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Research Article

FREE RADICALS SCAVENGING ACTIVITY AND PHYTOCHEMICAL COMPOSITION OF ARTEMISIA (*HERBA-ALBA*) EXTRACT GROWTH IN ALGERIA

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ABSTRACT

In this study, phenolic content, flavonoids, condensed tannins and antioxidant activities of *Artemisia (Herba-alba)* extract obtained by different extraction techniques (ultrasonic assisted, classical, microwave assisted and Soxhlet) method were evaluated. ABTS, DPPH, and FRAP scavenging methods were applied to test the antioxidant activities. The results obtained indicated that the maceration technique extract showed the highest antioxidant activities, total phenolic, and flavonoids content. The extracts obtained by Soxhlet, ultrasonic assisted and microwave assisted technique showed moderate phenolic content and antioxidant capacity. These results indicate that maceration can be considered the best technique for extract of phenolic compounds.

Keywords: *Artemisia Herba-alba*, Phenolic content, DPPH, ABTS, FRAP.

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1. INTRODUCTION

Artemisia is a wide genus of plants with approximately 500 species and attached to the Asteraceae family. Some varieties of this plant used in food as additives [1]. Recently, plant and their derivate used in medicines are getting an attention for the therapeutics market by their mild action and no negative effects registered. Environ 80% of African people prefers to use the plant in tradional medicine against synthetic drugs [2]. Some studies indicate that this plant used in traditional medicines with, anti-inflammatory, antitumor, antioxidant, antispasmodic, antimicrobial, insecticidal, antimalarial, antifungal antioxidant activity [3], hepatitis, malaria, cancer, and diarrhea [4]. While they have also used as drugs and treatment of several disease [5]. However, the bioactive compounds of this species exhibit some effects in liver disease remain unknown. Previously, we presented substantial progress in explain the chemical and phytotherapy properties of this species. Previous phytochemical studies reported the high level of phytochemical composition such as polyphenols, flavonoids, sterols, tannins and flavonols in *Artemisia* extract [6]. Other studies reported that Artemisia extract is rich on phenolic content [7] and glycosides [8]. This plant was characterized by the high concentration of phenolic compounds; these compounds manifest diverse biological activities. In addition, play diverse function such as their role as antioxidant, anti-allergic, anti-inflammatory and antimicrobial [9]. Dietary polyphenols is the most important types of natural antioxidants and chemo-preventive agents found in human diets including fruits, vegetables, grains, and their derivate food and beverages. Epidemiological, clinical and nutritional studies strongly support the evidence that dietary polyphenols enhance human health by lowering risk and preventing the beginning of degenerative malady including cancers, cardiovascular diseases and metabolic disorders [10]. Antioxidants were used as scavenging of free radicals, such as vitamins, Plant and their derivate play key role defensive to stop the generation of free radicals carotenoids and polyphenols. These compounds were scavenged reactive oxygen by inhibition the reaction chain, who give an electron to the free radical. The antioxidant defense system, supported by dietary antioxidants, protects the body from free radicals [11,12], and therefore possible to be deficiency in alleviating the malady caused by oxidative stress [13]. In this regard, plants it is principal source of natural drugs,

several recently studies reported that new drugs have been isolated from medicinal plants. Chemically, the free radicals it is unstable atoms and considerable as principal reason damage to the great molecules (lipid and proteins) because the disproportion between the generation ROS and the antioxidant enzymes. This phenomenon are known to be the underlying ROS, which is extremely related with pathogenesis of various malady such as cardiovascular diseases, metabolic syndrome and diabetes. These radicals can be inhibited by the defensive role of synthetic antioxidant and natural compounds. In addition, studies has been reported that synthetic antioxidant are toxic and caused several damage diseases, [14]. Recently, the attentiveness for the therapeutic use of natural compounds particularly from plants. In this research paper, influence of extraction technique on extracting natural products from *Artemisia Herba-alba* growth in Algeria and antioxidant properties is still not accessible. In this study, efficiency of extraction methods including microwave assisted extraction, classical, Soxhlet and ultrasonic assisted of *Artemisia Herba-alba* is investigate to obtain the best extraction technique. The phytochemical screening and the antioxidant activity of different extraction technique was estimated by in vitro methods (DPPH radical scavenging activity, ferric-reducing antioxidant power (FRAP) and ABTS radical).

2. RESULTS AND DISCUSSION

2.1. Total phenolic, flavanoids, and condensed tannins content

The different extracts obtained by different techniques of *Artemisia Herba-alba* used for quantification of total phenolic content, flavonoid and condensed tannins. The results it can be seen in Table 1. The level of total phenolic content in different extraction technique ranged from (24.27±0.6) mg GAE /g to (35.7±0.5) mg GAE /g. The content of flavanoids in catechin equivalent, varies from (10.8±0.1) mg CE /g to (16.5±0.2) mg CE /g, condensed tannins expressed in catechin equivalent varies from (4.8±0.1) mg CE /g to (6.5±0.09) mg CE /g. The highest amounts of phenolic compound, flavanoids, condensed tannins and anthocyanin are shown in the classical extraction and ultrasonic extraction. In addition, Soxhlet and microwave assisted technique extraction obtains the lowest amounts. Ultrasonic assisted technique is controlled (mechanical and chemical effects of acoustic cavitation collapse) and resulting in the disruption of cell walls. That make easy to release of their content into the

mixture, and generate high local temperatures and pressures [15]. This may be explain the phenolic content increase in the extract by the UE method. However, this phenomenon (local elevate temperature and pressure produced by cavitation) could induce the degradation of unstable compounds. This condition results the degradation of these natural compounds under high extraction pressure and temperature some phenolic compounds may be lost under longer extraction time of Soxhlet method [16].

Table 1. Total phenolic, flavonoid and condensed tannins content of different methods extract obtained by CE, SE, ME and UE

Extraction technique	Phenolic content mg GAE /g	Flavanoids content mg CE/g	Condensed tannins mg CE/g
CE	35.7±0.5	16.59±0.2	6.57±0.09
UE	14.27±0.5	10.87±0.1	3.89±0.07
SE	24.27±0.3	15.51±0.2	5.42±0.08
ME	17.27±0.4	12.87±0.1	4.18±0.08

2.2.1. DPPH assay

The DPPH˙ scavenging ability of ethanolic extract obtained by different methods from *Artemisia Herba-alba* is shown in Fig. 1. The strongest scavenging activity was observed in CE followed by SE with the IC_{50} values of 0.31 ± 0.02 and 0.51± 0.03 mg mg/mL respectively. The following order of free radical inhibition: Classical extract > Soxhlet extracts > microwave assisted extracts> ultrasonic extracts. The intermediary value are found for the ME (IC_{50}= 0.62 ± 0.02 mg/mL) and while UE displays the lowest response in this assay UE (IC_{50}=0.72 ± 0.05 mg/mL). The correlation DPPH˙ between scavenging ability and total phenolic content reported by several studies. In this case, correlation between free radicals inhibition and phenolic concentration are observed [17].

2.2.2. FRAP assay

Taking in account that involved reaction may determine two classifications reaction types: assays based on hydrogen atom transfer reactions and assays based on electron transfer [18]. Moreover, many tests used for elevated the scavenging capacity of biologically relevant

oxidants. Similar results for DPPH˙ found in FRAP assay, the reducing power of CE was the highest value for the analysis extracts, with an IC_{50}= 614.71± 7.5 mmol/mg dried plant, followed by SE, ME and UE (750.54± 8.5, 840.50± 7.4 and 975.75± 8.5 mmol/mg dried plant) respectively (Table.2).

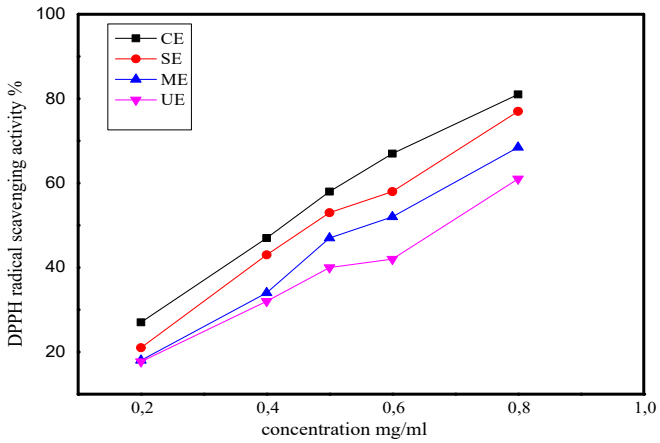

Fig.1. Antioxidant activity of CE, SE, ME and UE ethanolic extract from *Artemisia Herba-alba* against DPPH˙ radicals

2.2.3 ABTS assay

The scavenging activity from different technique was calculated. Table 2 and Fig.2 shows that all extracts analysis in this study inhibit ABTS radical potential. The IC_{50} of ABTS radical inhibition properties of different extracts were ranged to 24.55±0.4 ± mg/ml from 37.57±0.9 mg/ml, the highest scavenging activities was found in CE IC_{50}=24.55±0.7 µg/ml, the medium in SE 29.87 ± 0.5 mg/ml, ME 33.21±0.6 µg/ml. The lowest value in UE IC_{50}=37.04±0.65 µg/ml.

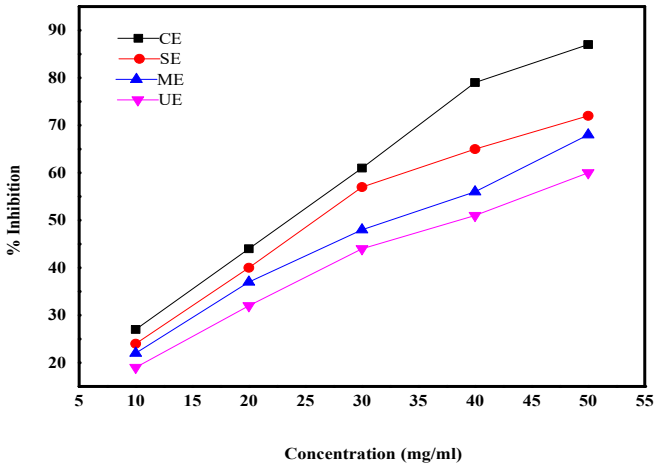

Fig.2. ABTS radical inhibition potential of CE, SE, ME and UE ethanolic extract from *Artemisia Herba-alba*.

Table 2. Scavenging activity of DPPH, FRAP, and ABTS of extracts obtained by different technique from *Artemisia Herba-alba*.

Extraction Methods	DPPH IC_{50}= (mg/ml)	FRAP IC_{50}= (mmol/ml)	ABTS IC_{50}= (mg/ml)
CE	0.31± 0.02	614.71± 7.5	24.55±0.4
SE	0.51±0.03	750.54± 8.5	29.87±0.5
ME	0.62±0.02	840.50± 7.4	33.21±0.6
UE	0.72±0.05	975.75± 8.5	37.57±0.9

3. EXPERIMENTAL

3.1. Plant material

The leaf, flowers and seed (aerial parts) from *Artemisia herba-alba* were collected from southeast of Algeria, state of El Oued on October 2016. The parts then separated, washed and dried at ambient temperature. All these organs were transformed to powder by grinder and stored. The obtain powder was put in a hot air for drying (oven at 60 °C). Depending on the parameters and characteristics of different parts, the time ranged from 24 at 30 h.

3.2. Classical extraction (CE)

Fifteen grams of powdered (50 g) and ethanol (500 ml) are put in Erlenmeyer flasks (1000 ml), and the ratio of plant material mass (g) to solvent volume (ml) was 1:10, no additional stirring used. The temperature of process is 30 °C and the time 24 h min. We obtained filtrate liquid, the filtrates recuperated and concentrated using rotary vacuum evaporator at 45 °C. Extracts were stored at +4 °C.

3.3. Ultrasonic assisted extraction (UE)

Ultrasonic apparatus from Branson was used for accelerated extraction. Fifteen grams (50 g) were extracted with ethanol (500 ml) for 60 min. The different extracts extract was filtered (Whatman No. 4) and concentrated by using rotary-evaporated under vacuum at 45 ◦C to dryness [19]. The different parts extracts and the ethanolic extracts were stored at +4 °C.

3.4. Soxhlet extraction (SE)

Fifty grams of powder were mixed with 500 ml ethanol and extracted with Soxhlet mounting for 6 h. The extracts recuperated by using the rotatory evaporated at 45 °C by [20]. The ethanolic extracts were stored at +4 °C.

3.5. Microwave assisted extraction (ME)

Fifty grams of powders, 500 ml of ethanol were mixed and put in microwave extraction (vessel. Yima Opto-Electrical Technology Co. Beijing, China). All the microwave extractions were performed under a set microwave irradiation power (1600 W). During the extraction operation, the mixture was stirred to obtain the homogenous exposure. The extract was filtered (Whatman No. 4 paper), and concentrated by rotary evaporation under vacuum at 45 °C and stored at +4 °C.

3.6. Total phenolic concentration

The concentration of phenolic contents in different extracts were estimated by the Folin-Ciocalteu method. Briefly. The reaction constituted 100 µl extract or the standard (gallic acid), 2.5 ml water and 0.25 ml of 1N Folin-Ciocalteu reagent. 2.5 ml of sodium carbonate aqueous solution (2%, w/v) was added after 5 min to the mixture. The reaction completed in 30 minutes in darkness at ambient temperature [20]. The absorbance was registered at 765 nm using a spectrophotometer. The phenolic content was calculated as mg gallic acid equivalent (GAE) per g of dry weight and the equation of calibration curve: $Y= 0.00778x$, $R^2 =0.991$.

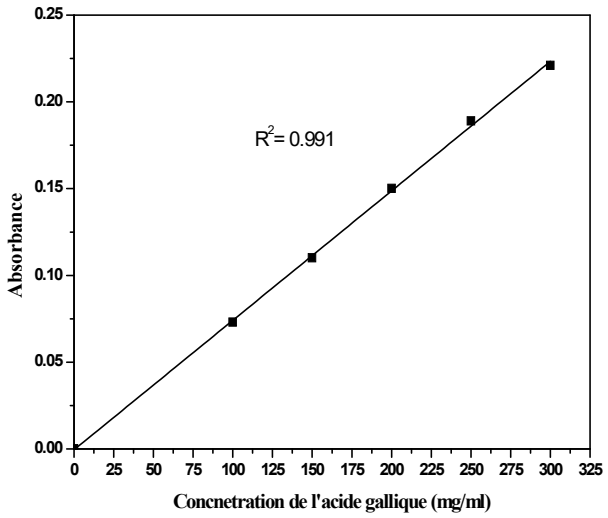

Fig.3. Calibration curve for determination of total phenolic content expresses as mg GAE/g DW

3.7. Total flavonoids

The total flavonoids quantification spectrophotometrically. 1 ml of different technique extract mixed with 4 ml of distilled water (4 ml). After, 0.3 ml of added (5% sodium nitrite solution) and 0.3 ml of (10% aluminum chloride solution) was added to the above mixture; the reaction incubated at room temperature for 5 min. 1 M NaOH (2 ml) was added. For 10 ml of reaction volume we completed by distilled water [21]. The reaction was vortexed and the absorbance readied at 510 nm. The total flavonoids were calculated as mg catechin equivalents (CE)/g of dry weight.

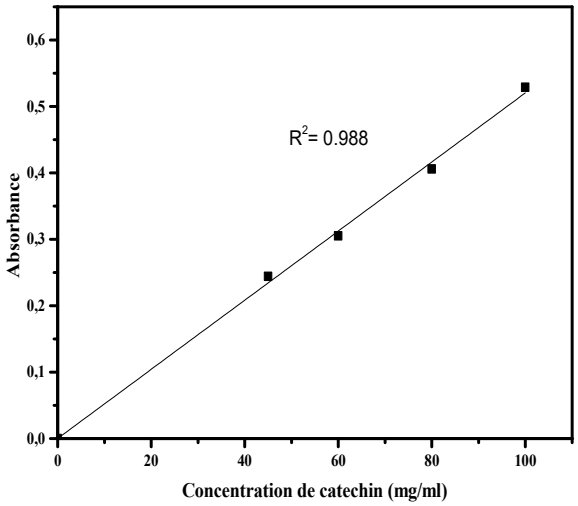

Fig.4. Calibration curve for determination of total flavonoids content expresses as mg CE/g

3.8. Quantification of condensed tannins

Proanthocyanidins content was quantified using a spectrophotometric method [22]. Ethanolic extract or standard (0.5 ml) was added to 4% vanillin methanol (3 ml) and 1.5 ml of hydrochloric acid, the reaction was allowed to stand at ambient temperature (15 mn). The absorbance of was measured at 500 nm. Total condensed tannins was calculated as mg catechin equivalent (mg CE/g).

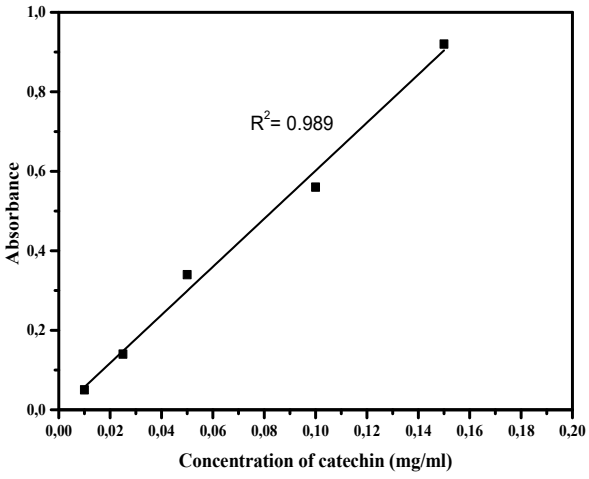

Fig.5. Calibration curve for determination of total phenolic content expresses as mg GAE/g DW

3.9. Antioxidant activity

3.9.1 DPPH Assay

Antioxidant activities and total phenolic contents of various extracts from *Artemisia Herba-alba* described by Zhu et al [23].

3.9.2. FRAP assay

The test inhibition of FRAP radicals by different extracts was determined according to the method of Rajkumar et al [24].

3.9.3. ABTS assay

The antioxidant activity of extracts against ABTS radicals was evaluated by the method of Rivero-Pérez et al [25].

3.10. Statistical Analysis

Experimental values are given as means ± standard error (SEM) of three replicates. Statistical were determined out by Origin Pro Version 8.0 software (Origin Lab Corporation). Values of $p < 0.05$ were considered significant.

4. CONCLUSION

The results of this study demonstrate the best extraction technique for the polyphenols and natural compounds. Phenolic content and free radicals scavenging activity of *Artemisia herba-alba* by different extraction techniques (CE, SE, UE and ME) were evaluated. We found that *Artemisia herba-alba* extract obtained by classical technique exhibited strongest antioxidant activity, after respectively Soxhlet, microwave and ultrasonic assisted technique. The extracts obtained by classical technique presented high amount of contents of phenolic, flavanoïds and condensed tannins against the other extraction technique. The relation between the percentage of free radicals scavenging and the concentration of extracts is shown. This result can be developed to use this plant as source of natural antioxidants and background for the new drugs.

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