Antioxidant Assay (MTT) of Watercress (*Nasturtium officinale*) Bioactive Compounds in Duhok / Kurdistan Region of Iraq

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***Abstract*—**Watercress has been used as a home remedy by different cultures as medicinal plant. The aim is to investigate its antioxidant activity. Hexane and methanol extracts of dried plant materials were collected from three different villages in Duhok/ Iraq. The relationship between secondary metabolites contents and antioxidants were tested by MTT assay. The highest antioxidant activity was observed in aerial parts at lowest altitudes at 459 m Zawa village. High amount of steroids compounds of methanol extract accompanied the least antioxidant activity in the areal parts that collected from high altitude 1340 m (Kanimasea village). The isolation and purification were done for the hexane and methanol aerial parts extracts resulting in obtained white crystalline powder which was conformed to physical, chemical and spectral identification by 1H-NMR as ß-sitosterol. Due to antioxidant potential watercreass extract, it might find application in the prevention of free radical related diseases.

***Keywords*—** Antioxidant, Functional food, Steroid compounds, Watercress.

I. Introduction

THIS *Nasturtium officinale* belong to the mustard family (Brassicaseae) is an emergent perennial herb native to Europe and has been introduced to the south of Eastern world. Normally, it is consumed as raw vegetable in salads, cooked as soup in European and USA and in other recipes is used for its spicy and peppery taste. The areal parts of watercress has historically been widely used as a home remedy for medicinal purposes which was dating back to Roman [62]as a depurative, expectorant, diuretic, odontalgic, stimulant and stomachic. It was also used to cure abdominal pain in traditional medicine [57] as well as its use for treating cardiovascular and hypertension diseases [18], [6]

Watercress; locally named (Peaz) which is considered as one of the most important herbaceous mountainous medicinal plant grown in gently flowing streams, in a moistly and around running water habitat sand in several parts of world. It had been used by rural healers as antioxidant, nutritive and anti-inflammatory agent. Watercress is a rich source of minerals, essential vitamins and phytochemicals such as zeananthin, lutein and steroids [65]. It is considered an excellent functional food for prevention of cancer related diseases which was reported to limit breast cancer risk, treat lung cancer [45] [23]. Also, it is stimulating the immune system; in addition to other health benefits [45].

Redox reactions are crucial for the success of many biological processes, but it can also damage the human body by oxidative and damaged cells which could be the factors that have important roles in the pathogeneses of several clinical disorders and the normal process of ageing[9] [43]. Low levels of antioxidant can result in high levels of reactive oxygen species, which cause oxidative stress and then cell death and ultimately causes many diseases such as heart diseases, cancer, cataracts and congestive disorders [63].

Potently reactive of oxygen, known as reactive oxygen species (ROS) including superoxide, hydrogen peroxide (H_2O_2) and hydroxyl radical (•OH) are continuously generated by regular metabolic process or from exogenous chemicals in the environment. ROS are normally neutralized by powerful antioxidant of the cells without any wayward effect. However, restrain the balance between ROS generation and antioxidant defense lead to the oxidative stress followed by a series of events including cellular disturbance. Physiological disorders might lead to several chronic ailments such as sclerosis, cancer, diabetes, arthritis, etc. Antioxidants appear their effect by direct reacting with ROS, quenching then their effect (the catalytic metal ion) [32].

Antioxidants compounds play an important role in preventing or delaying the beginning of degenerative diseases. The physiological role of antioxidants is to determine the oxidation chain reaction by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves. Synthetic antioxidant including (BHA), butylated hydroxyl anisol (BHT), butylated hydroxytoluene, tert-butyl hydroquinone (TBHQ), vitamin E (α tocopherol) and vitamin C are used as an excellent examples for this purpose, but they show toxic properties for human health in food industries, so scientists were tried to find new substances serving the same purpose from various sources such as medicinal plants [25] [12] [10].

Herbs have been used for medical treatments since ancient times [51]. Recently, the studies have shown the positive effective of bioactive components including flavonoids,

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bioflavonoids, alkaloids, steroids, bitters, saponins, glycosides, mucilage, phenols, phenolic acid and essential oils [58]. Phytosterols, subgroup of steroids are considered as an important class of bioorganic molecules; ß sitosterol is one of phytosterols that are common in plants and animals as well as fungi, and have structural similarity to cholesterol. Phytosterlos play essential role in physiology of eukaryotic organisms. European Food Safety Authority (EFSA) recommended to consume about (1.5-2.4 g/day) of phytosterol and/or stanols which were proved to decrease blood cholesterol level [30]

Moreover, EFSA confirmed the role of food containing phytosterol esters inside a low saturated fat and cholesterol diet in diminution the risk of heart disease, especially consuming of at least (1.3 g/day) sterols twice a day [19]. The natural foods with high phytosterols containing dietary have been constantly marketed for decades in different countries [53]. The antibacterial activities of ß sitosterol have been reported in many reports [56] [53]. Research had mentioned that ß sitosterol has significant role in prevention of certain cancers including prostatic, colon, breast, ovarian and leukemia. It is also possesses potent antioxidant activity, hypoglycemic and anti-inflammatory properties. ß sitosterol decreases the level of blood glucose as anti-diabetic agent.

The main purpose of this investigation is to extract, isolate, characterize by spectroscopic and evaluate the antioxidants activity of the areal parts of watercress, which had been collected from three areas in Kurdistan Region of Iraq that had been used as traditional remedy to revaluing nutritive treatment and functional food of the watercress plant.

II. MATERIALS AND METHODS

A. Collection of Plant Materials

Aerial portions of watercress plants *Nasturtium officinalis* in the vegetative stage were collected from March 1st - June 30th, 2014 from three different altitudes; Zawa as lowest altitude 459 m, Kanimasea as high altitude 1340 m villages, and (Sumeail) as a third location with low altitude 569 m, Duhok Governorate/ Kurdistan Region of Iraq. Watercress plants were collected from field in both villages were transferred to the greenhouse in the College of Agriculture, University of Duhok where they were grown at media containers, containing sandy loam soil. Shoot apex and single nodes from greenhouse watercress grown plants were used as explant's source for tissue culture propagation in the laboratory of Horticulture Department/ College of Agriculture and Forestry / University of Duhok.

The collected aerial parts (leaves and stems) from the three locations were shade dried in medicinal plants laboratory / Horticulture Department - College of Agriculture and Forestry / Duhok University. The plant materials were protected from direct sunlight and ground to a powder by mill and kept as a powder in dark glass containers for preparing them to hexane and methanol extraction as well as performing the phytochemical tests and establish the following experiments.

B. Extracts Preparation

The powder of dried plant materials were collected from the three locations; Kanimasea, Zawa and Sumeail were weighted (125 g), (10 g) and (10 g), respectively and transferred to Bioactive Natural Products and Phytochemical Laboratory, Department of Horticulture, Michigan State University, East Lansing, USA for extraction and antioxidant assay. All used solvents were of American Chemical Society reagent grade (Sigma–Aldrich Chemical Company, St. Louis, MO, USA). The plant materials extracted three times with hexane (1000 mL, 150 mL and 150 mL), respectively at room temperature overnight on automatic shaker. The extracts were concentrated under reduced pressure in a rotary evaporator to yield hexane extract (2.47 g, 2.02 g and 4.11 g), receptively. The residue was extracted three times with methanol (1000 mL, 150 mL, and 150 mL), respectively at room temperature overnight on automatic shaker, centrifuged and the supernatants concentrated under reduced pressure in a rotary evaporator. Lyophilizing was then preformed for 48 h to yield crude methanol extract (21.47g, 2.00 g, 2.6 g), respectively.

C. Thin-Layer Chromatography Analysis

To compare among the three samples of hexane crude and three sample of methanol crude, thin-layer chromatography (TLC) was done; 250 µm silica gel plates (Analtech, Inc., Newark, DE, USA) were used for thin-layer chromatography (TLC). TLC plates were viewed under UV light at 254 and 366 nm in Spectroline CX-20 ultraviolet fluorescence analysis cabinet (Spectroline Corporation, Westbury, NY, USA), and sprayed with 10% sulfuric acid solution.

D. MTT Antioxidant Assay

MTT [3-(4, 5-dimethylthiazole-2-yl)-2, 5-diphenyltetrazolium bromide], tert-butylhydroquinone (TBHQ) and (vitamin C) were purchased from Sigma–Aldrich Chemical Company (St. Louis, MO, USA). MTT assay was evaluated for the six crud extracts according to previous report [63]. The stock solution of test extracts (10 mg/mL) and positive controls (vitamin C and TBHQ at 1 mg/mL) were prepared in DMSO. An aliquot of 10 µ L of test samples and 190 µL of MTT water solution (1 mg/mL) were vortexed in a capped glass vial (2 mL) for 1 min, which was then incubated at 37° C for 24 h. To this, 200 µL of DMSO was added and vortexed again for 1 min. An aliquot (200 µL) of the reaction mixture was pipetted to 18-well cell culture plates and the absorbance was read at 570 nm on a Bio-Tek Elx800 universal micro plate reader. Each sample was tested in duplicate.

The [3-(4,5-dimethylthiazole-2-yl)-2,5diphenyltetrazolium bromide] (MTT) assay is an established colorimetric assay for measuring the activity of mitochondrial enzymes present in healthy cells by monitoring the absorbance of purple formazan (570 nm) formed as the enzymatic reduction product of MTT (410 nm). This method is used extensively to determine the cellular toxicity of natural and synthetic compounds. In

addition, the qualitative use of the MTT method has been reported in the evaluation of the antioxidant potential of other plant extracts, [63] [66]

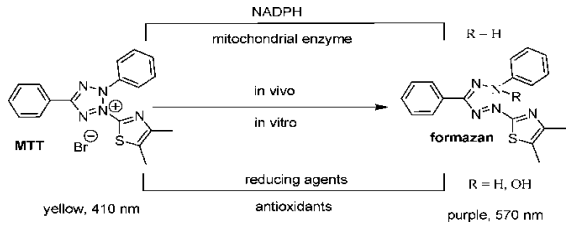


Figure (1):The [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) assay is an established colorimetric assay for measurement the activity of mitochondrial enzymes existent in healthy cells by observation the absorbance of purple formazan (570 nm) formed as the enzymatic reduction product of MTT (410 nm).

E. C-Fractionation Isolation and Identification Procedures

Based on the TLC profile and MTT assay that showed there were no differences among the three locations except in the quantity of secondary metabolites compounds, all the work was devoted to Zawa extract

F. Hexane Extract

An aliquot of the hexane extract sample 1 (1.993 g) were dissolved at (10 mL) of hexane, centrifuged three times and then get (1.685 g). An aliquot of this combined extract (1.685 g) was fractionated by (silica medium column) using hexane/ethyl acetate (8:1,4:1,2:1 and 1:1 v/v) and eluted by acetone (100 %) to yield fractions A1 189 mg , B1 249 mg, C1 236 mg, D 706 mg and E 233 mg. Fractions D590 mg and E 461 mg that collected from pervious column were fractionated again by MPLC (silica small column), repeated three times using chloroform: methanol (25:1, 15:1 and 5:1 v/v) to yield fractions F 229 mg, G 326 mg. The fraction (195 mg) that was collected from previous column fractionated again by MPLC (silica small column) using hexane: ethyl acetate (4:1 v/v, 2:1v/v, 1:1v/v ethyl acetate: hexane 2:1v/v) and eluted by ethyl acetate (100 %) to yield fractions a (16 mg), b (25 mg), c (12 mg), d (21 mg), e (13 mg), f (19 mg), g (36 mg), h (21 mg) and I (25 mg). An aliquot of fraction h (36 mg) was purified by preparative TLC (hexane/isopropyl alcohol, 10:1, v/v) to yield compound (18 mg) of white crystalline powder.

G.Methanol Extract

Twenty g of methanol extract was partitioned into four fractions (methanol, chloroform, ethyl acetate and equates) by adding (100 mL) of each solvent, stirred overnight, centrifuged and filtered continuously to yield chloroform soluble fraction (4.20 g), methanol soluble fraction (9.56 g), ethyl acetate soluble fraction (7.07 g) and equates (3.43 g) [11]. A small portion of chloroform fraction was dissolved in chloroform and the solution was spotted on TLC plates, then the TLC plates were run by specific solvent system and were viewed individually under UV light and also sprayed with sulfuric acid reagent [31]. Through several guide experiments, it was found that the compounds of chloroform fractions were separated by the solvent system ethyl acetate: methanol. An aliquot fraction weighted (2.18 g) was fractionated by media silica gel column using solvent system (20:1, 10:1, 4:1,1:1 v/v and eluted methanol) to yield five fractions. The third fraction weighted 386 mg into proportion of (4:1 v/v) was subjected to column chromatography on small silica gel column using solvent system hexane: acetone (2:1 and 1:3 v/v) to get five fractions. The second fraction that yielded from previous column weighted (38 mg). Preparative TLC using hexane: acetone solvent system (4:1v/v) was done to isolate pure compound (23.2 mg) white crystalline powder.

H.Oil Rat Evaluation

The essential oil was distillated from the plants by (Steam-distillation method) using Soxhlet - extraction method according to [7].

I. Steroid Test

Thin-layer Chromatography (TLC) was done to compare among the isolated compound and ß-sitosterol standards (ß-sitosterol) by dissolving a few crystals powder in chloroform and spotted on TLC silica gel plates (Analtech, Inc., Newark, DE, USA) using solvent system chloroform : methanol (30:1 v/v). TLC plates which were viewed under UV (ultraviolet) light at 254 and 366 nm in spectroline CX-20 ultraviolet fluorescence analysis cabinet (Spectroline Corporation, Westbury, NY, USA) and sprayed with 10% sulfuric acid solution.

J. Spectroscopic Characterization

1H-NMR spectroscopic method was used to elucidate the structure of isolated compound. 1H-NMR spectra recorded at 31.4 MHZ using CDCl3 as solvent with Totramethylsilan (TMS) as an internal standard, β-sitosterol (2): white crystal. 1HNMR (400 MHz,CDCl3) δ: 5.34 (1H, d, J 5.2 Hz, H6), 3.51 (1H, m, H3), 1.00, 0.67 (3H, s, H19 and H18), 0.92 (3H, d, J 6.0 Hz, H21), 0.85 (3H, d, J 8.0 Hz, H29), 0.83 (3H, d, J 7.2 Hz, H26) and 0.79 (3H, d, J 7.2 Hz, H27).

III. RESULTS AND DISCUSSION

Based on TLC profile (Figure 2) that detected, the crud extract of Kanimasea plants contained high levels of secondary metabolites compared with the plants that collected from Zawa and Sumeail. The different extracts were showed no clarified variation among the plant materials that taken from Zawh (459 m), Kanimasea (1340 m) villages and Sumeail (plants produced from tissue culture technique laboratory) (569 m) , especially in the quality of secondary metabolites (Figure 3). In order to detect the TLC profile, there was a highest quantity of secondary metabolites contents appeared in both hexane and methanol extracts of the dried plant materials that collected from Kanimasea village, where we are aware that that the largest amount was in the methanol extract. The hexane and methanol extracts from different areas (subject of study of the watercress plant) were evaluated for antioxidant activity using MTT assay [63] [16] [15].

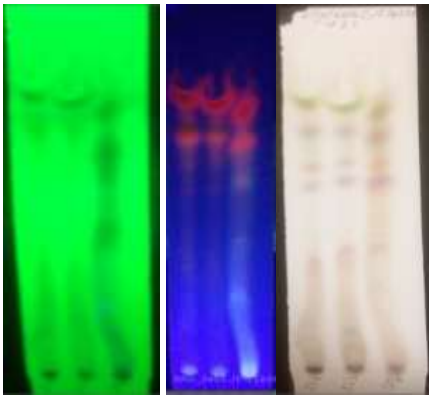


Fig 2: TLC profile to compare among three crud extracts showed that Kanimasea village extract contain high quantity of photochemical compounds more than

MTT assay detected the compounds that have a role in reducing or removing oxidative agent. In MTT assay, hexane extracts of aerial parts from Kanimasea showed strongest activity with absorbance value between (0.4 – 0.5) compared with Zawa that had the weakest antioxidant activity with the absorbance values between (0.1– 0.2) at 570 nm (Figure 2). On the other hand, the methanol extract showed the same behavior as hexane extract for the three areas. The methanol extract that yielded from Kanimasea showed the highest absorbance between (0.5 – 0.6) and higher antioxidant activity compared with the other two areas. Among these six extracts, methanol extract of Kanimasea showed highest antioxidant activity, compared to the other hexane and methanol extracts and also compared to the absorbance displayed by the positive controls.

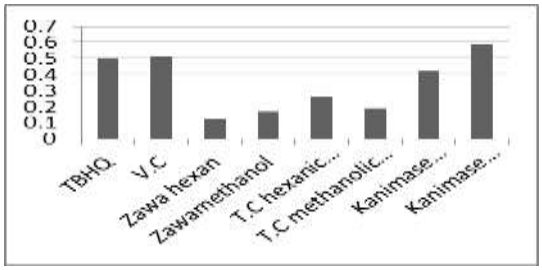


Fig (3): Absorbance values at 570 nm of Hexane and Methanol extracts of 6 samples of aerial parts of watercress plant at 250 µg/mL obtained after reaction with MTT at 37° C. Vitamin C and THHQ were used as positive control at 250 µg/mL. Vertical bars represent the standard deviation data point (*n*=2)

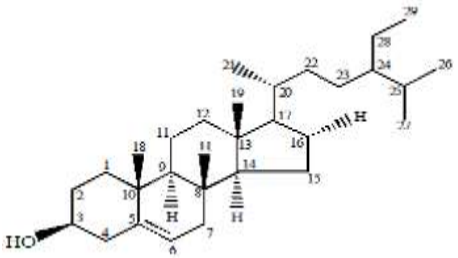
Fig (4): TLC profile showed the comparison among the isolated compound and ß-sitosterol standers, the solvent

The results mentioned in Table 1 showed that the aerial parts of *Nasturtium officinale* collected from high altitude produced higher oil yield than that collected from low altitudes (12.52 % , 8.91 % (V/W), respectively.

TABLE (1)
EFFECTS OF VARIOUS GEOGRAPHICAL LOCATIONS IN KURDISTAN REGION ON THE OIL RAT % (V/W).

Factors	Locations		
	Kanimasea	Sumeail	Zawa
Oil rat %	12.524 a	9.001 b	8.919b
Elevation	1340 m	569 m	459 m

*Means followed by the same letter for each factor do not differ significantly from each other's according to (T) Test LSD.



β-sitosterol

These results were matched with those of [36] when they approved that highest secondary metabolites contents such as total phenolic and flavonoids in the aerial parts of *Nasturtium officinale* were observed in plants materials that were collected from high altitude areas Touskacheshmesh (1400 m) compared with the lowest amounts of these compounds from the plant material that were collected from low altitude (200 m) Nosrat Abad. Also, these results acceptant with [21]when they concluded that the quality and quantity yield of thyme (*Origanum syriacum*) essential oils were increased with altitude and reached its maximum in Majdal Selem 650 m which gave the highest yield, then followed by Kfar Sier 400 m and then Houmine Al Tahta and Deir Kanoum Al Nahr 250 m and 200 m, respectively. These results also are similar to those reported by [40]to confirm the effects of different geographical and environmental regions, to compare the essential oils yield where the obtained quantity of essential oils from high altitude 920 m is greater (5.4%) than that obtained from Jeresh (low altitude 540 m) of *Thymus serpyllum*.

The hexane and methanol extracts from different areas (subject of study of the watercress plant) were evaluated for antioxidant activity using MTT assay [63] [15] [16]. MTT assay detected the compounds that have a role in reducing or removing oxidative agent. In MTT assay, hexane extracts of aerial parts from Kanimasea showed strongest activity with absorbance value between (0.4 – 0.5) compared with that of weakest antioxidant activity, Zawa with absorbance values between (0.1 – 0.2) at 570 nm (Figure 2). On the other hand, the methanol extract showed the same behavior as hexane extract for the three areas; the methanol extract of the plant materials that collected from Kanimasea showed the highest absorbance between (0.5 – 0.6) and the highest antioxidant

activity compared with the other two areas. Among these six extracts, methanol extract of Kanimasea showed highest antioxidant activity compared to the other hexane and methanol extracts and also compared to the absorbance displayed by the positive controls. These results agreed with [14] when they refereed that methanol extract of different varieties of date fruits Kkashram, Ruthana and Luban showed the highest antioxidant activity. Also, these results match with [36]when they approved that the highest antioxidant activity and radical scavenging effect were observed in the extract of material plant that collected from high altitude (1400 m) Touskacheshmes area of watercress compared with plant materials that were collected from low altitude (200 m) Nosrat Abad area.

Stigmasterol, β-sitosterol and camestanol are plant sterol or phytosterol, which are structurally similar to cholesterol and exist in several forms in plant [47].The quantitative analysis of the hexane extract revealed that the predominant compound in black truffle *Terfezia claveryi* is stigmasterol at the highest concentration of 31.10 % followed by β-sitosterol 15.73 % [52] [4],isolated white crystalline powder from petroleum ether extract of aerial parts of *Ageratum conyzoides*, which was subjected to physical, chemical and spectral identification by IR,13 C-NMR,1H-NMR and GC-MS, as stigmasterol and β-sitosterol.

Other researches [36]showed that the methanol extraction of dried root barks powder of *Calotropis gigantea* gave two pure compounds; their structures determined as stigmasterol and β-sitosterol by physical, chemical and spectral characteristics (D-NMR and mass spectrometry). Also, β-sitosterol was isolated from ethyl acetate extract of root bark *Terminalia glucescens* where it's colorless powder compound had been isolated, which was further characterized by UV, IR, 1HNMR and 13 C-NMR [33],[64],isolated two steroid compounds from hexane extract of *Saurauia roxburghii* plant leaves; and IR structures were defined them to be stigmasterol and β-sitosterol. Phytochemicals screening of the *Odontonema strictum* plant leaves extract indicated the presence of steroids where the isolation and purification refers to white crystalline powder which was later determined physically, chemically and spectral identification by1HNMR, 2D-NMR, IR and 13 C-NMR as a mixture of stigmsterol and β-sitosterol [35].

Fruits and vegetables contain different antioxidant substances, whose activities have been well proved recently. The existence of phenolic compounds, phenolic acids, flavonoids, carotenoids and phytosterols including steroids participate to the beneficial effects of these foods[20] [3] [13]. The results of multiple studies indicated that *Nasturtium officinale* might be evaluable food industry. [2]indicated the antioxidant activity of crud extract, dichloromethane, ethyl acetate and butanolic fraction of *Nasturtium officinale*. The investigation was done using (1-1-diphenyle -2-picryl-hydrazyl and thiobarbitaric acid reaction). The antioxidant and radical scavenging activity of the crud extract and fractions were found in the following decreasing order: butanolic fraction > ethyl acetate fraction > dichloromethane fractio > crud extract. The principle of antioxidant activity is based on the availability of electrons to neutralize any free radical.

It has been noticed the antioxidant activities of *Nasturtium officinal* oils and methanol extracts by 2,2-diphenylhyrazyl(DPPH) assay radical scavenging activity (RSA) and β- carotene linoleate model system, that the leaves methanol extract afforded highest antioxidant activities which is close to synthetic antioxidant TBHQ [24]The accumulative results of study conducted by [54] clearly indicated that *N. officinalle* aqueous – ethanol extract had potent antioxidant properties through direct trooping of free radicals and also reducing power. [48]by DPPH assay concluded that therapeutic application of *N. officinalle* has been attributed to its antioxidant capacity which is mostly tested by means of cell-free assay 2,2-diphenylhyrazyl (DPPH) and ferric reducing antioxidant power (FRAP). On the other hand, [36]reported that the aerial parts of *N. officinalle* R.Br. in vegetative period contain the highest quantity of total phenolic compounds with antioxidant activity which could be valuable natural antioxidant source confirming traditional application of this plant by the natural healers as nutritive in inflammation problems. [57] reported that *N. officinalle* ethanol extract had antioxidant activity or reduction power more than aqueous extract especially in DPPH method.

Environmental conditions are important factors affecting plant growth, yield and chemicals compounds levels [42] as an instance, [44] reported that light and day lengths affected plants essential oils content. Other studies had also reported the influence of mineral nutrients, drought, light intensity and altitude on plants growth and essential oil contents [41] [37]. Also, (Boz et al., 2014) confirmed that the differences in the chemical composition are affected by various altitudes due to the environmental factors such as altitude and geographical position. The researchers concluded that the ecological factors of habitat such as altitude and physiochemical properties of soil [59] [46] ,not only can effect on plant vegetative growth, but also they can change the quality and quantity of essential oils and chemical compounds in aromatic and medicinal plants.

As it is known in upland, the humidity is less and more sunshine rays especially ultra-violate rays which is powerful to make dense the plant physiological fluids as essential chemical composition of *Phlomis cancellata* has followed this version. A number of researchers have reported that altitude increasing levels can decrease the amount of the chemical compounds of essence that they were [34] [22]. Environmental conditions are changes due to altitude and latitude; as known the plants in upland expose to hard environmental conditions more than the plants that grown in down land which affect its growth and development. Development period usually characterized by low temperature, less humidity and more sun shine rays, especially UV rays; so more morphological, anatomical and physiological changes will happened to resist the environmental stress; for instance, vegetative and root system

[39]In general, reducing the number of stomata cause a reduction in water loss resulting in a reducing chlorophyll synthesis that effect photosynthesis efficient and hence decrease the produced compounds that used for plant survival, growth and development. The latter authors mentioned that UV rays are powerful to make spurs and dwarf shoots. They added that less expose leaves area to the light led to less nutrients absorption that are necessary to plants photosynthesis, growth and development. Low temperatures in high land effect on biological processes in the plant such as photosynthesis and respiration led to decrease the primary metabolites that are essential for growth and development of plants but increase the secondary metabolites[67] .

Plant growth changes guided to plant chemicals contain changes such as more essential oils and total essential oil yield by increasing the oil glands in plant leaves [60] [46]. In high altitude, [46] plants produced high terpenes, phenolic compounds as a results of a lack carbon amounts needed for growth and increase the quantity of carbon allocated to resist unfavorable environmental conditions, leading to produce more essential oils and more metabolites compounds; for instance, antioxidant compounds and additionally produce more chemical compounds as essential oils. β-sitosterol is an important phytosterol found in plant food; it had been shown anti-proliferative effect on breast, colon and prostate cancer. Also, its effect on stomach cancer cells *in vitro* was well known [55], [33], mentioned that β-sitosterol also showed anti-inflammatory, anti-arthritic, antipyretic, anti-ulcer, insulin releasing and estrogenic effects and inhibition of spermatogenesis. β-sitosterol is mainly used for its cholesterol lowering property. According to [57],phytosterols including β-sitosterol are involved in various mechanisms of action as inhibiting cancer–cell apoptosis. [5], isolated β-sitosterol from *Hyprophila spinosa* chloroform extract which was proved to have anti-inflammatory and cholesterol lowering properties. Also, several studies had shown that the phytochemicals may have other health benefits including easing symptoms of being prostatic enlargement as well as reducing risk of cancer and finally prevention of oxidative damage through its antioxidant activity. [52],reported that the chemical analysis of *Terfezia claveryi* were indicated to have some phytosterols including β-sitosterol; the antioxidant and anticancer effect were emitted to phytosterols including β-sitosterol, therefore, this finding provide evidence of watercress health beneficial, owing its antioxidant activities and nutrients properties which may be used in nutraceutical or pharmaceutical industries.

IV. CONCLUSION

In conclusion, based on the *in vitro* antioxidant activities of six extracts of watercress aerial parts that showed it contained components that can play a role as a reducing agent and scavenging the free radical in cellular reactions *in vivo*. The results supported the health beneficial of watercress more above its nutritional value. Based on the TLC profile, there is no a clear difference among the plant materials that collected from Zawh, Kanimasea and Sumeail in the quality secondary metabolites, but the high quantity of secondary metabolites was found in plant materials collected from Kanimasea area. The MTT antioxidant assay considered as a rapid and inexpensive because it requires only one reagent and universal solvents including water and DMSO. Since the reaction time is short and the absorbance is read by using plate reader or by a UV-via spectrophotometer, antioxidant potential of several samples can be generated in a short period of time. The method is multilateral and overcomes the solubility issues related with extracts. Finally, this method could be developed as a high –throughput assay for evaluating the antioxidant potential of large volume of sample.

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