botanical Garden among the cultivated species by the onset of autumn until the onset of the next summer season. It was observed by practice that natural weed control is

not effective against *E. maculata* more than *E. peplus*, this may be attributed to the hidden nature of the former underneath the cultivated species.

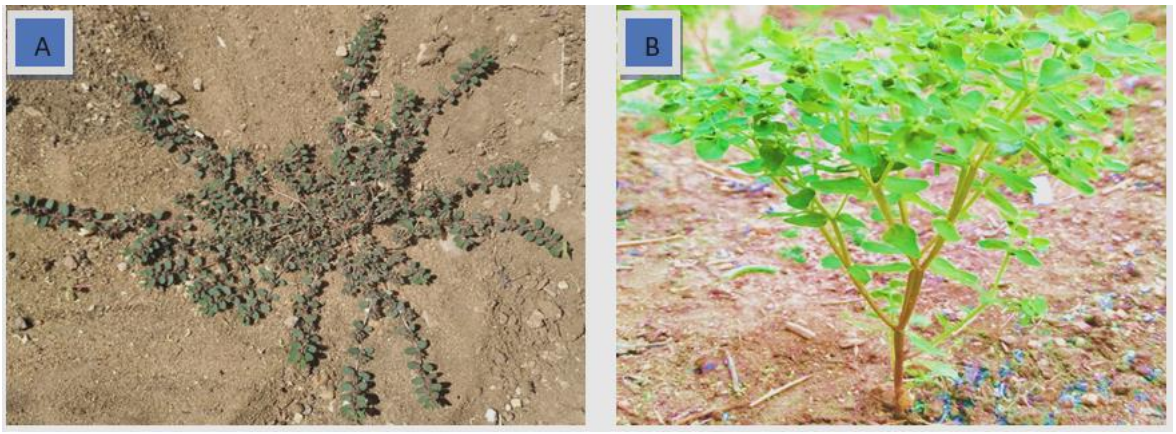

Fig. 1: Natural habit specimens of (A) *Euphorbia maculata* and (B) *Euphorbia peplus* at Benha University Botanical garden**.**

By comparing the water contents of the two herbs shoots and roots (Table, 1) it was found that *E.maculata* has a significant shoot and nonsignificant root water content evaluated by 72.17 and 49.08 % against 82.17 and 57.50 % for *E.peplus* shoot and root water content in the same order. Besides the shoot/root length ratio of *E.maculata* (1.5) is significantly reduced in respect to that of *E. peplus* (3.07).

The water content and shoot /root length ratio data could inspire that *E.maculata* which creeps in the sly below the cultivated plants is more adaptive to the stress conditions than *E. peplus* and this lends the former a more competitive nature. The shoot /root length ratio is an indicator of the survival of plants since it reflects the extent of root expansion inside the soil. The reduced values of the shoot root ratio indicate that roots extensively. This situation is a normal adaptation in competing species like the invasive Euphorbs.

Table 1: Growth biomarkers of *Euphorbia maculata* and *Euphorbia peplus*. Each value is a mean of 5 replicas.

Plant sample	Average Shoot length/cm	Average shoot fresh weight/g	Average shoot dry weight /g	Average Shoot water content %	Average root length/ cm	Average Root fresh weight/g	Average root dry weight/ g	Average root water content %	Average shoot/ root ratio
E. maculate	$11.32^{b} \pm 1.60$	$2.52^{b} \pm 1$	$0.52^{b} \pm 0.25$	$72.17^{b} \pm 1.4$	$7.82^{a} \pm 1.35$	$0.12^{b} \pm 0.07$	$0.04^{b} \pm 0.03$	$49.09^{a} \pm 8.53$	$1.52^{b} \pm 4.66$
E. peplus	$27^{a} \pm 2$	$17.34^{a} \pm 6$	$4.76^{a} \pm 0.93$	$82.17^{a} \pm 1.13$	$9.34^{a} \pm 2.31$	$0.49^{a} \pm 0.18$	$0.33^{a} \pm 0.07$	$57.50^{a} \pm 3.69$	$3.07^{a} \pm 7.75$

2. Qualitative Analysis of Phytochemical Components Qualitative Screening:

The qualitative screening of bioactive secondary constituents of both *E. maculata* and *E. peplus* (Table 2) revealed the presence of 9 natural phytochemicals namely terpenoids, steroids, saponins, phenolics, flavonoids, tannins, coumarins, quinones, and glycosides in both studied *Euphorbia* species but varied degrees in some cases. On the other hand, alkaloids were reported only in *E. peplus* only.

Table 2. Qualitative analyses of phytochemical components of *Euphorbia maculata* and *Euphorbia peplus.*

Phytochemical compound	*E. maculata*	*E. peplus*
Terpenoids	++	+
Steroids	++	+
Saponins	+	+
Phenols	+	++
Flavonoids	+	++
Tannins	+	++
Coumarins	+	+
Quinones	+	+
Alkaloids	-	+
Glycosides	+	+

++: intensely present, +: Present, -: Absent

These results were supported by the findings of Matsunaga *et al.,* (1988), Agata *et al*., (1991), Amakura *et al*., (1997), and Luyen *et al*., (2014) who reported the existence of tannins, a flavonol glycoside, and triterpenoids in *E. maculata*

In accordance Khafagy *et al*., (1975), Cateni *et al.* (2010) and Ali *et al.,* (2013) documented the occurrence of phytochemicals such as diterpenes, triterpene alcohols, sterols, cerebrosides, dihyroflavonol, rutin, quercetin, kaempferol, myricetininside,- and O-Glucosides and 3-O-monoglycosides, in *E. peplus.*

Terpenoids, Steroids, and Saponins:

The data represented by Table 2, demonstrated the presence of terpenoids, steroids and saponins in *E. maculata* as well *as E. peplu*s. In support, Shamsabadipoura *et al.*, (2013) and Zhao (2015) isolated a number of triterpenes and steroids from extract of aerial parts of *E. denticulate* and *Euphorbia hypericifolia.*

Terpenoids are a group of significant secondary metabolites in plants with a variety of forms and are the most prevalent molecules in plant-based products. Terpenoids are crucial contents of the food, cosmetic, and pharmaceutical industries. Terpenoids have anti-inflammatory, antibacterial, antiviral, antimalarial, anticancer, possess hypoglycemic properties, promote transdermal absorption, and protect and treat cardiovascular diseases. Moreover, terpenoids were discovered to offer a wide range of potential applications, including insecticidal, immunity control, antioxidation, antiaging, and neuroprotection agents (Yang *et al.,* 2020).

Plant sterols/ phytosterols (PS) are C28 and C29 carbon steroid alcohols (Otaegui-Arrazola *et al*., 2010). They have been demonstrated to be vital constituents of plant plasma membrane microdomains (Roche *et al*., 2008), and may also do similar tasks in animal and human cells. Humans are unable to synthesize sterols themselves inside their bodies and must have them ready-made through the diet, where they are abundant in lipid-rich plant products (Weihrauch, 1978). The pharmacological characteristics of plant sterols/stanols could be summarized as cholesterol-modulating actions which may intersect with their anti-cancer properties against breast cancer (Bruce, 2013) and, colon cancer and prostate hyperplasia (Moghadasian, 2000).

Saponins are an extensive group of natural compounds that are found extensively in various plant species. Saponins are chemically categorised as triterpenoid glycosides. Saponins' roles as effective antioxidants, antiviral/antibacterial agents, and anti-neoplastic

pharmacophores are among their most potent pharmacological functions (Biswas and Dwivedi, 2019).

In an experiment to evaluate the Therapeutic role of saponins as potent antioxidant agents, Bigoniya and Rana (2010) induced acute hepatotoxicity in rats by CCL_4. The authors pretreated rats with saponin isolated from *Euphorbia neriifolia.* The authors reported that pretreatment with saponins protects cellular phospholipid protection from deleterious peroxidation brought on by extremely reactive harmful intermediate radicals created during CCl_4 biotransformation.

Phenolics, Flavonoids, Tannins, Coumarins, and Quinones

The phytochemical screening for secondary metabolites of *E. maculata* and *E. peplus* proved that, both these Euphorbes contain phenols, flavonoids, tannins coumarins, and quinones in varied degrees. Generally, these phenolic compounds were more apparent in *E. peplus* (Table 2). In accordance, Tostes, *et al.,* (2019) isolated a number of polyphenols (flavonoids) from an aqueous ketone extract from *E. heterophylla* leaves.

Plants produce phenolic compounds, which are further classified into phenolic acids and polyphenols, as a major class of secondary metabolites. These substances can exist as derivatives, such as ester or methyl esters, or they can be found in combination with mono- and polysaccharides, linked to one or more phenolic groups.

One of the most prevalent phenolics in medicinal plants is polyphenol. They can be divided into phenolic acids, flavonoids, tannins, coumarins, quinones, lignans, phenanthrenes, stilbenes, etc. based on their molecular structure. Due to their distinctive molecular structure, they are widely dispersed in almost all plants and demonstrate significant antioxidant activity. They are synthesized from phenylalanine or tyrosine via the phenylpropanoid pathway.

In addition to offering Plants defence against herbivores and pathogens, polyphen ols also have a variety of advantageous pharmacological effects. These potent effects include the ability to regulate key cellular enzyme activity, cardiovascular illness, diabetes, arthritis, neurodegenerative diseases, and many other conditions. They act as anti-oxidative, anti-inflammatory, anti-mutagenic, antiviral, antibacterial, and anti-carcinogenic agents among others (Panche *et al*., 2016, Sekowski *et al.*, 2018, and Sinha, 2019).

Quinones are a group of biological and synthetic origins with a number of valuable properties. They are significant factors for plant life as they act as electron transporters during photosynthesis reactions. As vitamins, quinones can prevent and treat conditions including osteoporosis and cardiovascular disorders. Quinones enhance overall health through their antioxidant activity. Many of the cancer medications that have been or are now undergoing clinical studies are quinone-related compounds (El-Najjar *et al.,* 2011).

Alkaloids and Glycosides:

The qualitative assay of phytochemicals (Table 2) ascertained the presence of glycosides in both *E. maculata and E. peplus* whereas, alkaloids are detected in *E. peplus* only.

One of the greatest classes of natural products, plant alkaloids, is composed of a wide variety of chemical compounds. Alkaloids have incredibly powerful pharmaceutical effects. For instance, famous plant alkaloids include morphine and codeine, apomorphine, papaverine, a muscle relaxant, and sanguinarine and berberine, antimicrobials. Additionally, some effective anti-cancer medications have been produced from plant alkaloids (O'Connor 2010 and Salminen *et al*., 2011).

Raji *et al.,* (2019) conducted an experiment to determine the bioactive components responsible for the antimicrobial activity of four tested medicinal plants. The authors extracted alkaloids, flavonoids, tannins and saponins from *Cassia alata, Thespesia populnea, Euphorbia hirta and Wrightia tinctoria, and* examine the extracted metabolites

against *Pseudomonas aeruginosa*. The authors documented that, the alkaloids and saponins of *C. alata, E. hirta, T. populnea* and *W. tinctorial* showed dose-response antibacterial activity. They recorded the highest antibacterial activity of 1.9 cm for the tannin of *W. tinctoria* and saponin of *E. hirta* at 16µg concentration. The authors also added that the extracted metabolites especially alkaloids were able to restrict the movement of microorganisms to a confined space which was evident from the swarming test.

The plant glycosides are plant secondary metabolites that can be decorated with sugars, or glycosylated (Wink, 2015). Liu, *et al*., (2007) isolated some flavonol glycosides namely afzelin, quercitrin, and myricitrin from the MeOH extracts of *E. hirta* Linn shoots. The isolated glycosides exhibited inhibition against *Plasmodium falciparum* growth and caused little cytotoxic effect on human epidermoid carcinoma cells.

3. The Carotenoids Contents (TC):

Concerning, the carotenoids, as a photosynthetic pigment that also act as an antioxidant that protects the chlorophylls from photo-oxidation, E.*maculata* has a significantly higher content of carotenoids estimated by 240.29 µg /g leaves F.Wt. of leaves versus 160.83 µg/g leaves F.Wt. of *E. peplus* (Table,3). Moreover, these carotenoid contents of *E.maculata* and *E. peplus* could be considered as high contents as it was reported by Yaacob *et al.* (2019) that, the carotenoids content of 24.62 to 114.13 µg/g dry weight is high level in the traditional vegetables, which he scheduled as rich in carotenoids.

Table 3: Carotenoids, Total flavonoids and Total phenolics contents and the Total antioxidant activity of *Euphorbia maculata* and *Euphorbia peplus*. Each value is a mean of 3 replicas, the mean values are expressed as µg /g F.Wt.

	Carotenoids	**Total flavonoids**	**Total phenolics**	**Total antioxidant capacity**
E. maculate	240.28 ^{a}±12.4	2018.8^{b}±6.2	4078.2^{b}±476.3	5155.2^{b}±211.6
E. peplus	160.8^{b}±13.7	3889.3^{a}±93.2	7465^{a}±182.5	6685^{a}±119.7

One of the most prominent categories of naturally produced pigments, carotenoids come in several isomeric forms. These substances, which are also present in many dark green vegetables, are mostly responsible for the red, yellow, and orange hues of fruits and vegetables. The carotenoids β-carotene, α-carotene, γ-carotene, lycopene, lutein, beta-cryptoxanthin, zeaxanthin, and astaxanthin are the most naturally occurring isomers. The precursor of vitamin A, -carotene, is a vital component for human health and can support a variety of body processes, such as cellular differentiation and growth, reproduction, inhibiting atherosclerosis, and enhancing vision (Alam *et al*., 2016 and Yaacob *et al*., 2019). Besides, acting as ROS scavenger (Yaacob *et al*., 2019).

4.1. Total Flavonoids Content (TFC) and Total Phenolics Content (TPC):

On contrary to the carotenoids, the data illustrated by Table (3) showed that the total flavonoids and phenolics contents of *E.peplus* 3889.3 and 7465 µg /g shoot F.Wt surpasses significantly these of *E.maculata* 2018.8 and 4078.2 µg /g shoots F.Wt .

Frezza *et al.,* (2018) screened the phytochemical profile of the ethanolic extract of *E. peplus* L. and recognized the existence of fourteen compounds categorized under five different classes of natural compounds i.e. peculiar diterpenoids (jatrophanes and pepluanes), triterpenoids (pentacyclic and saponin), flavonoids (flavonols), caffeoyl-quinic acids and rare disaccharides.

Total phenolic content (TPC) and total flavonoid content (TFC) are significant parameters of total antioxidant capacity (TAC) and are frequently employed for the

evaluation of antioxidant extracts, such as those from cereals, legumes, fruits, and spices. Phenolic compounds are produced from plant-derived shikimic acid and pentose phosphate pathways. Phenolic compounds are thought to acquire their antioxidant activity through the structural presence of benzene rings with one or more hydroxyl substituents (Lin *et al*.,2016). These various hydroxyl substituents give phenolic compounds the redox characteristics needed to counteract ROS (•OH- and O2 •) produced during regular cellular metabolism. (Alam *et al*., and 2016 and Yaacob *et al*., 2019).

Phenolics are classified as primary antioxidants because they serve as hydrogen donors or free radical acceptors and promote the production of more stable radicals, which prevents the chain reaction of oxidation. The inhibitory reaction produces stable molecules that do not produce additional free radicals or cause rapid oxidation through a chain reaction and is thought to compete with the propagation phase of lipid oxidation (Nawar, 1996).

Being antioxidants, flavonoids are extremely essential. The Fenton reaction, as well as the breakdown of lipid hydroperoxides into highly reactive forms peroxyl and alkoxyl radicals, are recognised to be the mechanisms by which transition metal ions promote lipid oxidation. Strong chelators of metals such as flavonoids can quickly neutralise prooxidant metal ions, delaying or even inhibiting the oxidation of lipids brought on by transition metal ions. When a compound is established between the antioxidant and the transition metal, such that metal ions can no longer act as a promotor of lipid oxidation, and this is the implication of the anti-oxidant capability of metal chelators (Wettasinghe & Shahidi, 2002; Karawita *et al*., 2005; Wijeratne *et a.l*, 2006; Chandrasekara &Shahidi, 2010; Zhong *et al.,* 2012).

4.2. The Total Antioxidant Activity (TAC):

Referring to the Total antioxidant activity, *E. peplus* (6685µg /g shoots F.Wt) significantly proceeded *E. maculata (*5155.2 µg /g shoots). This is may be attributed to the natural habit of both plants where, *E. peplus* is more exposed to sun light than *E. maculata*

It was previously mentioned that the carotenoid content was higher in *E. maculata*. Meanwhile, the total phenolics and flavonoid contents were higher in *E. peplus*. These findings may interpret why both plants have a high level of antioxidant capacity.

The total antioxidant activity is a common analyte for determining the antioxidant state of biological materials and is capable of assessing the antioxidant defence mechanism against the free radicals generated by a specific cellular disease. It is regarded in biochemistry, medicine, and food science as a biomarker of disease (Kusano & Ferrari, 2008 and Rubio *et al.,* 2016).

Antioxidants are compounds that, when consumed or present in the body in very small amounts, delay, regulate, or even stop oxidative processes (Shahidi & Zhong, 2007). Consequently, antioxidants are crucial for food preservation and supporting the health-promoting (Shahidi and Zhong 2015).

Free radical scavengers, singlet oxygen quenchers, inactivators of peroxides and other reactive oxygen species (ROS), metal ion chelators, quenchers of secondary oxidation products, and inhibitors of pro-oxidative enzymes are some examples of antioxidants that fulfil this classification (Shahidi & Zhong, 2007).

These chemicals work to prevent the oxidation processes through a variety of mechanisms and actions. They are typically categorised as primary and secondary antioxidant reactions based on their mode of action (Nawar, 1996). Phenolics, flavonoids, and carotenoids are natural antioxidants, which offer health advantages and guard against chronic degenerative disorders (Alam *et al*., 2020). These antioxidants are recognised for their abilities to prevent and inhibit reactive oxygen species (ROS) and offer a potent line of defence against oxidative stress (Podsędek 2007, Mertz *et al*., 2009, and Alam *et al.,* 2020).

In the previously mentioned experiment of Raji *et al*. (2019) on *C. alata, T. populnea, E. hirta* and *W. tinctoria*, the authors assessed the antioxidant activities of these medicinal plants and documented that, flavonoid of *E. hirta, C. alata* and *W. tinctorial* exhibited respectable percentage of free radicals inhibition in DPPH scavenging assay. They recorded the highest inhibition percentage of 88.75% for the tannin of *E. hirta*.

5. The Antimicrobial Activity (AA):

The methanolic extracts of *E.muculata* and *E. peplus* shoots were investigated against the number of bacteria and fungi using the agar well diffusion method to evaluate the potential of both plants as alternative antimicrobial natural agents. The results of antimicrobial inhibition activities (Table 4) revealed that the methanolic extracts of both plants have varied degrees of antimicrobial activities according to plant species and the tested microorganism.

Table 4: The total antimicrobial activity of *Euphorbia maculata* and *Euphorbia peplus* against *Staphylococcus aureus, Bacillus subtili Escherichia coli and Salmonella typhimurium, Saccharomyces cerevisiae* and *Aspergillus flavus*. Each value is a mean of 3 replicas and values of inhibition zones are expressed in cm.

Tested Micro-organism / Plant Extract	Gram –ve Bacteria		Gram +ve Bacteria		Fungi	
	Escherichia coli	*Salmonella typhimurium*	*Bacillus subtilis*	*Staphylococcus aurerus*	*Saccharomyces cerevisiae*	*Aspergillus flavus*
E. maculata	$1.5^{a}\pm0.125$	$1.6^{a}\pm0.094$	$1.3^{a}\pm0.163$	$1.6^{a}\pm0.163$	$1.1^{b}\pm0.094$	$0.9^{a}\pm0.082$
E. peplus	$1.2^{b}\pm0.082$	$1.5^{a}\pm0.125$	$1.4^{a}\pm0.125$	$1.4^{a}\pm0.082$	$1.6^{a}\pm0.163$	$0.7^{b}\pm0.047$

As a general speech, *E. maculata* exhibited higher antibacterial activity for all the examined bacterial strains except for the Gram +ve *Bacillus subtilis*. Furthermore, *E. maculata* progressed *E. peplus* as an antifungal agent against *Saccharomyces cerevisiae* whereas the opposite was true with respect to *Aspergillus flavus.*

In this investigation, the enhancement of antibacterial activity of *E. maculata* over *E. peplus,* may be attributed to the occurrence of higher content of carotenoids in *E.maculata*. This explanation got supported by the findings of Szabo *et al.* (2019) who extracted and estimated the carotenoids and phenolic contents of 10 tomato varieties and investigated their antimicrobial activities against Gram-positive bacteria. The authors revealed that the ¸Tărăn, es, tiroz variety which had the higher total carotenoids content was also the most effective extract against the Gram-positive bacteria. Even though, this variety extract contained a significantly lower phenol content compared to other extracts tested.

Moustafa *et al.* (2017) examined five solvent extracts of dried leaves of *E. prostrata, E. peplus,* and *E. terracina* against five pathogenic organisms, including *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa, Staphylococcus aureus,* and *Shigella sp*. The authors reported that five solvent extracts displayed varying degrees of antibacterial activity. *Pseudomonas aeruginosa* was more sensitive to the methanol and petroleum ether extracts obtained from *E. prostrata* than to *E. peplus* and *E. terracina*. While *Klebsiella pneumoniae* and *Shigella sp*. effectively stopped growth by ethanol extracts from *E. peplus*, methanol extract from *E. prostrata*, and ethanol extract from *E. terracina*.

Furthermore, in a separate study done by Kirbag *et al*., (2013), eight different species of *Euphorbia*, including *E. aleppica, E. szovitsii, E. falcata, E. denticulata E. macroclada, E. cheiradenia, E. virgata,* and *E. petiolate*, were examined for their

antimicrobial properties against *Staphylococcus aureus*, *Bacillus megaterium*, *Proteus vulgaris*, *Klebsiella pneumonia*. The findings showed that all of the examined microorganisms were inhibited by extracts of *Euphorbia* species in a variety of ratios, and it was noted that the MIC values of the extracts ranged from 31,2 to 1000 g.

Conclusion

From the outward comparison done in this work, we could conclude that *Euphorbia maculata* and *Euphorbia peplus* are worthy biological resources for ethno-medicinal purposes. Our findings indicate that both *E. maculata* and *E. peplus* are favorable organic antioxidants and antimicrobials that can be used instead of the hazardous conventional synthetic agents, even though, and Due to the noticeable chemical diversity of *Euphorbia spp*. We recommend further work should be done to offer applicable and reliable analytical procedures. Besides, an intensive investigation should be done for evaluating the toxicity of extracted metabolites and solvents, the stability of the fractioned extracts under various environmental conditions and to examine various types of extracts against multidrug-resistant microorganisms. These suggestions as a whole serve as useful gears in the calibration of plant resources as safe medications in the pharmaceutical industry.

Competing Interests: The authors have declared that there are no competing interests (financial or otherwise).

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