Phytochemical Analysis and Antioxidant Activity of Wormwood (*Artemisia absinthium* L.) as a Comparative Study Between *in vitro* and *in vivo* Plants

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Abstract

Artemisia absinthium is a wild edible species presence in Iraqi Kurdistan region the local people has used the plants as a part of their traditional medicine and their daily life. Nowadays, these plants become more attractive and interested due to their phytochemicals and antioxidant activities. Therefore, This study sought to evaluate various phytochemicals of this plant's aerial section generated by micropropagation *in-vitro* at tissue culture Laboratory of College of Agricultural Engineering science/University of Duhok, and with identical plants harvested from the field from Gali Ali Bak (*invivo*), including the difference in total phenolic compound and flavonoid content and antioxidant activity using DPPH test. The results showed that *A. absinthium* plants were rich with the high concentration of phenolic components in the Invivo plant extract was high (1.65 mg gallic acid equivalents/g of dry extract, eq.$100g^{-1}$), while it was lowest in the *in-vitro* plant extract (0.56 mg gallic acid). and (5.2 and 2.9 g of quercetin equivalents/g of dry extract, eq.$100g^{-1}$) for the *Invivo* and *in-vitro* plants, respectively, were the determined amounts of flavonoids extracted in the *Invivo* plant extract compared to the *in-vitro* plant extract. The anti-oxidative activity was tested by measuring their ability to scavenge stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical, the results displayed that the different quantities of plant extract of *Invivo* and *in-vitro* ranged between (150-300 g/ml) concentrations had radical-scavenging activity as comparative with the positive control (ascorbic acid),however the best value was at 200 g/ml for *Invivo*(118) as compared with positive control (ascorbic acid) (80.8). Thus, the evidence of this study shown that Artemisia can enhance natural medicines for human being.

Keywords*: Artemisia absinthium;* medicinal plants, flavonoid, Phenol, DPPH Radical Scavenging.

Introduction

Scientists are interested in investigating medicinal plants which are commonly used by public and derived from folklore or anecdotal information (16). Some famous selected examples used to represent the importance of those plants based on human observation, trial and error, religious advices and from various generations' accumulated experiences, which should never neglected or classified as unscientifically based treatment (3). Herbal or 'botanical' medicines, recorded in developing countries with ancient civilizations, such as Egypt and China, provide an abundant Pharmacopoeia of products that have been prescribed for many diseases over many centuries. The natural products underlying traditional medicines have received increased scientific attention lately (15). Traditional healers employed the plant, which later served as a model for scientific investigations into the development of novel and beneficial medicines for both humans and animals. (20 and 11). *Artemisia absinthium*, also known as Sweet Annie, Sweet Sage belongs to the family Asteraceae, is a common type of wormwood that grows throughout the world. It is a plant for the production of antimalarial and possibly antibacterial agents and natural pesticides. *Artemisia absinthium* is native to China and a widely naturalized and cultivated medicinal plant (12). The plant is a source of Artemisinin, a sesquiterpene lactone compound that is produced in the glandular trichomes of leaves and floral parts (13). Artemisinin is a vital antimalarial medicine effective against drug resistant Plasmodium falciparum. Artemisinin combination therapies (ACTs) are recommended as a first-line treatment for drug-resistant malaria that no longer responds to quinine-derived drugs such as chloroquine or mefloquine. Globally, the World Health Organization (30) attributed an estimated 212 million new cases and 429,000 deaths to malaria in 2015. At the start of 2016, nearly half of the world's population was at risk of malaria. An important additional feature is that *A. annua* compounds also exhibit anti-inflammatory, antibacterial, antitumor, antiviral, and anthelmintic activities (8). *Artemisia absinthium* (Asteraceae, formerly Compositae), also known as wormwood, is an annual herb native of Asia. This plant has been used for many centuries in traditional Chinese medicine for the treatment of fever and malaria. 300 million illnesses and at least one million deaths are caused by malaria in a year.

Softwood cuttings are typically used to grow *Artemisia absinthium*, however this traditional form of propagation has a number of drawbacks, including poor roots, lost time, and costly expenses. This significant ornamental and medicinal plant was propagated toward mass production using the plant tissue culture technique because of its well-known benefits, such as improving root formation and producing healthy and disease-free plant material in a very short period of time throughout the year with cost-effective protocols. In order to produce a large number of plants, it has already been demonstrated that certain Artemisia species may micropropagate and organogenetically reproduce. (5). An efficient in vitro method for multiple bud induction and regeneration has been developed in *Artemisia absinthium*, using leaf, stem, shoot tip, explants or by using young inflorescence segments (19). Tissue culture uses standard protocols with shoot tips of mature field grown plants (14). Shoot-tips and lateral buds of *A. absinthian* produced numerous shoots on MS medium and formed 100% roots on half strength Murashige and Skoog minimal organic medium. The medicinal use of *A. absinthium*

in the tropics should be emphasized. There is therefore an urgent need for the conservation and rapid propagation of the seedlings using tissue culture techniques.

Antioxidants are a group of substances that are useful for fighting cancer and other processes that potentially lead to diseases such as atherosclerosis, Alzheimer's, Parkinson's, diabetes and heart disease (29). To combat these radicals, living organisms produce enzymes (for example, catalase, superoxide dismutase and peroxidase) or rely on nonenzymatic molecules, such as glutathione, cysteine, ascorbic acid, flavonoids and vitamin K for protection (27).

Harvesting wild edible plants has a close connection to the rural and native people in the Kurdistan Region of Iraq. Nonetheless, *Artemisia absinthium* is well recognized for its unrecorded or unstudied phytochemical capabilities. The purpose of this study is to examine various phytochemicals in the plant's aerial component, including reducing sugar, carbohydrates, flavonoids, saponin, glycosides, steroids, and phenolic compounds, as well as the impact of altitude on their overall quantity and antioxidant capacity.

Materials and Methods

For this experiment, *Artemisia absinthium* L. the plantlets that had been taken out of the controlled environment would be exposed to the outside environment that produced from micropropagation the shoot tips and nodal segments were taken as explants. At the laboratory the cultures were maintained at 25 ± 2Cº and 60 ± 5% relative humidity in a room with a 16h light and 8h dark photoperiod furnished by white fluorescent lamps, the illumination is 1000 lux. An automated timers was set maintained the photoperiod. On the other hand the aerial parts were collected from the field Gali Ali Bak aria for (*in vivo*) experiment.

Total phenolics and Flavonoids content analysis

Plant aerial parts preparation and extraction

the dry powder with 500 ml of 96% ethanol in a conical flask to create the employed. This extraction took roughly twenty-four hours. Subsequently, using a Rotary Vacuum Evaporator, ethanol extracts were concentrated at a temperature of 40 °C after being filtered through layers of folded filter paper. A rotary evaporator was used to eliminate the solvent. Vacuum-assisted solvent removal under close observation was necessary for this technique. The water bath was heated to 40 degrees Celsius while the distillation flask was spun between 150 and 200 revolutions per minute while containing 50% ethanol plant extracts. Following the evaporation of ethanol, the condensed crude plant material was collected and scraped with a spatula off the wall of the distillation flask. Using a tiny quantity of ethanol solvent, the remaining plant stock was scraped off the flask's wall and added to the mixture before being left to evaporate overnight at room temperature. The weight of the resulting crude plant material was then recorded and kept in a freezer at -5 °C for later examination.

Total phenol content (TPC)

According to Folin-technique, Ciocalteu's the total phenolic content of the ethanolic extract of *Artemisia absinthium* was determined (17; and 2). In a test tube containing 1 mL of each, Folin-phenol Ciocalteu's reagent and concentrated extract material solution were combined. The mixture was then kept in the dark for approximately 5 minutes before 10 ml of a 7% sodium carbonate (Na2CO3) solution and 13 ml of deionized distilled

water were added. The mixture was then gently agitated until well combined. The mixture was kept in the dark for 30 minutes at room temperature (20–23 °C) to allow the reaction to finish, after which the absorbance of its blue hue was measured at 760 nm using an ultraviolet spectrophotometer. The total phenol content was determined using a gallic acid solution standard curve, and the findings were expressed in milligrams of gallic acid equivalents per 100 grams of extracted dry sample (mg GAE/100 g). The estimation of total phenolic compounds was done in triplicate.

Total flavonoid content (TFC) determination

The total flavonoid content was calculated using the technique outlined by (Saeed *et al*., 2012). Consequently, a half milliliter of each extract (0.1 g/ml) in methanol was combined with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of purified water. The mixture remained at room temperature for thirty minutes. A UV/visible spectrophotometer was utilized to measure the absorbance of the reaction mixture at 415 nm. Each sample was evaluated twice. Following the same procedure for all standard quercetin solutions in methanol (12.5–100 g/ml), a standard curve was generated. The results were expressed as milligrams of quercetin equivalent per gram of extract (mg QE/g).

DPPH Radical Scavenging Activity for *Artemisia absinthium* Plant Extract.

The donating ability of the obtained *Artemisia absinthium* plant extracts was evaluated by bleaching the purple solution of 1, 1-diphenyl-2 picrylhydrazyl radical (DPPH) using the technique of (26). In the dark, one milliliter of plant extract at various concentrations (0, 50, 100, 150,200,250, and 300 g/ml) was added to one milliliter of newly made 0.2 mmol/l DPPH ethanolic solution. The mixture was then violently mixed before being kept at room temperature for 30 minutes. At 517 nm, the absorbance of the samples was measured. The capacity to scavenge DPPH radicals was represented as IC50 ($mg.ml^{-1}$), the quantity necessary to inhibit DPPH by 50%. (28). Completely Randomized Design (CRD) used for each experiment, the tissue culture experiments were developed, the comparison between means was carried out according to Duncan's multiple range tests (DMRT) at the ($p < 0.05$). The data were evaluated and the means compared. SAS, a computer program, was used for all statistical analyses (SAS, 2001).

Results and Discussion

The amount of phenolic compounds was greatly impacted by the plant resource employed, according to the study's findings, which are depicted in Fig. 1. The in-vitro plant model exhibited the lowest quantity of phenolic compounds (0.56 mg gallic acid equivalents/g of dry extract, eq.100g-1), which is interesting because Invivoshowed a high level of phenolic compounds (1.65 mg gallic acid equivalents/g of dry extract, eq.100g-1). These findings strongly suggested that the resources used had an impact on the quantity of phenolic compounds overall. These results are consistent with the study of Asma'u Mahe *et al*, (6) showed the presence of phenol compound and was positive in *Artemisia absinthium* plant. Furthermore, (11) the highest level of phenol compound was achieved (0.60g gallic acid eq.100g-1), while the lowest value was (0.39mg gallic acid eq.100g-1) from watercress plant. Moreover, (10) observed for all tested cultivated garlic *Allium sativum* L extracts. Various levels of

phenolics (0.05–0.98 mg gallic acid equivalents/g of dry extract). Also, agreement with Beato *et al.*(7) when they found that the total phenolic content in cultivated garlic *Allium sativum* L. varied from 3.4 mg gallic acid equivalents (GAE)/g of dry matter (dm) to 10.8 mg GAE/g of dm with a mean value of 6.5 mg GAE/g of dm in the bulbous parts. The metabolic products are known as polyphones and phenolic compounds are found in plant-based meals. These compounds had numerous biological and pharmacological properties that could offer protection from chronic illness (18). They are more active antioxidants than vitamins. They have the capacity to neutralize oxidative free radicals (22).

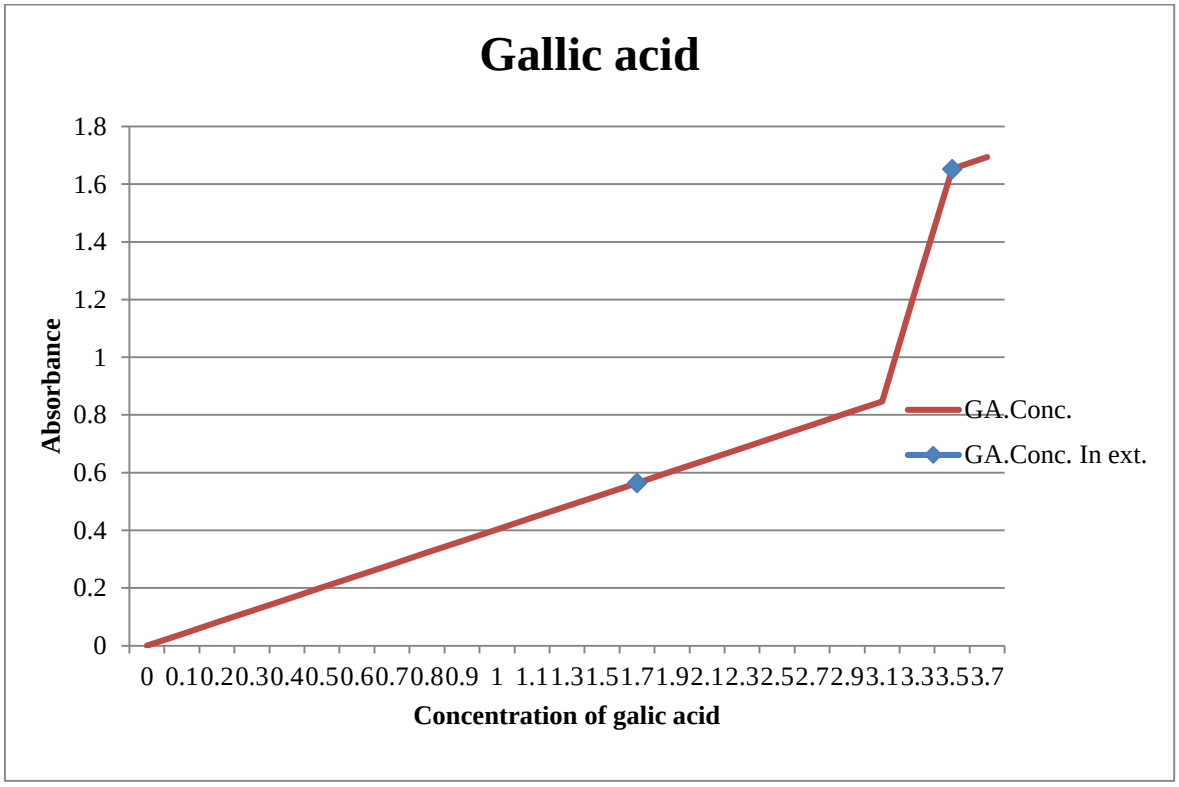

Figure 1. Comparison of phenolic compound found in the *in-vitro* and *Invivo* extracts of *Artemisia absinthium* plant.

(Fig. 2) showed the *Artemisia absinthium* the quantity of flavonoids extracts were calculated the in the extract of the *Invivo* plant was more than that of the extract of the in *vitro*; the results were (5.2 and 2.9 g quercetin equivalents/g of dry extract, eq.100g-1) for the Invivoand in vitro plants, respectively. The result was in agreement with the result of Asmau *et al*. (6) whom recorded the elimination of all phytochemical compound including Flavonoids were 81.84% and 70.59% both of control and plant extract respectively. Flavonoids are reported to be anti-activities of parasite against various strains of malaria parasite (2). The bioactive exract compounds which were found in the plants extract might be acting synergy or singly with one to another from

the *Artemisia absinthium* leaves (6). According to the research by (Asma'u Mahe *et al.*, 2019), there is evidence that the *Artemisia absinthium* leaf extract has antiplasmodial properties. Flavonoids, which are regarded as a subclass of plant metabolites known as vitamins, are the source of the easily digestible yellow pigment as well as other plant pigments (11).These results are comparable with those revealed by (11) recorded the presence of flavonoids compounds in watercress valued by (5.39mg qurcen eq.100g-1).

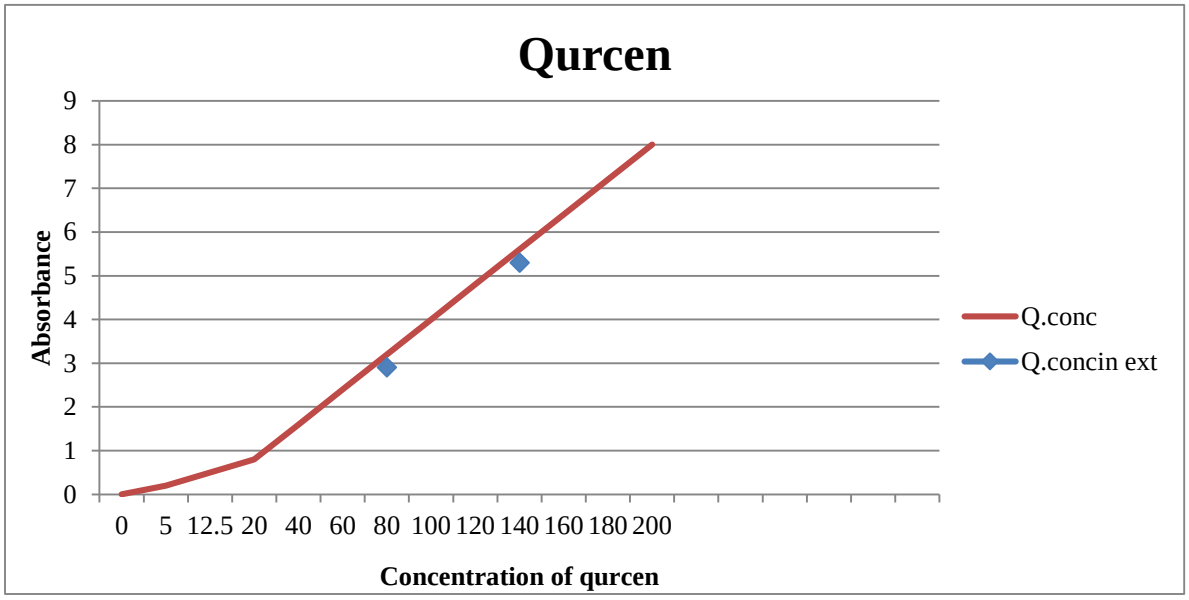

Figure 2. A comparison of the flavonoids compounds found in the *in-vitro* and *Invivo*extracts of *Artemisia absinthium* plant.

The outcomes of the DPPH test using different quantities of *Artemisia absinthium* plant extract and a positive control (ascorbic acid) are displayed in Fig. 3. For in-vitro plants, the extract at a concentration of 50 200 g.ml^{-1} showed the lowest antioxidant activity, whereas the extract at a concentration of 200 g.ml^{-1} demonstrated the same radical-scavenging activity for *Invivo* plants. The IC50 values for extracts 150, 200, 250 and 300 g/mL of *Artemisia absinthium* ranged from (85.50 to 118.02), which was highest than Ascorbic acid. However, *Artemisia absinthium* plant extract exhibited extraordinary scavenging properties, where a lower IC50 value suggests a greater antioxidant activity. In addition, the radical scavenging activity of plant extracts is lower than that of the control at 50 g/mL (64.9 and 73.39) and 100 g/mL (72.62 and 79.64) respectively for both *in-vitro* and *Invivo* plant extract compared to Ascorbic acid (80.08). The antioxidant capacity of the plant extract was tested, and it was discovered that a considerable number of phytochemicals with the ability to share a hydrogen atom or an electron exert an antioxidant effect. The antioxidant activity of garlic was closely associated with its phenol concentration,

which also possessed antioxidant capabilities in conjunction with its flavonoid level.

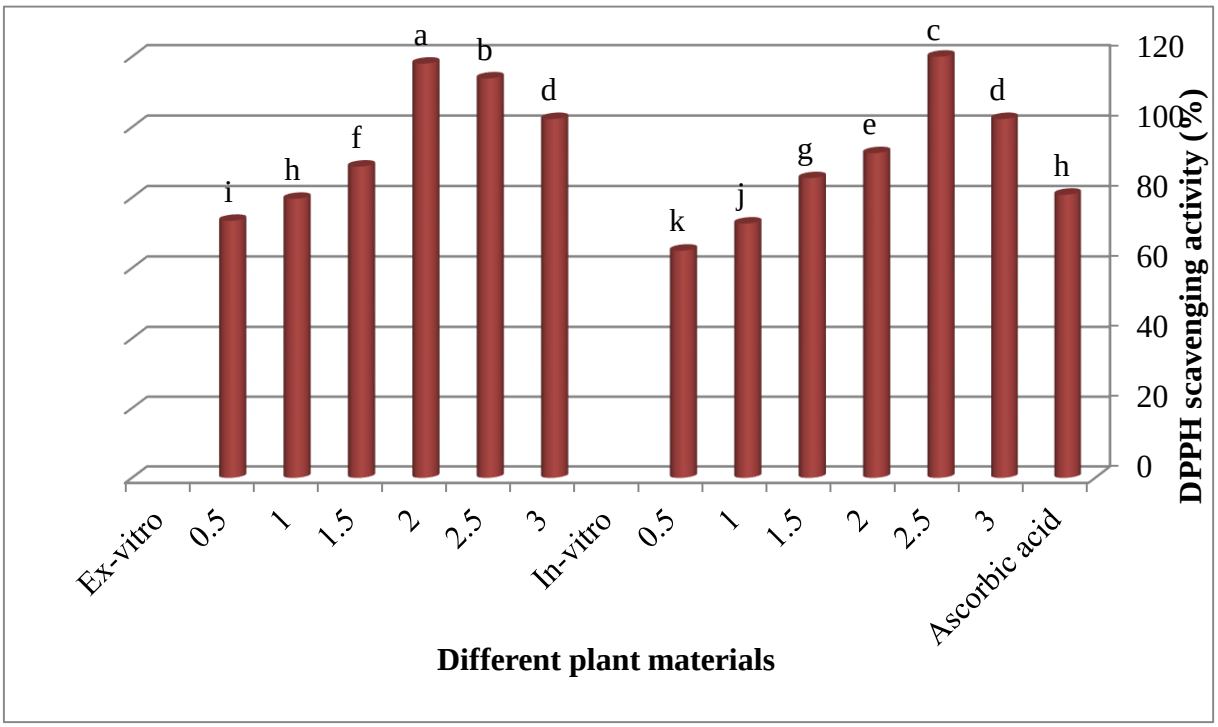

Figure 3. Free radical scavenging activity by 2,2 -diphenyl-1-picrylhydrazyl(DPPH) assay of *Artemisia absinthium* extracts as comparative study between *in-vitro* plantlet and *Invivo* plants compared to the positive control(Ascorbic acid).

These results are in agreement with those of (11), who determined the total phenols and total flavonoids content and antioxidant activity of *Nasturtium officinale* plant from different location the best values were obtained from kanimase village when compared with plants produced from tissue culture technique, (21) who determined the phytochemical compounds of aerial parts of watercress *Nasturtium officinale*, the results showed that the total phenols content and total flavonoids content were obtained.

Environmental influences have a significant impact on plant development, yield, and chemical component concentrations. The amount of essential oils in plants was influenced by day length and light. In other studies, the effects of drought, light intensity, altitude, and mineral nutrients on plant growth and essential oil content were also discussed (4). The changes in chemical composition are influenced by different altitudes due to environmental factors including height, according to (9).The researchers came to the conclusion that ecological environment parameters like height and the physiochemical characteristics of soil (20). Additionally to having an impact on plant vegetative growth, essential oils and chemical compounds in aromatic and medicinal plants can also change in terms of quality and quantity. Environmental circumstances are affected by changes in

latitude and altitude; as is well known, plants that grow in uplands are more exposed to harsh environmental conditions than those that do so in lowlands, which have an impact on their growth and development. Low temperatures, low humidity, and higher sun exposure, particularly UV radiation, are typical characteristics of the development phase. In order to resist environmental stress, greater morphological, anatomical, and physiological changes will take place, such as in the vegetative and root systems (23). Low temperatures on high terrain have an impact on plant biological processes like respiration and photosynthesis.

Conclusion

Researchers are interested in investigating medicinal plants which are commonly used by public and derived from folklore or anecdotal information. Some famous selected examples used to represent the importance of those plants based on human observation, trial and error, religious advices and from various generations' accumulated experiences. However, *Artemisia absinthium*is well known from its phytochemical properties that has not been documented or analyzed . The findings recorded that *A. absinthium*is L .was enriched with the high content of phenolic compound (1.65 mg gallic acid equivalents/g of dry extract, eq.100g-1) and flavonoids (5.2 g of quercetin equivalents/g of dry extract, eq.100g-1) were achieved from the *Invivo*extract compared to the *in-vitro* phenolic compound ((0.56 mg gallic acid equivalents/g of dry extract, eq.100g-1) and flavonoids compound (2.9 of quercetin equivalents/g of dry extract, eq.100g-1). The results of the scavenge stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical, displayed that the different quantities of plant extract of *Invivo* and *in-vitro* ranged between 150-300 g/mL concentrations had radical-scavenging activity as comparative with the positive control (ascorbic acid),however the best value was at 200 g/mL for *Invivo*(118) as compared with positive control (ascorbic acid) (80.8). Thus, the evidence of this study shown that Artemisia can enhance natural medicines for human being. Nevertheless, further research are necessary to investigate the *in vivo* and *ex vivo* toxicity of the plant.

Conflict of interest

The authors have no conflict of interest.

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