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Assessment of total polyphenols, flavonoids and anti-inflammatory potential of three *Apiaceae* species grown in the Southeast of Morocco

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Abstract

Plants have been frequently used for centuries in the Southeast of Morocco to heal various diseases such as inflammatory disorders. In this investigation, total phenols and flavonoids contents were examined in stems and leaves of hydromethanolic extracts of three *Apiaceae* species, namely Parsley (*Petroselinum crispum*), Celery (*Apium graveolens*) and Coriander (*Coriandrum sativum*) along with their anti-inflammatory activity. Phenols amount and flavonoids level were measured by the Folin-Ciocalteu's reagent and Aluminium chloride solution, respectively. The anti-inflammatory potential was evaluated via nitric oxide (NO) scavenging potential, inhibition of protein denaturation and membrane stabilizing effect. Results revealed that parsley, which is the richest in polyphenols (21.63 mg GAE/ g DW) and flavonoids (15.73 mg RE/g DW) contents, exhibited the highest anti-inflammatory activity based on protein denaturation inhibition assay ($IC_{50} = 118.92 \mu\text{g/mL}$), NO free radical scavenging potential ($IC_{50} = 142.45 \mu\text{g/mL}$) and membrane stabilizing effect ($IC_{50} = 192.08 \mu\text{g/mL}$). The strong correlation found between flavonoids and anti-inflammatory assays ($R^2 \geq 0.915$) suggests that flavonoids were the key providers of this anti-inflammatory effect. These findings could validate the traditional use of these plants to treat various inflammatory diseases.

Keywords: *Anti-inflammatory; Parsley; Celery; Coriander; Flavonoids; Polyphenols*

1. Introduction

Inflammation is a multifaceted physiological response to aggression triggered by xenobiotic agents [1]. Inflammatory reaction requires physiological levels of free radicals to ensure its mediation [2]. In fact, during acute inflammation process, different categories of immune cells are recruited to the site of damage where they produce an arsenal of pro-inflammatory molecules such as cytokines, COX byproducts and various reactive oxygen species (ROS) like singlet oxygen and nitric oxide free radical, with the objective to reestablish the physiological integrity [1]. This process induces an overproduction of free radicals, a key source of oxidative stress, which eventually leads to chronic disorders such as cancer, diabetes, atherosclerosis and chronic inflammation-associated disorders [3]. The current anti-inflammatory drugs used for decades have revealed many side effects including osteoporosis, renal failure, peptic ulcers and gastrointestinal hemorrhage among others [4].

It was demonstrated that polyphenols can prevent both the production and action of pro-inflammatory mediators, resulting in anti-inflammatory capacity [5]. Moreover, plants synthesize polyphenol compounds as secondary metabolites to defend themselves or to ensure optimal growth during stress and to terminate free radicals [6]. In this way, numerous plant extracts have displayed effective anti-inflammatory potential [7]. Thus, developing safer and efficient anti-inflammatory drugs is needed, especially from folk medicine known for their efficacy in the management of many human illnesses in recent years.

Parsley (*Petroselinum crispum*), Celery (*Apium graveolens*), and coriander (*Coriandrum sativum*) are members of the *Apiaceae* (Umbelliferae) family, which encompasses various vegetables and spices with some of them possessing medicinal properties. In the Moroccan cuisine, celery, parsley and coriander are employed to flavor various popular foods, including salads, desserts and almost all strong, salty cooked dishes. Despite their culinary uses, they have shown many therapeutic effects. Celery proved its beneficial powers in asthma and bronchitis [8]. Parsley herb and roots were often recommended in both folklore medicine for their advantageous outcomes for urinary, gastric and menstrual disorders [9] and also in conventional therapy for its anti-diabetic, antihypertensive, nephroprotective, antihyperlipidemic and antibacterial properties [10]. In Moroccan folk medicine, coriander and its products have been in use to cure hyperglycemia [11]. Moreover, coriander has been investigated to be responsible for therapeutic properties against diabetes, inflammation, hypercholesterolemia and hepatorenal toxicity [12]. Nevertheless, these plants still need more targeted studies to highlight their biological activities.

This study aims to examine total polyphenols and flavonoids contents along with anti-inflammatory activity of hydromethanolic extracts of three *Apiaceae* species. The results can be an argument for the traditional use of these plants in the treatment of different inflammatory diseases.

2. Materials and Methods

2.1. Plant material

The aerial parts (stems and leaves) of three plants belonging to the *Apiaceae* family known as Coriander (*C. sativum*), Parsley (*P. crispum*) and Celery (*A. graveolens*) were collected on April 14/2019 from Tinghir region located in the South East of Morocco. The plants were authenticated using voucher specimens for Parsley plant (FSTE-19/315), Celery plant

(FSTE-19/316) and Coriander plant (FSTE-19/317) deposited at the Faculty of Sciences and Techniques, Errachidia Morocco. The plant material was dried and then stored at ambient temperature (25 °C) in the dark before extraction.

2.2. Preparation of rich polyphenol extracts

The rich polyphenol extracts of different plants were prepared following the process established by Bouhlali et al. [13] with slight adjustments. First of all, thirty grams of powdered aerial parts of plants were extracted with 150 mL of Methanol - water (80/20, v/v), at 35 °C for 12 hours employing an orbital shaker-incubator. The mixture was then filtered and the filtrate was concentrated under minimal pressure at 40 °C by total evaporation of solvent, using a rotary evaporator. Then the resulting crude extracts were stored at -20 °C in dark glass bottles until further use. The extracts were dissolved in known dilution of water (1%) to determine phenolic and flavonoids levels plus their anti-inflammatory capacity.

2.3. Measurement of total phenol compounds

Quantification of phenolic compounds in plant extracts was performed colorimetrically using the Folin-Ciocalteu's reagent, following the method described by Obeng et al. [14]. One hundred microliter of the dissolved plant extract was transferred to a 5 mL container to which was added 500 µL of 1/10 dilution of Folin-Ciocalteu's reagent. After one minute, 400 µL of sodium carbonate solution (7.5% w/v) was added and the volume was filled up to 5 mL with water. The mixture was left to stand for 60 minutes at ambient temperature and its absorbance versus control was measured at 765 nm. Polyphenol concentrations (six-fold measurement) were presented in milligram of gallic acid equivalents per gram of dry weight (mg GAE/g DW) through the calibration curve with gallic acid.

2.4. Measurement of flavonoids content

Quantification of flavonoids was fulfilled according to the method described by Hmidani et al. [15]. Summarily, one milliliter of each extract was stirred with 4 mL of distilled water. Then, 0.3 mL of sodium nitrite solution (5%) was added, followed by 0.3 mL of aluminum chloride solution (10%). Thereafter, the tubes were left to stand at room temperature for 5 min, by then 2 mL of sodium hydroxide (1 M) were poured into each test tube and filled with distilled water up to 10 mL. The mixtures were vortexed thoroughly and their turbidity versus control was read at 510 nm. Total flavonoids content (six fold measurement) was

depicted in mg of rutin equivalents per gram DW (mg RE/g DW) by means of the calibration plot with rutin.

2.5. Nitric oxide radical scavenging activity

Nitric oxide scavenging ability was measured using the Griess reaction based on the procedure described by Bouhlali et al. [16]. Briefly, five hundred microliter of sodium nitroprusside (10 mM) made within phosphate-buffered saline (PBS) (pH 7.4) was stirred up with 1 mL of each plant extract at various concentrations (100 – 1500 µg/mL) and incubated at 25 °C for 150 min. The Griess reagent was prepared using 2.5% phosphoric acid, 1% sulfanilamide and 0.1% naphthylethylenediamine dihydrochloride, and a volume of 1.5 mL of this reagent was added to each plant extract mixture after incubation. The same process was repeated using similar volumes of the PBS and Trolox (20 - 120 µg/mL) as control and standard, respectively. The turbidity was determined at 546 nm and the scavenging ability of NO free radical by plant extract and standard was deduced manipulating the following equation:

$$NO \text{ scavenging activity (\%)} = ((Abs \text{ Control} - Abs \text{ Sample}) / (Abs \text{ Control})) \times 100$$

Abs Control is the turbidity of control, and Abs Sample is the turbidity of sample extract/standard. The concentration required to scavenge 50% of nitric oxide free radical (IC₅₀) was determined graphically using a calibration plot of NO scavenging effect percentages against various concentrations.

2.6. Inhibition of protein denaturation

The potential of plant extracts to inhibit albumin denaturation was evaluated using the procedure of Bouhlali et al. [17]. The reactants consisted of one milliliter of bovine serum albumin (1%) made within phosphate-buffered saline, pH 6.4 and 1 mL of each hydromethanolic extract in variable concentrations (20 - 1000 µg/mL). The mixtures of different extracts were given 20 min to stand at ambient temperature and later on heated at 70 °C for 5 min. After cooling, each mixture's absorbance was read at 660 nm. The same protocol was performed six fold using double-distilled water and diclofenac sodium as negative and positive controls, respectively. The inhibition percentage (IP %) of protein denaturation was deduced using the formula :

$$IP \% = (Abs.control - Abs.sample) \times 100 / (Abs.control)$$

Abs. control is the absorbance without plant material; Abs. sample is the absorbance of plant extract or standard. The IC₅₀ was deduced graphically using a plot of inhibition percentages against different concentrations.

2.7. Membrane stabilizing effect

The membrane stabilizing ability was investigated by applying the procedure established by Hmidani et al. [18]. The sterilized Alsever's solution (prepared via dissolving in distilled water several components comprising sodium chloride (0.42%), dextrose (2%), sodium citrate (0.8%) and citric acid (0.05%)) was mixed up with equal blood volumes obtained from healthy human volunteers who did not take in any non-steroidal anti-inflammatory drugs for 15 days before the blood sampling. Later on the obtained blood solution was centrifuged at 3000 rpm and the cell pellets were rinsed with an isotonic saline (9 g/L) which was used later on to prepare a suspension containing 10% of cell pellets. Hence, the assay mixture composition was as follows: 2 mL of hypotonic saline (3.6 g/L), 1 mL of phosphate buffer, 0.5 mL of plant extract at various concentrations ranging between 20 to 1000 µg/mL and 0.5 mL of blood suspension (10%). After incubating the different mixtures for a half an hour at 37 °C, they were centrifuged at 3000 rpm. The amount of hemoglobin in supernatant was deduced measuring turbidity at 560 nm. Distilled water was used as the negative control. The positive control was diclofenac sodium at various concentrations starting from 20 to 1000 µg/mL. The percentage of membrane stabilization (MS %) was calculated using the equation:

$$MS \% = (Abs.control - Abs.sample) \times 100 / (Abs.control)$$

Where Abs. control is the turbidity without plant material; Abs. sample is the turbidity of extracts or standard. The IC₅₀ was deduced from a plot of membrane stabilizing percentages against different concentrations.

2.8. Statistical analysis

The experimental data were processed using Statistical Package for the Social Sciences (SPSS) version 23 software. The concentrations were presented as the mean of six repetitions for all assays ± SE (standard error). One-way Analysis of variance (ANOVA) was performed to point out the differences among the experimental groups. The two-group variations were highlighted using the *post hoc* Bonferroni tests at the level of significance α

= 0.001. Pearson's square correlation coefficient (R^2) was also used to evaluate the relationship between pairwise variables.

3. Results and Discussion

3.1. Determination of total phenolic contents

Polyphenols are secondary metabolites generated by whole plants. They include phenols, phenolic acids, quinones, flavonoids, tannins and phenylpropanoids among others [19]. A large variety of these phytochemicals has antioxidants. The polyphenol level was measured using the colorimetric method of Folin–Ciocalteu, which is a simple, rapid, reproducible and low-cost procedure. This method implicates the reduction of phosphomolybdic–phosphor tungstic acid by polyphenols in an alkaline medium. As revealed in Table 1, the overall analysed plant extracts showed significant differences ($p < 0.001$). The highest value of total polyphenols was displayed by parsley (21.63 ± 1.81 mg GAE/g DW) and the lowest level was found in coriander (13.72 ± 1.13 mg GAE/g DW). Our results are lower than those found by Tang *et al.* [20] who measured a total phenolic content in parsley of 42.31 ± 0.50 mg GAE/g DW. Nevertheless, El-Sayed *et al.* [21] reported that parsley exhibited higher polyphenols level (121.95 ± 2.15 mg GAE/ g extract). Alternatively, Msaada *et al.* [22] recorded lower levels of phenolic content in Egyptian coriander (0.94 ± 0.05 mg GAE/g DW), Tunisian coriander (1.00 ± 0.06 mg GAE/g DW) and Syrian coriander (1.09 ± 0.02 mg GAE/g DW). Moreover, Uddin *et al.* [23] obtained higher polyphenol content in celery (63.46 ± 12.00 mg GAE/g DW). These variations in total polyphenols level may be related to genetic variations, conditions of cultivation, extraction time or the extracting solvent.

3.2. Determination of total flavonoid contents

Total flavonoids content was determined by a mixture of reactants including aluminium chloride, which bind to flavonoids thus form aluminium-flavonoid complexes having maximum turbidity at 430 nm. Table 1 summarized that flavonoids content in all analysed plants extracts varied largely ($p < 0.001$) and ranged from 8.14 to 15.73 mg RE/g DW. Parsley exhibited the highest flavonoids level and celery showed the lowest level. Our results are largely lower than those established by Priecina and Karlina [24] who observed that flavonoids content in parsley and celery were 120.56 mg CE/100 g DW and 352.99 mg CE/100 g DW, respectively. However, our results concerning celery and coriander are higher than those reported by Chandra *et al.* [25] (14.35 mg QE/g

DW) and Msaada et al. [22] (2.51 mg CE/g DW), respectively. These differences can be explained by plant genotype, environmental conditions and extraction procedure. Flavonoids are renowned for their herbal synthesis when microbial attacks occur. It should therefore not be surprising that flavonoids have been identified in vitro as effective antimicrobial agents against a wide variety of microbial pathogens [26]. Several studies claimed that flavonoids possess abundant health benefits such as anti-allergic, anti-inflammatory, analgesic, cardio protective, hepatoprotective, antiviral and anti-oncogenic properties. This diversity of therapeutic effects can be related to variant chemical structures of flavonoids [26].

3.3. Nitric oxide radical scavenging activity (NOSA)

During an immune response, activated neutrophils, macrophages and other immune cells overproduce NO mainly through the overexpression of NO synthase [27]. Despite its physiological role as a vasodilator and protein modulator [28], NO free radical may cause toxic reactions against tissues around inflammation site and since it is generated at high amounts in some categories of inflammatory conditions, for example asthma, it has been entitled as a pro-inflammatory agent [27]. It has been demonstrated that non-steroidal anti-inflammatory drugs can scavenge NO free radical and inhibit its synthesis [29]. For this reason, NO free radical quenching capacity is an important way to handle inflammatory diseases. As presented in Table 2, all analyzed plant extracts showed significant NO free radical scavenging activities ($p < 0.001$). Celery ($IC_{50} = 270.38 \mu\text{g/mL}$) recorded the weakest NO free radical scavenging ability while parsley ($IC_{50} = 142.45 \mu\text{g/mL}$) showed the strongest NO free radical scavenging potential, but it remains lower than that of Trolox ($IC_{50} = 83.37 \text{ mg/mL}$). These three *Apiaceae* species possess the potential of quenching NO free radical and thus can be of remarkable importance in the prevention of NO free radical's deleterious outcomes in the human body. This scavenging capability is enabled by some classes of polyphenols in plant extracts, particularly flavonoids, as suggested by a strong correlation between NO scavenging potential and flavonoid level ($R^2 = 0.915$) on this hand and a low correlation between NO scavenging potential and polyphenols ($R^2 = 0.356$) on the other hand.. As a result, total polyphenols and flavonoids may be responsible for the observed variations between the plants studied.

3.4. Inhibition of protein denaturation (IPD)

Type III hypersensitivity response is one of the main factors involved in many diseases, such as serum disease, lupus erythematosus and many other inflammatory conditions. [30]. Moreover, the primary mechanism of anti-inflammatory drugs was thought to be by inhibiting protein destruction. [31] but Vane [32] has figured out their inactivation effect on cyclo-oxygenase. Therefore, as part of their anti-inflammatory properties, the capacity of different *Apiaceae* plant extracts to prevent protein denaturation was assessed. As revealed in Table 2, all plant extracts examined have been able to prevent protein denaturation in a dose-dependent aspect and significant differences have been observed among the studied plant extracts ($p < 0.001$). The potency order was as follows: coriander < celery < parsley. Only parsley showed strong anti-denaturation potential compared to diclofenac sodium ($IC_{50} = 225.04 \mu\text{g/mL}$) which is the regular anti-inflammation drug. Various works proved that interactions among polyphenols and proteins enhance thermal protein stability [33]. Indeed, Ali et al. [34] confirmed the improvement of thermal stability triggered by phenolic compounds in soybean glycinin, bovine serum albumin (BSA) and bovine p-lactoglobulin. Phenolic interactions strongly affect the secondary structure of proteins [34]. In fact, a strong relationship was concluded between inhibition of protein denaturation and flavonoids ($R^2 = 0.956$) (Table 3). As a result, variations in phytochemical composition may explain the different inhibition of membrane denaturation potential.

3.5. Membrane stabilizing effect (MSE)

During the inflammation response, active immune cells release lysosomal components such as proteases, which cause a chain reaction associated with many disorders such as glomerulonephritis, systemic lupus erythematosus and rheumatoid arthritis [35]. Various anti-inflammatory drugs are reported to disallow the release of enzymes from lysosomes and to stabilize their membranes [36]. As a result plant extracts may prevent inflammation development which makes them certainly a crucial source of anti-inflammatory remedies. Given the similarity between erythrocyte and lysosomal membranes, the prevention of erythrocyte lysis caused by hypotonia was used to assess the anti-inflammatory potential of plant material. Based on data presented in Table 2, all plant extracts showed tough erythrocyte membrane protection and a significant difference ($p < 0.001$) has been evidenced. Parsley showed the highest membrane stabilizing potential ($IC_{50} = 192.08 \mu\text{g/mL}$) followed by coriander ($IC_{50} = 247.18 \mu\text{g/mL}$) and celery ($IC_{50} = 273.52 \mu\text{g/mL}$). The membrane stabilizing potential of these

extracts is slightly higher than that of diclofenac sodium ($IC_{50} = 304.88 \mu\text{g/mL}$) used as a reference anti-inflammatory drug. Polyphenols especially flavonoids can manage interactions with hydrophilic heads of cytoplasmic membranes, hence lower fluidity and strengthen rigidity [37]. These hydrogen bond-enabled interactions protect the membrane from harmful molecules, such as pro-oxidants, consequently ensuring membrane physiological function and integrity. Some studies have actually shown that plant flavonoids have anti-inflammatory properties [38]. The analysis of correlation (Table 3) showed a high link between membrane stabilizing activity and flavonoids ($R^2 = 0.927$) followed by a weak correlation concerning polyphenols ($R^2 = 0.435$). These specific findings may be attributable to different amounts and nature of phenolic compounds in which flavonoids play an important role in preventing erythrocyte membranes from lipid peroxidation and stabilizing their membranes against hypotonic lysis [18].

4. Conclusion

Parsley, coriander and celery are very common medicinal and aromatic plants with various uses in folk / traditional medicine. The main results of the current study concluded that parsley had the richest phenolic and flavonoid levels thus demonstrated potent anti-inflammatory properties through NO free radical scavenging potential, membrane stabilizing effect and prevention of protein denaturation. Thus, the current findings may explain the traditional use of extracts from these *Apiaceae* species in the treatment of inflammatory diseases.

Ethical approval

This article does not contain any studies involving animals performed by any of the authors. This research involving human material (blood) has been performed in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards, and this study protocol was approved by the Ethics Committee for Biomedical Research, Faculty of Sciences and Techniques of Errachidia (FSTE), Morocco, under reference number FSTE-19/2016.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Funding Statement

No funding was received for this work.

Tables and Figures

Table 1: Total phenolic and flavonoid contents of different plants (mg/g DW)

	Total Phenolic content mg GAE/g DW	Total Flavonoid content mg RE/g DW
<i>Coriandrum sativum</i>	13.72 ± 0.96 ^c	10.24 ± 0.81 ^b
<i>Petroselinum crispum</i>	21.63 ± 1.04 ^a	15.73 ± 0.83 ^a
<i>Apium graveolens</i>	17.39 ± 0.89 ^b	8.14 ± 0.61 ^c

Values in average (n = 6) ± SE. Means in the same column with different superscripts (a-c) are significantly different according to *post hoc* Bonferroni tests (p < 0.001).

Table 2: Effect of plant extracts on nitric oxide scavenging activity, membrane stabilizing activity and inhibition of protein denaturation (µg/mL)

	Membrane stabilizing activity (IC ₅₀) µg/mL	Inhibition of protein denaturation (IC ₅₀) µg/mL	Nitric oxide radical scavenging activity (IC ₅₀) µg/mL
<i>Coriandrum. sativum</i>	247.18 ± 5.81 ^c	247.18 ± 5.81 ^b	218.63 ± 6.41 ^b
<i>Petroselinum crispum</i>	192.08 ± 4.98 ^d	118.92 ± 5.84 ^d	142.45 ± 5.59 ^c
<i>Apium graveolens</i>	273.52 ± 5.16 ^b	227.07 ± 5.16 ^c	270.38 ± 5.25 ^a
Standards	304.88 ± 5.67 ^{a#}	225.04 ± 5.96 ^{a#}	83.37 ± 3.20 ^{d##}

Values in average (n = 6) ± SE. #: Diclofenac sodium; ##: Trolox. Means in the same column with different superscripts (a-d) are significantly different according to *post hoc* Bonferroni tests (p < 0.001).

Table 3: Correlation between phenolic content, flavonoids level, IPD, MSE and NOSA

	MSE	IPD	NOSA	Polyphenols	Flavonoids
MSE	1				
IPD	0.948	1			
NOSA	0.972	0.970	1		
Polyphenols	0.435	0.396	0.356	1	
Flavonoids	0.927	0.956	0.915	0.491	1

MSE: Membrane stabilizing effect; IPD: Inhibition of protein denaturation; NOSA: Nitric oxide scavenging activity.

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