Progress in Microbes and Molecular Biology

HH PUBLISHER

Original Research Article

Radical-Scavenging Effect, Ferric Reducing Ability and Phytochemical Analysis of *Urtica urens* (L.) and *Mercurialis annua* (L.)

Doukkali Zouhra[1], Meryem El Jemli[1,2], Abdelhakim Bouyahya[3]*, Khang Wen Goh[4], Ganesh Sritheran Paneerselvam[5]*, Belatar Bahia[6], Yahia Cherrah[1], Katim Alaoui[1]

Article History

Received: 26 March 2023;

Received in Revised Form: 21 May 2023;

Accepted: 28 May 2023;

Available Online: 07 June 2023

[1]Laboratory of Pharmacology and Toxicology, Faculty of Medicine and Pharmacy, Mohammed V University, Rabat, Morocco; d.zouhra@yahoo.fr (DZ); eljemli.meryem@gmail.com (MEJ); cherrahy@yahoo.fr (YC); alaouikma@yahoo.fr (KA)

[2]Mohammed VI University of Health Sciences (UM6SS), Casablanca, Morocco

[3]Laboratory of Human Pathologies Biology, Faculty of Sciences, Department of Biology, and Genomic Center of Human Pathologies, Faculty of Medicine and Pharmacy, Mohammed V University in Rabat, Morocco

[4]Faculty of Data Science and Information Technology, INTI International University, Nilai 71800, Malaysia; khangwen.goh@newinti.edu.my (KWG)

[5]School of Pharmacy, Taylor's University, 47500 Subang Jaya, Malaysia

[6]Research Unit of Cerebral Monitoring in Neuro-Reanimation, Faculty of medicine and pharmacy, University Mohamed V of Rabat, Morocco; belatar.belatar80@gmail.com (BB)

*Corresponding author: Abdelhakim Bouyahya, Laboratory of Human Pathologies Biology, Faculty of Sciences, Department of Biology, and Genomic Center of Human Pathologies, Faculty of Medicine and Pharmacy, Mohammed V University in Rabat, Morocco; a.bouyahya@um5r.ac.ma (AB); Ganesh Sritheran Paneerselvam, School of Pharmacy, Taylor's University, Jln Taylors, 47500 Subang Jaya, Selangor; ganeshsritheran.paneerselvam@taylors.edu.my (GSP)

Abstract: Important Moroccan medicinal plants known for their food and medicinal potential include *Urtica urens* and *Mercurialis annua*. We examined the antioxidant activity of the methanolic and aqueous extracts by several *in vitro* systems of the assay, namely the ferric reducing/antioxidant power (FRAP) assay, the DPPH radical-scavenging activity, and the ABTS free radical-scavenging capacity. In addition, all active extracts were subjected to phytochemical screening to determine the key groups of chemicals implicated in their therapeutic activity. Total phenolic and flavonoid concentrations were also measured to see how they affected the antioxidant properties of the plant extracts. The extracts of *Urtica urens* and *Mercurialis annua* were found to have different levels of antioxidant effect in the systems tested. Based on the three different antioxidant assays, the *Mercurialis annua* extracts showed the highest values of antioxidant ability, based on the three used assays.

Phytochemical testing of studied extracts revealed the presence of flavonoids, anthocyanins, and tannins. Total phenol and flavonoid contents were shown to provide the highest association with different antioxidant methods. The present study provides evidence that the extracts of *Urtica urens* and *Mercurialis annua* are a potential source of natural antioxidants, and this justifies their uses in Moroccan traditional medicine.

Keywords: *Urtica urens*; *Mercurialis annua;* Phytochemical screening; Phenolic and flavonoid contents; antioxidant effects.

1. Introduction

Free radicals produced in the body during normal physiologic functions or introduced from the entourage are highly unstable products capable of inducing several side health effects and diseases [1–5]. Medicinal herbs are known to have the ability to reduce this damage and thus prevent oxidative stress-related diseases [6–9]**.** *Urtica urens* and *Mercurialis annua* are the most common species worldwide, extensively used for diverse medicinal purposes.

Urtica urens (Urticacea), locally known as "Hurriyqa", is a perennial plant with stinging hairs frequently used as decoctions, infusions, or powders to treat anxiety, arthritis, rheumatism, cancer, toothache, scabies, and pruritus [10–13]**.** Seeds soaked in milk are frequently used against cough, kidney stones, cystitis, and oliguria [10]**.** The aerial parts are known for diuretic, galactogenic, and aphrodisiac properties [10], and are used to treat cancer and sciatica [14], and as a styptic [15], the leaves are frequently used against burns, rheumatism, and urinary diseases [14–16]. Several pharmacological and phytochemical works have confirmed their traditional uses in folk medicine [11,17,18]**.** The methanolic extract of the *Urtica urens* aerial part has proved to have anxiolytic activity [11], free radical scavenging activity, and antimicrobial potential [19]. The ethanol and aqueous extracts of the leaves of *Urtica urens* have been tested on *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* and show considerable antibacterial activity [17]**.** Many studies have shown that the biological properties of *U. urens* are especially related to its richness on various bioactive compounds such as polyphenols [20], caffeic acid [21] , and chlorogenic acid [22]**.**

*Mercurialis annua (*Euphorbiaceae), popularly known in Moroccan folk medicine as "Hurriyqa l-melsâ", is largely used in the treatment of a range of disorders [11,23,24]**.** In Morocco, the plant is extensively used as a purgative, anxiolytic, and also in treating Female Infertility[10,11,25]**.** In Portugal, the whole plant is known for its diuretic effect and is traditionally used to treat internal parasitosis and oral inflammation [26]**.** The Italians widely use leaf infusions against diuresis and constipation [24]**.** One cup of a leaf decoction is taken

three times/day to treat diabetes and cancer in Israeli folk medicine [27]. The plant has also been used as a laxative [28], anti-warts, and antibronchial [29]. The dried, powdered plant also promotes wound healing [30]. Several studies have confirmed the various traditional reputations [18,23,31]. *Mercurialis annua* is plenty of biological effects, such as anti-cancer [23], anti-inflammatory [23], anti-microbial [23], anti-anxiety [18], and anxiolytic effects [31]. Many reports have demonstrated that pharmacological activities of *M. annua are* related to their rich content of phenolic compounds such as rutin, narcissin, flavonol glycosides [32–34].

Despite the large flow of data of *Urtica urens* and *Mercurialis annua* uses, and to the authors' knowledge, no works have so far been performed to evaluate the antioxidant effect of the Moroccan *Urtica urens* and *Mercurialis annua*. Accordingly, this study mainly tested the ABTS and DPPH radical-scavenging abilities, the ferric reducing power (FRAP) effect, the phenolics contents of *Urtica urens* and *Mercurialis annua* methanolic extracts.

2. Materials and Methods

2.1. Plant Materials

Mercurialis annua and *Urtica urens* aerial parts were manually collected from Wazzan town (Jaaouna el Basra), Morocco, and were identified by Pr. M. Ibn Tatou and Pr. H. Khammar of the Scientific Institute of Rabat, Mohammed V University, Rabat, Morocco, where voucher specimens have been deposited at its herbarium (voucher number RAB78984 and RAB78983 respectively). The samples were thoroughly dried at laboratory ambient temperature, grounded into a fine powder, and kept at room temperature until further use.

2.2. Extracts preparation

Methanolic extract of *Mercurialis annua* (MAM) and *Urtica urens* (UUM) *were* separately performed by cold maceration using 100 g of Dried and powdered aerial parts in 1000 mL of methanol at room temperature 24 h. Aqueous macerate of *Mercurialis annua* (MAA) and *Urtica urens* (UUA) were prepared by the same procedure. The macerates were filtered, and the filtrates obtained were evaporated under reduced pressure and at a temperature lower than 65 °C.

2.3. Phytochemical Screening

Mercurialis annua and *Urtica urens* extracts were evaluated for the qualitative determination of major phytoconstituents, i.e., alkaloids, flavonoids, tannins, saponins, and cardiac glycosides following these methods [35,36].

2.4. Determination of Total Phenolic Content

Total phenolic content (TPC) was spectrophotometrically determined according to the Folin-Ciocalteu method [37] . A total of 20μL aliquot of the tested extract was mixed with 1.16mL of distilled water, 100μL of Folin Ciocalteu reagent, and 300μL of Na_2CO_3 solution (20%). The tubes were kept at 40∘C for 30 min, and the absorbance was measured at 760nm utilizing UV–visible spectrophotometer. The total phenolic content was obtained from the extrapolation of the calibration curve, which was made by various concentrations of gallic acid solution in methanol. TPC was expressed as µg of gallic acid equivalents (μg GAE)/mg dry extract.

2.5. Determination of Total Flavonoid Content

The overall quantity of flavonoids (TFC) of *Mercurialis annua* and *Urtica urens* extracts was investigated using the method described by Ordonez et al., 2006 [37] with slight modifications. Briefly, 0.5mL of the extracts was mixed with 0.5mL of 2% $AlCl_3$. After 1 hour of incubation at room temperature, the absorbance was measured at 420 nm. TFC was expressed as microgram quercetin equivalent per milligram dry plant extract (µg QCE/mg dry extract).

2.6. Antioxidant Activity

The antioxidant effects of *Mercurialis annua* and *Urtica urens* extracts were tested using three complementary standard tests: DPPH free radical scavenging, ABTS scavenging, and ferric-reducing antioxidant power (FRAP) assays. All samples were analyzed in triplicate.

2.7. DPPH Free Radical-Scavenging Activity

The DPPH radical scavenging effect of *Mercurialis annua* and *Urtica urens* extracts was evaluated according to Sahin method [38]. A mixture containing 0.50 mL of various extracts concentrations and 2 mL of the DPPH methanolic solution (60μM) was incubated in the dark for 20 min. The absorbance was then measured at 517 nm and the percentage of DPPH radical scavenging performance was calculated as following Eq. (1).:

$$\text{Percentage (\%) of DPPH radical scavenging ability} = [(A_0\text{-}A_1)/A_0] \times 100$$

Where, A_0 was the absorbance of blank, and A_1 was the absorbance of the studied extract. Quercetin (QC) and Butyl hydroxytoulene (BHT) were taken as standards. The extract

concentration providing 50% of inhibition (IC_{50}) was calculated from the plotted inhibition curve (%) against concentrations.

2.8. ABTS Free Radical-Scavenging Assay

The ability of water and methanolic extracts of *Mercurialis annua* and *Urtica urens* to scavenge the ABTS radical was estimated using the method described by Pukalskas et al. [39]. A radical solution was produced by combining 10 mL of ABTS (7 mM), and 10 mL of potassium persulphate (70 mM) was kept in the dark at room temperature for 16 h. After the incubation period, the ABTS solution was diluted to obtain an absorbance of 0.700 ± 0.002 at 734 nm. A total of 100μL of the diluted extract was added to 2mL of ABTS solution freshly prepared, and the absorbance was measured at 734 nm after 1 min. The percentage inhibition and the half-maximal inhibitory concentration (IC_{50}) of the extract were calculated with the same procedure described above.

2.9. Reducing Power Determination

The ferric-reducing ability of *Mercurialis annua* and *Urtica urens* extracts was investigated using the method of Tundis [40]**.** A mixture containing 0.2mL of each extract at various concentrations, 2.5 mL of potassium ferricyanide (1%), and 2.5 mL sodium phosphate buffers (0.2M, pH 6.6) was incubated at 50 °C for 20 min. After the incubation was completed, 2.5 mL of trichloroacetic acid (10%) was added, and the resulting mixture was centrifuged at 1000 r/min for 10 min; then, 2.5 ml of the supernatant was mixed with 2.5 mL of distilled water and 0.5 ml of $FeCl_3$ (0.1%). The absorbance was measured at 700 nm, and the IC_{50} was calculated by plotting the absorbances versus the sample concentrations.

2.10. Statistical Analysis

All analyses were carried out in triplicate, and these values were then presented as mean values ± their standard derivations (SD). Data were analyzed using the Graph pad prism 6.0. Statistical comparisons were performed with one-way analysis of variance (one-way ANOVA), and ($p < 0.05$) were regarded as significant. The correlation coefficients (R) between DPPH, ABTS, FRAP, TPC, and TFC were calculated to determine their relationship.

3. Results and Discussion

3.1. Extraction Yield

The extraction yields of *Mercurialis annua* and *Urtica urens* aerial parts in methanol and water were determined and are shown in Figure 1. Depending on the species and the extraction solvent, the dry weight yield in the studied samples was remarkably affected. *Mercurialis annua* aqueous extracts showed the highest extraction yield (32.7%), followed by the methanolic extract from the same plant (21.23%). While *Urtica urens* samples extracted with water and methanol gave extract yields of 12.12% and 11.92%, respectively. The difference observed can be explained by the solvents' polarity and the solubility of bioactive compounds [41].

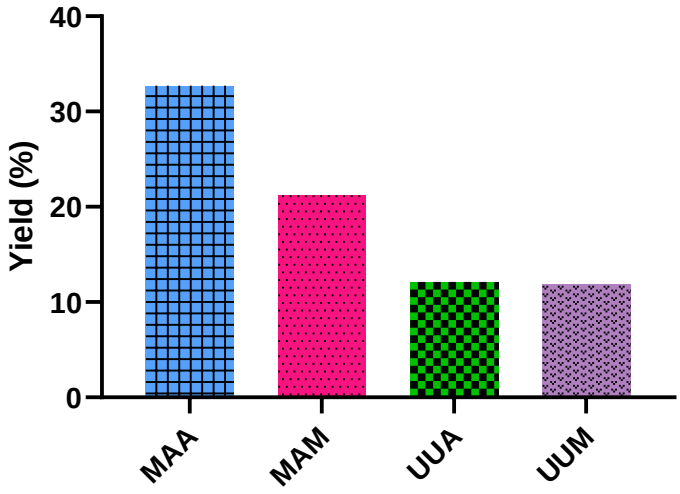

Figure 1. Yield (%) of *Mercurialis annua* and *Urtica urens* extracts.

3.2. Phytochemical Screening

Phytochemical screening of the crude extracts of *M. annua* and *U. urens* revealed the major classes of compounds present to be polyphenols, flavonoids, and tannins (Table 1). These constituents are known to possess various pharmacological activities including antioxidant, anticancer, antifungal, antibacterial, anti-inflammatory, and antidiabetic [42–46]. Thus, the presence of these compounds may be responsible for the antioxidant abilities of the extracts.

Table 1. Phytochemical screening of crude extracts of *Mercurialis annua* and *Urtica urens*.

	MAA	MAM	UUA	UUM
Total polyphenols	++	+++	++	++
Flavonoids	++	++	+	+
Anthocyanins	+	+	-	-
Tannins	++	++	+	+
Alkaloids	-	-	-	-
Terpenes	-	-	-	-
Saponins	+	+	+	+
Quinones	+	+	+	+

Total Phenolic Content

Mercurialis annua and *Urtica urens* extracts were characterized by a considerable amount of phenolic content (Figure 2). The highest TPC was found in MAM with (223.7 ± 1.59) μg GAE/mg edw, followed by MAA (168.274 ± 2.64) μg GAE/mg dry plant extract (edw), UUM (130.816 ± 2.82) μg GAE/mg edw, and UUA (109.768 ± 3.60) μg GAE/mg edw. The richness of *Mercurialis annua* and *Urtica urens* on phenolic compounds agrees with several reports [33,47]. However, the variation in total phenolic content of the different studied extracts can be explained by various parameters and conditions such as genetic factors and type of extract.

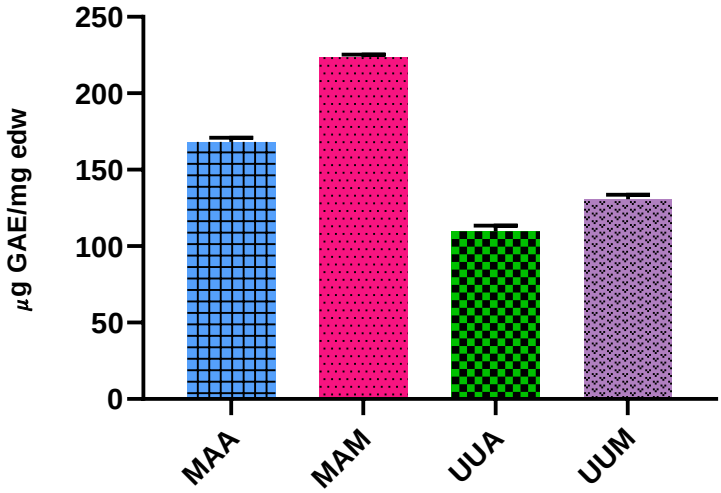

Figure 2. Total Phenolic Content expressed as gallic acid equivalents (μgGAE)/mg plant extracts of *Mercurialis annua* and *Urtica urens*. Data are expressed as mean ± SD (n=3).

3.3. Total Flavonoid Content

Flavonoid content in investigated extracts ranged from 10.98 to 80.71 μg (QE)/ mg edw (Figure 3). The high flavonoids concentration was determined in MAM extract (80.71 ± 2.27μg (QE)/mg edw) followed by UUM (43.79 ± 2.10μg (QE)/mg dry plant extract (edw)), MAA (29.51 ± 0.51 μg (QE)/mg edw). The lowest total flavonoid content was determined for UUA extract (10.98 ± 0.39μg (QE)/mg edw). The richness of *Mercurialis annua* and *Urtica urens* extracts on flavonoid compounds is in agreement with several studies [33,47]**.** However, the variation in total flavonoid Content between the studied extracts can be explained by various parameters and conditions such as genetic factors and type of extract.

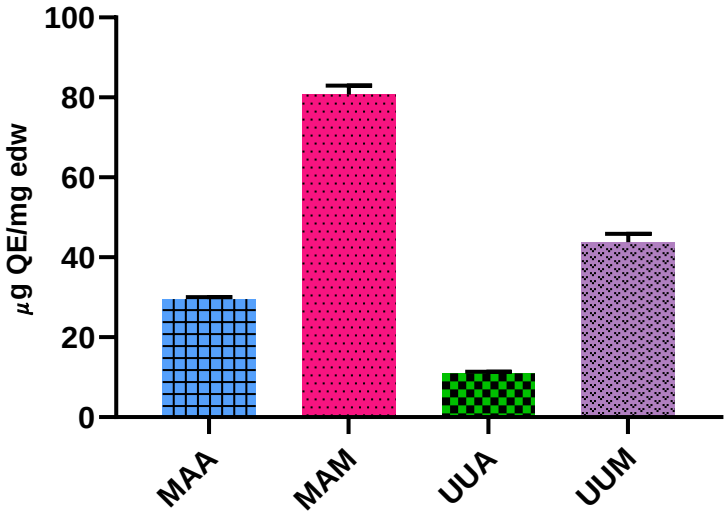

Figure 3. Total flavonoid content expressed as quercetin equivalents (μgQE)/mg plant extracts of in *Mercurialis annua* and *Urtica urens*. Data are expressed as mean ± SD (n=3).

3.4. Antioxidant Activity

The antioxidant activity of the *Mercurialis annua* and *Urtica urens*, evaluated by the three assays varied significantly among the extracts (Table 2**).** The MAM and MAA extracts presented the most substantial reducer effect in the FRAP assay (IC_{50} = 79.15 ± 0.14 and 91.52 ± 1. 30 μg/mL, respectively) and the better antioxidant capacity in the DPPH (IC_{50} = 51.16 ± 0.09 and 61.32 ± 0.65 μg/mL respectively) as well as in the ABTS assays (IC_{50} = 59.00 ± 1.48 and 73.61 ± 0.88 μg/mL respectively). The lowest capacities were recorded for the *Urtica urens* extracts with an $IC_{50} \geq$ 227.11 μg/mL. However, all the tested extracts exhibited low antioxidant capacities compared to Quercetin, Trolox, and BHT with IC_{50} from 1.29 to 7.02 μg/mL. The antioxidant potential of *Mercurialis annua* and *Urtica urens* might

be explained partly by their richness in polyphenols such as flavonoids previously revealed in our study (Table 1, Figure 1, Figure 2). The antioxidant activities of these compounds have mainly been reported to have strong antioxidant power. Various reports have been on the antioxidant effects of several *Mercurialis* and *Urtica* species [48,49]**.** *Urtica urens* aerial parts extract exhibited great antioxidant activity which has been strongly associated with its richness in phenolic compound [49]**.** Aerial parts of *Urtica membranacea* also showed a potent antioxidant effect [49]**.** The whole plant of *Urtica dioica* showing a strong antioxidant activity in the cupric reducing antioxidant capacity (CUPRAC) and the ferric reducing/antioxidant power (FRAP) assays which mainly due to its richness on phenolic compounds such as ursolic acid and quercetin [50]**.** Stinging nettles provide high antioxidant activity and richness in phenolic compounds [51]**.** The antioxidant ability of the nettle (*Urtica dioica*) has mainly been attributed to phenolic compounds such as rutin, quercetin 3-O-glucoside, chlorogenic acid, 2-O-caffeoylmalic acid, isorhamnetin 3-O-rutinoside, kaempferol 3-O-rutinoside, caffeic acid derivatives [26,52,53]**.** In the same sense, several studies have shown the richness of *Mercurialis* spp. on phenolic compounds widely known for their potent antioxidant power, such as kaempferol, squalene, and cycloartenol [54–57]**.**

Table 2. IC50 values (μg/mL) of *Mercurialis annua* and *Urtica urens* extracts and of quercetin, BHT, and Trolox.

Assays	Plant extracts				Positive controls		
	MAA	**MAM**	**UUA**	**UUM**	**QC**	**BHT**	**Trolox**
DPPH	61.32 ± 0.65	51.16 ± 0.09	335.1 ± 2.18	282.58 ± 3.38	1.29 ± 0.01	4.20 ± 0.02	-
ABTS	73.61 ± 0.88	59.00 ± 1.48	376.53 ± 4.85	289.90 ± 1.13	-	-	1.93 ± 0.01
FRAP	91.52 ± 1.30	79.15 ± 0.14	372.35 ± 1.94	227.11 ± 0.89	2.06 ± 0.01	7.02 ± 0.02	-

3.5. Relationship between Antioxidant Assays

To evaluate the reliability and suitability of the three antioxidant assays used to determine the antioxidant abilities of *Mercurialis annua* and *Urtica urens* extracts, we performed a correlation analysis of the three used tests. As shown in Table 3, the values of DPPH were highly correlated with ABTS (R=0.997; p>0.05) and FRAP (R=0.955; p>0.05). The relation between ABTS and FRAP also showed a high and insignificant correlation

(R=0.976; p p>0.05). This suggests that DPPH, ABTS, and FRAP can be predictive assays for each other. The relationship between the three used assays has been largely reported [58,59].

Table 3. The correlation coefficient among antioxidant tests and total phenolic and flavonoid contents.

	DPPH	**ABTS**	**FRAP**
ABTS	0.997	-	-
FRAP	0.955	0.976	-
TPC	-0.903	-0.908	-0.879
TFC	-0.622	-0.656	-0.710

3.6. Relationship between Antioxidant Effect and Phenolic Content

The relationship between the antioxidant effect and phenolic content of *Mercurialis annua* and *Urtica urens* extracts is described in Table 3. The TPC was negatively but highly correlated with DPPH ($R=-0.903$; $p<0.05$), ABTS ($R=-0.908$; $p<0.05$), and FRAP ($R=-0.879$; $p<0.05$). The negative relationship is due to the antioxidant activity in terms of IC_{50} decreases, the TPC increases. The results show that high TPC indicates a better antioxidant effect in terms of DPPH, ABTS, and FRAP assays of *Mercurialis annua* and *Urtica urens* extracts. The TFC was correlated with DPPH ($R=-0.622$; $p<0.05$), ABTS ($R=-0.656$; $p<0.05$), and FRAP ($R=-0.710$; $p<0.05$). The results of flavonoid content were quite similar to those of phenolic content; thus, both of them can be a measure to assess the antioxidant effects of *Mercurialis annua* and *Urtica urens* extracts, but still, the role of other phenolic compounds and metabolites present in the plants. Generally, individual flavonoid compounds cannot be as effective as in combination with other phenolic compounds. The high correlations between the phenolic content (TPC and TFC) and antioxidant potential have been reported for other medicinal plants such as *Hedychium spicatum*, *Azadirachta indica*, *Juniperus thurifera*, *J. oxycedrus*, *J. phoenicea* and *Tetraclinis articulate* [58,60,61]. Therefore, total phenolic and flavonoid content can be used to assess medicinal plants' antioxidant ability.

4. Conclusion

The results obtained in this work support the therapeutic uses of *Mercurialis annua* and *Urtica urens.* Their phenolic content contributed an increased antioxidant activity of those Moroccan species In addition, the study suggests that the water and methanolic extracts

from the *Mercurialis annua* and *Urtica urens* constitute a valuable source of antioxidant metabolites. Further investigations are required to identify the bioactive metabolites. The study also confirms that phenolic content can predict antioxidant assays like ABTS, DPPH, and FRAP.

Author Contributions: Author Contributions: Conceptualization, D.Z., A.B., L.C.M., K.A.; methodology, D.Z., M.E.J., A.B., K.A.; software, D.Z., K.W.G., K.A.; validation, D.Z., M.E.J., K.A.; resources, A.B., K.W.G., L.C.M., K.A.; writing—original draft preparation, D.Z., K.A.; writing—review and editing- D.Z., A.B., L.C.M., B.B., Y.C., K.A.; visualization, D.Z., K.W.G., K.A.; supervision, A.B., K.A,; project administration, D.Z., K.A.; All authors have read and agreed to the published version of the manuscript.

Funding: All the materials and working tools for my thesis are available in the laboratory of pharmacology and toxicology, Faculty of medicine and pharmacy, University Mohammed V Rabat.

Acknowledgments: We thank Hayat Aouari, for their technical assistance throughout the studies. We also thank Professor Amina Zellou for his advice regarding the analysis of the experiments presented in this paper.

Competing interests: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Mccord JM. Human disease, free radicals, and the oxidant/antioxidant balance. Clin Biochem 1993; 26(5): 351–357.
2. Moskovitz J, Yim MB, and Chock PB. Free radicals and disease. Arch Biochem Biophys 2002; 397(2): 354–359.
3. Selvaraja M, Abdullah M, Shah AM, *et al*. Systemic Lupus Erythematosus (SLE): A review on the prevalence, clinical manifestation, and disease assessment. Prog Microbes Mol Biol 2020; 3(1): a0000106.
4. Ser HL, Yin WF, Chan KG, *et al*. Antioxidant and cytotoxic potentials of Streptomyces gilvigriseus MUSC 26T isolated from mangrove soil in Malaysia. Prog Microbes Mol Biol 2018; 1(1): a0000002.
5. Smith MA, Perry G. Free radical damage, iron, and Alzheimer's disease. Journal of the neurological sciences 1995; 134(suppl.): 92–94. org/10.1016/0022-510X(95)00213-L.
6. Bouyahya A, Belmehdi O, El Jemli M, *et al*. Chemical variability of Centaurium erythraea essential oils at three developmental stages and investigation of their in vitro antioxidant, antidiabetic, dermatoprotective and antibacterial activities Industrial. Crops and Products 2019; 132 ():111–117. doi.org/10.1016/j.indcrop.2019.01.042.
7. Elouafy Y, Mortada S, El Yadini A, *et al*. Bioactivity of Walnut: Investigating the Triterpenoid Saponin Extracts of Juglans regia Kernels for Antioxidant, Anti-diabetic, and Antimicrobial Properties. Prog Microbes Mol Biol 2023; 6(1): a0000325.
8. Jemli M E, Khattabi N, Lachqer K, *et al*. Antifungal and insecticidal properties of Juniperus thurifera leaves. Nat Prod Commun 2018; 13(8): 1934578X1801300831.
9. Marmouzi I, Kharbach M, El Jemli M, *et al*. Antidiabetic, dermatoprotective, antioxidant and chemical functionalities in Zizyphus lotus leaves and fruits Industrial. Crops and Products 2019; 132:134–139.
10. Bellakhdar J, Claisse R, Fleurentin J, *et al*. Repertory of standard herbal drugs in the Moroccan pharmacopoeia. J Ethnopharmacol 1991; 35(2): 123–143.
11. Doukkali Z, Bouidida H, Srifi A, *et al*. Les plantes anxiolytiques au Maroc. Études ethnobotanique et ethno-pharmacologique. Phytothérapie 2015; 13: 306–313.

12. Friedman J, Yaniv Z, Dafni A, *et al*. A preliminary classification of the healing potential of medicinal plants, based on a rational analysis of an ethnopharmacological field survey among Bedouins in the Negev Desert, Israel. J Ethnopharmacol 1986; 16(2-3): 275–287.
13. Sargın S A, Akçicek E and Selvi S. An ethnobotanical study of medicinal plants used by the local people of Alaşehir (Manisa) in Turkey. J Ethnopharmacol 2013; 150(3): 860–874.
14. Tuzlacı E and Erol M K. Turkish folk medicinal plants. Part II: Eğirdir (Isparta) Fitoterapia 1999; 70(6): 593–610.
15. Al-Qura'n S. Ethnopharmacological survey of wild medicinal plants in Showbak, Jordan. J Ethnopharmacol 2009; 123(1): 45–50.
16. Thibane VS, Ndhlala AR, Abdelgadir HA, *et al*. The cosmetic potential of plants from the Eastern Cape Province traditionally used for skincare and beauty. S Afr J Bot 2019; 122:475–483.
17. Abdallah L, Almanasrah M, Taradeh M, *et al*. Antibacterial activity of camel whey in combination with various medicinal plant extracts. J Med Plant Stud 2017; 5(3):50–55.
18. Doukkali Z, Kamal R, El Jemeli M, *et al*. Anti-anxiety effects of Mercurialis annua aqueous extract in the elevated plus maze test. Pharm. Bioprocess 2016; 4(4), 056–061.
19. Maaroufi L, Hossain M S and Tahri W. New insights of Nettle (Urtica urens): Antioxidant and antimicrobial activities. J Med Plants Res 2017; 11(4):73–86.
20. Jimoh F, Adedapo A, Aliero A, *et al*. Polyphenolic and biological activities of leaves extracts of Argemone subfusiformis (Papaveraceae) and Urtica urens (Urticaceae). Revista de Biología Tropical 2010; 58(4): 1517–1531.
21. Budzianowski J. Caffeic acid esters from Urtica dioica and U. urens Planta Med 1991; 57(5):507–507.
22. Bucar F, Britzmann B, Streit B, *et al*. LC-PDA-MS-profiles of phenolic compounds in extracts of aerial parts of Urtica species Planta Med 2006; 72(11): P 152.
23. Assaf AM, Haddadin RN, Aldouri NA, *et al*. Anti-cancer, anti-inflammatory and anti-microbial activities of plant extracts used against hematological tumors in traditional medicine of Jordan. J Ethnopharmacol 2013; 145(3): 728–736.
24. Bruni A, Ballero M and Poli F. Quantitative ethnopharmacological study of the Campidano Valley and Urzulei district, Sardinia, Italy. J Ethnopharmacol 1997; 57(2): 97–124.
25. Slighoua M, Mahdi I, Amrati FE, *et al*. Ethnopharmacological survey of medicinal plants used in the traditional treatment of female infertility in Fez region, Morocco Phytothérapie 2020; 18: 321–39.
26. Neves VA, Picchi DG and Silva MA. Some biochemical properties of polyphenoloxidase from spearmint (Mentha arvensis). Braz Arch Biol Technol 2009; 52(4):1001–1010.
27. Said O, Khalil K, Fulder S, *et al*. Ethnopharmacological survey of medicinal herbs in Israel, the Golan Heights and the West Bank Region J Ethnopharmacol 2002; 83(3): 251–265.
28. Pieroni A, Quave C, Nebel S, *et al*. Ethnopharmacy of the ethnic Albanians (Arbëreshë) of northern Basilicata, Italy. Fitoterapia 2002; 73(3): 217–241.
29. Darias V, Bravo L, Barquin E, *et al*. Contribution to the ethnopharmacological study of the Canary Islands. J Ethnopharmacol 1986; 15(2):169–93.
30. Hudaib M, Mohammad M, Bustanji Y, *et al*. Ethnopharmacological survey of medicinal plants in Jordan, Mujib Nature Reserve and surrounding area. J Ethnopharmacol 2008; 120(1): 63–71.
31. Doukkali Z, Taghzouti K, El Jemli M, *et al*. Anxiolytic activity of aerial part of Mercurialis annua aqueous extract in mice using light/dark and hole board tests. J Basic Pharmacol Toxicol 2017; 1(2): 9–13.
32. Aquino R, Behar I, D'agostino M, *et al*. Phytochemical investigation on Mercurialis annua. Biochem Syst Ecol 1987;15(6): 667–669.

33. Blanco-Salas J, Vazquez FM, Hortigón-Vinagre MP, *et al*. Bioactive phytochemicals from Mercurialis spp. used in traditional Spanish Medicine. Plants 2019; 8(7): 193.
34. Lorenz P, Hradecky M and Berger M. Lipophilic constituents from aerial and root parts of Mercurialis perennis L. Phytochemical Analysis. Int J Plant Chem Biochem Techniques 2010; 21(3): 234–245.
35. Harborne AJ. Phytochemical methods a guide to modern techniques of plant analysis. springer science & business media 1998.3(11).
36. Trease G E and Evans W C. Textbook of pharmacognosy 13th ed London, Balliere Tindall 1989.
37. Ordonez A AL, Gomez JD and Vattuone MA. Antioxidant activities of Sechium edule (Jacq.) Swartz extracts. Food Chem 2006; 97(3): 452–458.
38. Şahin F, Güllüce M, Daferera D, *et al*. Biological activities of the essential oils and methanol extract of Origanum vulgare ssp. vulgare in the Eastern Anatolia region of Turkey. Food Control 2004; 15(7): 549–457.
39. Pukalskas A, van Beek TA, Venskutonis RP, *et al*. Identification of radical scavengers in sweet grass (Hierochloe odorata). Journal of Agricultural and Food Chemistry 2002; 50 (10): 2914–9.
40. Tundis R, Loizzo MR, Menichini F. Natural products as α-amylase and α-glucosidase inhibitors and their hypoglycaemic potential in the treatment of diabetes: an update. Mini Rev Med Chem 2010; 10(4): 315–331.
41. Bi HM, Zhang SQ, Liu CJ, *et al*. High hydrostatic pressure extraction of salidroside from Rhodiola Sachalinensis. J Food Process Eng 2009; 32: 53–63.
42. Elsalam RM, Goh KW, Mahadi M, *et al.* The Antibacterial Activities of Secondary Metabolites Derived from Streptomyces sp. Prog Microbes Mol Biol 2022; 5(1); a0000281
43. Lee HT, Loh HC, Ramlee SNL, *et al*. Oral Dietary Supplements Use Among Healthcare Workers During the COVID-19 Pandemic in Malaysia. Prog Microbes Mol Biol 2021; 4(1). a0000236.
44. Tan LKS, How CW, Foo JB, *et al*. Resveratrol as a potential broad-spectrum compound for cancer treatment. Prog Microbes Mol Biol 2020; 3(1). a0000098.
45. Saravanan S and Parimelazhagan T. In vitro antioxidant, antimicrobial and anti-diabetic properties of polyphenols of Passiflora ligularis Juss. fruit pulp. Food Sci Hum Wellness 2014; 3(2): 56–64.
46. Zarin MA, Wan HY, Isha A, *et al*. Antioxidant, antimicrobial and cytotoxic potential of condensed tannins from Leucaena leucocephala hybrid-Rendang. Food Sci Hum Wellness 2016; 5(2): 65–75.
47. Mzid M, Badraoui R, Khedir SB, *et al*. Protective effect of ethanolic extract of Urtica urens L. against the toxicity of imidacloprid on bone remodeling in rats and antioxidant activities. Biomed Pharmacother 2017; 91: 1022–1041.
48. Baştürk A, Ceylan MM, Çavuş M, *et al*. Effects of some herbal extracts on oxidative stability of corn oil under accelerated oxidation conditions in comparison with some commonly used antioxidants. LWT 2018; 89: 358–364.
49. Carvalho AR, Costa G, Figueirinha A, *et al*. Urtica spp.: Phenolic composition, safety, antioxidant and anti-inflammatory activities. Food Res Int 2017; 99(1): 485–494.
50. Bourgeois C, Leclerc ÉA and Corbin C. Nettle (Urtica dioica L.) as a source of antioxidant and anti-aging phytochemicals for cosmetic applications. Comptes Rendus Chimie 2016; 19(9): 1090–100.
51. Shonte TT, Duodu KG and de Kock HL. Effect of drying methods on chemical composition and antioxidant activity of underutilized stinging nettle leaves Heliyon 2020 ;6(5):e03938.
52. Haghju S, Beigzadeh S, Almasi H, *et al*. Chitosan films incorporated with nettle (Urtica dioica L.) extract-loaded nanoliposomes: I. Physicochemical characterisation and antimicrobial properties J Microencapsul 2016; 33(5): 438–448

53. Pinelli P, Ieri F, Vignolini P, *et al*. Extraction and HPLC analysis of phenolic compounds in leaves, stalks, and textile fibers of Urtica dioica L. J Agric Food Chem 2008; 56(19): 9127–9132.
54. Devi KP, Malar DS, Nabavi SF, *et al*. Kaempferol and inflammation: From chemistry to medicine. Pharmacol Res 2015; 99: 1–10.
55. Imran M, Rauf A, Shah ZA, *et al*. Chemo-preventive and therapeutic effect of the dietary flavonoid kaempferol: A comprehensive review. Phytother Res 2019; 33(2):263–275.
56. Lou-Bonafonte JM, Martínez-Beamonte R, Sanclemente T, *et al*. Current insights into the biological action of squalene. Mol Nutr Food Res 2018; 62(15), 1800136.
57. Zhang ZL, Luo ZL, Shi HW, *et al*. Research advance of functional plant pharmaceutical cycloartenol about pharmacological and physiological activity. China J Chinese Mat Med 2017; 42(3): 433–437.
58. El Jemli M, Kamal R, Marmouzi I, *et al*. Chemical composition, acute toxicity, antioxidant and anti-inflammatory activities of Moroccan Tetraclinis articulata L. J Tradit Complement Med 2017; 7(3): 281–287.
59. Rawat S, Bhatt ID, Rawal RS, *et al*. Geographical and environmental variation in chemical constituents and antioxidant properties in Roscoea procera wall. J Food Biochem 2017; 41 (2). e12302.
60. Rawat S, Bhatt ID and Rawal RS. Total phenolic compounds and antioxidant potential of Hedychium spicatum Buch. Ham. ex D. Don in west Himalaya, India. J Food Compos Anal 2011; 24(4-5): 574–579.
61. Vats S. Effect of initial temperature treatment on phytochemicals and antioxidant activity of Azadirachta indica A. Juss. Appl Biochem Biotechnol 2016; 178(3): 504–512.

Author(s) shall retain the copyright of their work and grant the Journal/Publisher right for the first publication with the work simultaneously licensed under:

Creative Commons Attribution-NonCommercial 4.0 International (CC BY-NC 4.0). This license allows for the copying, distribution and transmission of the work, provided the correct attribution of the original creator is stated. Adaptation and remixing are also permitted.