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R Saranya
PG & Research, Department of Biotechnology, Mohamed Sathak College of Arts and Science, Sholinganallur, Chennai, Tamil Nadu, India

M Syed Ali
PG & Research, Department of Biotechnology, Mohamed Sathak College of Arts and Science, Sholinganallur, Chennai, Tamil Nadu, India

V Anuradha
PG & Research, Department of Biochemistry, Mohamed Sathak College of Arts and Science, Sholinganallur, Chennai, Tamil Nadu, India

M Safia
Department of Forensic Biology, College of Forensic Science, Naif Arab University for Security Sciences, Riyadh, Kingdom of Saudi Arabia

Correspondence
M Syed Ali
PG & Research, Department of Biotechnology, Mohamed Sathak College of Arts and Science, Sholinganallur, Chennai, Tamil Nadu, India

Pharmacognosy and radical scavenging potential of different plant parts of *Anastatica hierochuntica*

R Saranya, M Syed Ali, V Anuradha and M Safia

Abstract
The present study was accomplished to explore the proximate analysis and mineral composition in stem, seed and leaves of *Anastatica hierochuntica* while quantification of secondary metabolites and free radical scavenging were assayed on various extracts of the Anastatica hierochuntica plant material. Proximate analysis exposed the presence of high protein (14.95%) and fat (8.24%) content in seeds, whereas high ash (17.32%) and fibre (13.5%) content in Stems, while Leaves was reported with highest carbohydrate (42.68%) and moisture (14.87%) contents. Results of elemental analysis by ICP shows that leaf has documented to be the highest source of Na(198 ppm), K(395ppm), Ca(346 ppm), Fe(8 ppm), Ba(1069 ppb), Co(25.11 ppb) and Mn(917.43 ppb)while seed for Mg(25 ppm), Ni (66.3 ppb)and Zn(602.2 ppb) whereas Cu(286.2 ppb) was recorded highest in stem. Gallic acid (GA) was taken as standard for phenol, tannin, non-tannins and Quercetin (Q) for flavonoid content. The total phenol was higher in aqueous extracts of leaf (32.25 GAE/g), while non-tannins in aqueous extract of seed (19.84 GAE/g) whereas tannins in aqueous extract of stem (17.36 GAE/g) and flavonoid in methanol extract of leaf (1108.552 QE/g). Trolox and Ascorbic Acid were used as standards for DPPH and ABTS free radical scavenging assays, IC_{50} value of DPPH assay for seeds methanolic extract was 293 µg/ml which was comparatively lower than trolox (362µg/ml) but Ascorbic acid IC_{50} value(205 µg/ml) for ABTS assay was lower compared to methanolic extract of seeds(380 µg/ml). These outcomes legitimizes its application in folkloric medicine and scientifically recommends the beneficial use of the plant in the treatment/management of human ailments.

Keywords: Pharmacognosy, secondary metabolites, free radical scavenging, *Anastatica hierochuntica*

1. Introduction
In this modern age herbs are recommended more common to treat the illness rather than medicine as they were considered to be safe, efficient with minimal side effects and often suggested in cultural practices. However a general awareness about the elements present in the herbs are important for human wellbeing as their ingestion in surplus or restricted amount can lead to severe health complications [1]. Therefore World health organization (WHO) has established a set of regulations to guarantee quality and safety for the use of herbal products [2]. In this context, proximate and mineral analyses are often analyzed for herbal drug's standardization. Moreover herbs that satisfies all the proximate composition parameter are considered to be safe for human consumption [3] as well as preliminary screening and quantification for chemical groups have been mandatory for the purpose of standardization as herbal formulation/ supplements based on these physicochemical data of the plant [4].
Previous studies have been done on herbs and showed that incidence of oxidative-stress related diseases has been reduced by the consumption of herbs due to the presence of functionally active antioxidants present in them [5]. Reactive oxygen species (ROS) react easily with free radicals which are produced in normal or pathological cell metabolism during cellular and signaling pathways at physiological concentrations[6] can become radicals. Short-come in the balance between ROS and antioxidant competence in the cells leads to a variety of pathophysiological processes such as inflammation, diabetes, genotoxicity, cancer, aging and neurodegerative disease [7, 8]. Therefore external source of antioxidants primarily from plant sources can resolve the imbalance and restore the equilibrium of normal functioning of the cell[9]. Synthesized antioxidants such as butylated hydroxytoluene and butylated hydroxyanisole have recently been reported to be toxic to mankind [10, 11]. As a result, the hunt for effective, harmless natural compounds with antioxidative property from plant-derived phenolic and flavonoids compounds [12, 13] have gained significance in modern ages.
Anastatica hierochuntica is a desert plant with a characteristic of tumbleweed and resurrection property belonging to Brassicaeae family.

It is a monotypic species of Anastatica genus commonly called as Kaff Maryam (Mary's hand), Rose of Jericho, Genggam Fatimah and widely distributed in Middle East and North Africa [14]. This herb has been well regarded for its ethano medicinal application to ease childbirth during delivery and reduce uterine hemorrhage [15] and aids in the management of various ailments [16, 17] as well as pharmacologically prominent as it has demonstrated the biologically important properties like antimicrobial [18, 19, 20], hypolipidaemic [21, 22], hypoglycaemic [21, 23], anti-melanogenesis [24], nitric oxide inhibitor [25], hepatoprotective [26, 27], gastro protective [28], anti-inflammatory [29, 30] and Immunostimulatory action [31].

Despite the fact that many researchers have studied on the secondary metabolite and anti-oxidant property of the plant [17, 29, 32, 33] there is an insufficient data lagging behind the knowledge about these metabolites and anti-oxidant potential specific to the plant parts, while the current study has been taken to assess the proximate and elemental composition on different parts of the plant viz. stem, seed and leaf material using standardized techniques and estimate the quantity of secondary metabolites and free radical scavenging prospective in different part of the plant extracts.

2. Materials and Methods

2.1 Reagents and Standards

2,2' -azino-bis-3- ethylbenzthiazoline-6-sulphonic acid(ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Ascorbic acid, Quercetin, Polyvinylpolypyrrolidone (PVPP), Folin–Ciocalteu's phenol reagent, Gallic acid, Sodium nitrate, Aluminum chloride, and Sodium hydroxide of analytical grade were purchased from Sisco Research Laboratories Pvt. Ltd.(India).

2.2 Sample Preparation

Anastatica hierochutica was collected from Saudi Arabia in western parts of Mecca during February to April 2018 in dried condition and was authenticated by local herbalist. The whole plant material was separated as stem, seeds and leaf and blended to fine particles using a mechanical grinder. About 100 g of each powder plant material were sequentially extracted with 300 ml of Hexane, Ethyl acetate, methanol and water at room temperature under continuous shaking for 3 days [34]. Each filtrate was concentrated using rotary evaporator under reduced pressure and low temperature. The yield of each extract was weighed and stored at 4 °C until used.

2.3 Proximate analysis

Proximate analysis of the powdered *A. hierochuntica* Stem, Seed and Leaf were estimated for the percentage of moisture content, ash content, crude fiber, crude fat, protein, carbohydrate content and energy value in Kcal/g

2.3.1 Determination of moisture content

Two grams of each plant material were placed in the pre-weighed crucible and heated at 105 ˚C for 24 h, was cooled and reweighed. The moisture content of each plant material was calculated as loss in weight of the original sample and expressed as percentage moisture content [35].

2.3.2 Determination of ash content

Two grams of the pulverized plant samples was placed in a crucible and ignited in a muffle furnace at 550 °C for 6 hours. It was then cooled in a desiccator and weighed at room temperature to get the weight of the ash. The total ash content of a substance is the percentage of inorganic residue remaining after the organic matter has been ignited [35].

2.3.3 Determination of crude fibre

Five grams of the powdered plant material was mixed with 200 ml of 1.25% H_2SO_4 and heated for 30 min. The filtrate was washed with distilled water until it was acid free. The residue was put in a pre-weighed crucible and dried at 105 °C in an oven overnight. After cooling in a desiccator, it was incinerated in a muffle furnace at 550 °C for 90 minutes to obtain the weight of the ash. The crude fiber was calculated as the difference in weight of the digested sample and the ignited sample to the weight of the sample [35].

2.3.4 Determination of crude lipid

One gram of the powdered plant sample were weighed and placed in a thimble. The thimble was placed in the extraction column connected to a condenser. 200 ml of petroleum ether was used to extract the lipid and the process lasted for 8 hr., the residue were dried in an oven for 2 h and then weighed. Difference in weight of the sample and defatted lipid gives percentage of crude lipid [35].

2.3.5 Determination of Protein

Three grams of sample was digested with 12 ml of concentrated H_2SO_4 for 112h in kjeldahl flask. Gradually 90 ml water, 25 ml sodium sulphate solution and 80 ml of 40% sodium hydroxide solution were added. The flask was connected to the distillation unit, to collect the liberated ammonia which was titrated against 0.04 N H2SO4 from green to a deep red end point. The crude protein percentage is calculated by multiplying titer value and nitrogen factor (6.25) to the weight of the sample [35].

2.3.6 Determination of carbohydrate

The carbohydrate content was determined by subtracting the summed up percentage compositions of moisture, protein, lipid, fibre, and ash contents from 100 [36].

2.3.7 Determination of gross energy value

Energy values were expressed as Kcal/g obtained by multiplying the percentage compositions of carbohydrate, protein and fat by the Atwater conversion factors 4,4 and 9 respectively [37].

2.4 Elemental Analysis by ICP-OES

Approximately 1gram of *A. hierochuntica* including stem, seed and leaf was digested with 6 mL of 1% nitric acid (v/v) and 2 mL of 35%hydrogen peroxide(v/v) in ETHOS ONE, High performance microwave digestion system from Milestone in two stages with 1000 W power for 180 °C at 10 and 30 minutes on 350 psi pressure. The resulting solutions were cooled and diluted to 50 mL and 15ml with Ultrapure water obtained by a Milli Q system, Millipore for macronutrients and micronutrients. All samples were digested in triplicate.

All measurements were performed using an inductively coupled plasma optical emission spectrometer ICP OES 5110 configured with SPS 4 auto sampler from Agilent Technologies. The operating conditions employed for ICP-OES determination were 1200W RF power, 12 L min-1 plasma flow, 1.0 L min-1 auxiliary flow, 0.7 L min-1 nebulizer flow, 2 mL min-1 sample uptake rate. Radial view was used to measure the analytical signal for macro elements

(Na, K, Ca, Fe, Mg) and micro elements (Cu, Mn, Zn, Co, Ni, Ba) present in the digested sample, while 2-point per peak and fitted background correction were applied. A multi-elemental standard solution of 1000 mgL-1 was used for calibration. The calibration standards in the range of 0.01 to 1.0 mg L-1 were prepared by diluting the multi-elemental standard stock solution with 1% nitric acid (v/v). The calibration fit remained to be linear weighted for all the studied elements.

2.5 Quantitation Secondary Metabolites

2.5.1 Total Phenol content

Total phenolics content of the extracts was measured by Folin–Ciocalteu's phenol reagent [38]. Briefly, 200 µL of diluted sample or gallic acid standard were added to 2.8 mL of distilled deionized water. Then, 500 µL of Folin–Ciocalteu's phenol reagent was added. After 5 min, 2.5 ml of 7% (w/v) Na_2CO_3 solution was added and mixed. After incubation for 90 min at room temperature, absorbance was measured at 725 nm versus a blank. Gallic acid concentrations of 20, 40, 60, 80 and 100 mg/L were used as a calibration curve. The content of total phenolics was expressed as mg gallic acid equivalent (GAE)/ g of sample. All samples were analyzed in triplicate

2.5.2 Total Tannin content

Tannin content of the extracts was determined using insoluble polyvinyl-polypirrolidone (PVPP), which binds to tannins [39]. Briefly, 1 ml of sample or standard(gallic acid) was dissolved in the respective solvent, was mixed with100 mg PVPP and vortexed was incubated for 15 min at 4 °C and then centrifuged for 10 min at 3,000 rpm. In the clear supernatant the non-tannin phenolics were determined the same way as the total phenolics using Folin–Ciocalteu's phenol reagent. Difference in total phenolic and non-tannin phenolic content was calculated as Tannin content. The non-tannin phenolic and tannins content was expressed as mg gallic acid equivalent (GAE)/ g of sample. All samples were analyzed in triplicate

2.5.3 Total Flavanoids

Total flavonoids content of the extracts was determined using a spectrophotometric method [40]. Briefly, 500 µL of sample or standard (Quercetin) were mixed with 3.2 mL of distilled deionized water. 150 µL of 5% (w/v) sodium nitrite ($NaNO_2$) solution were added and mixed. After 5 min, 150 µL of 10% (w/v) aluminum chloride ($AlCl_3$) was added and mixed. After 6 min, 2 mL of 4%(w/v) sodium hydroxide (NaOH) was added and mixed. Absorbance was measured at 510 nm versus a blank. Quercetin concentrations of 20, 40, 60, 80 and 100 mg/L were used as a standard curve. Total flavonoid content was expressed as mg Quercetin equivalent (QE)/ g sample. All samples were analyzed in triplicate.

2.6 Free Radical scavenging assays

Methanol and aqueous extracts of the plant material were assayed for free radical scavenging activity. Ascorbic acid and Trolox served as a standard for ABTS and DPPH assays respectively.

2.6.1 ABTS assay

The ABTS assay was accomplished based on the defined method [41]. Briefly, the radical ion was prepared by mixing equal volume of 7.5mM ABTS stock solution with 2.45 mm potassium persulphate. The mixture was kept in the dark at room temperature for 12 h for complete radical generation, and then diluted with 95% ethanol to read the absorbance at 734 nm to be 0.70 ± 0.02. Then, 0.6 mL of standard or sample (100-500 µg/ml) or water for control were mixed with 0.4 mL of prepared ABTS solution and incubated for 10 min at 37 °C. The decrease of absorbance was monitored at 734 nm

2.6.2 DPPH assay

The DPPH assay was performed according to the described method [42]. A solution of 1 mm DPPH was prepared in 80% (v/v) methanol and absorbance of the solution was adjusted to 0.65±0.02 at 517 nm. Then, 2mL of standard or sample (100-500 µg/ml) or water for control were mixed with 2mL of DPPH solution and incubated for 30 min in the dark at room temperature. Decrease of absorbance was monitored at 517 nm

The percentage of ABTS and DPPH inhibition of the extracts was calculated as

Inhibition % = (C-T)/C × 100, where C is the absorbance of the blank, and T is the absorbance of the extracts/standard. IC_{50} of the all extracts and standard was calculated by graphical plotting % inhibition versus concentration.

3. Results

3.1 Proximate analysis

The proximate composition of *A. hierochuntica* plant materials are presented in Table 1. The result showed a relatively high percentage of carbohydrate content in all the plant parts when compared with other compositions analyzed. Stem's ash, protein and fiber content outlined leaves and seed composition. Conversely percentage of fat and moisture content was reported higher in seed and leaf respectively. However seed contained the highest energy value of 301.04 Kcal/g which are approximately equal in leaf (284.19 Kcal/g) and stem (261.67 Kcal/g).

Table 1: Proximate analysis for different plant parts of *A. hierochuntica*

Proximate Analysis	Stem % dry wt	Seed % dry wt	Leaf % dry wt
Moisture content	12.96±0.22	10.54±0.58	14.87±0.36
Ash content	17.32 ±0.82	15.47 ± 0.11	12.13±0.52
Crude protein	12.36±0.29	14.95±0.45	10.84±0.27
Crude fiber	13.59±0.43	9.03±0.37	11.29±0.14
Fat content	5.59±0.65	8.24±0.41	7.79±0.23
Carbohydrate content	40.48	41.77	42.68
Nutritive/energy value*	261.67	301.04	284.19

Each value represents the mean ± S.D. (n = 3); *Values are expressed as Kcal/g

3.2 Elemental Analysis

The essential macro compositions of *A. hierochuntica* plant materials were measured in terms of ppm are shown in table 2. Except for magnesium, leaf has rated to have the highest concentration of all the studied macroelememts particularly potassium concentration was about 395±1.96 ppm. Similarly Seed's magnesium were reported to be highest with 25±1.88 ppm.

Table 2: Macroelememts Analysis for different plant parts *A. hierochuntica* by ICP-OES

Macroelememts (ppm)	Sodium	Potassium	Calcium	magnesium	Iron
Stem	52±2.36	161±3.21	48±5.03	12±2.58	1±0.36
Seed	62±1.05	334±3.58	176±3.44	25±1.88	5±5.11
Leaf	198±4.32	395±1.96	346±5.26	16±2.52	8±3.69

Each value represents the mean ± S.D. (n = 3)

The microelements of *A. hierochuntica* plant materials are shown in table 3 were expressed in ppb are presented in table 6. Copper concentration was higher in Stem with 286.2±7.25 ppb while seed possess higher concentration of nickel and zinc at 66.30±5.32 ppb and 602.2±6.58 ppb correspondingly. Barium, Cobalt and manganese were reportedly higher in leaves compared to stem and seed.

Table 3: Microelements Analysis for different plant parts *A. hierochuntica* by ICP-OES

Macroelememts (ppb)	Barium	Cobalt	Copper	Manganese	Nickel	Zinc
Stem	218.58±6.37	0.42±0.05	286.2±7.25	206.63±6.38	35.49±6.10	247.6±4.22
Seed	671.21±5.89	3.5±1.14	207.19±5.29	592.62±7.24	66.30±5.32	602.2±6.58
Leaf	1069±11.42	25.11±2.57	180.88±2.16	917.43±5.33	52.59±2.96	298.09±4.39

Each value represents the mean ± S.D. (n = 3)

3.3 Quantitation of Secondary metabolite

Among the secondary metabolites that were quantified, the total flavanoid content was the highest for methanol extract of leaf (1108.52±1.23) mg of QE/g followed by aqueous extract of stem(1075.65±0.89) mg of QE/g and methanol extract of seed (1009.86±1.09) mg of QE/g. Similarly tannins content was reported to be higher in aqueous extract of stem (17.36±1.69) mg of GA/g trailed by methanol extract of leaf (16.53±0.59) mg of GA/g and seed (14.05±1.39) mg of GA/g, while higher phenol content were observed in aqueous extract of leaf (32.25±1.14) mg of GA/g compared to seed (31.42±1.62) mg of GA/g and stem (27.29±0.97) mg of GA/g. The results are tabulated in table4.

Table 4: Quantitation of Secondary Metabolites for different plant parts *A. hierochuntica* extracts

Plant	Solvents	Phenols mg/g dry weight	Non tannins mg/g dry weight	Tannins mg/g dry weight	Flavonoids mg/g dry weight
Stem	Hexane	23.982±2.34	16.539±3.98	7.442±2.66	648.026±1.24
	Ethyl Acetate	18.193±1.65	8.269±1.08	9.923±3.18	845.394±5.82
	Methanol	25.636±3.58	13.231±4.01	12.404±2.64	944.078±6.22
	Aqueous	27.290±0.97	9.923±4.32	17.366±1.69	1075.65±0.89
Seed	Hexane	23.155±4.36	14.885±2.11	8.2699±2.49	549.342±5.34
	Ethyl Acetate	18.193±1.25	13.231±3.47	4.961±2.43	713.815±2.37
	Methanol	28.944±3.33	14.885±4.53	14.058±3.67	1009.861±1.09
	Aqueous	31.425±1.62	19.847±1.10	11.577±2.96	911.184±3.45
Leaf	Hexane	19.020±2.47	14.885±3.04	4.1349±3.14	615.131±2.57
	Ethyl Acetate	22.328±4.35	11.577±2.69	10.750±2.65	779.605±5.29
	Methanol	29.771±3.62	13.231±3.58	16.539±0.59	1108.552±1.23
	Aqueous	32.252±1.14	18.193±2.14	14.058±1.39	976.973±5.94

Each value represents the mean ± S.D. (n = 3)

3.4 Free Radical scavenging assay

Five concentration of plant material extracts and standard ranging from 100-500 µg/ml were tested for the free radical scavenging assays. The extracts scavenged the free radical in increasing concentrations. The maximum inhibitory concentration (IC_{50}) of free radicals by 50% has been used to measure scavenging activity. Methanol and aqueous extracts of *A. hierochuntica* seed out performed free radical scavenging activity compared to stem and leaves extarcts.

The results of DPPH radical scavenging activity of *A. hierochuntica* plant material's methanol and aqueous extract along with trolox are presented in table 5. The percentage inhibition of the DPPH radical by the aqueous and methanol extract of seed at 500 µg/ml was 73.72% and 70.44%, while the IC_{50} values were 315 µg/ml and 293 µg/ml respectively which are comparatively lower than Standard trolox 362 µg/ml.

Table 5: Inhibition Percentage of DPPH radical scavenging activity for different plant parts of *A. hierochuntica* extracts

Concentration (µg/ml)	Trolox%	Aqueous extracts			Methanol extracts		
		stem %	seed %	leaf %	stem %	seed %	leaf %
100	25.12	25.94	28.57	21.18	26.1	26.92	22
200	31.69	31.03	35.13	26.92	33.49	40.88	28.57
300	42.93	36.78	49.09	33.49	44.99	55.66	35.13
400	50.57	44.17	54.84	37.6	54.84	61.41	45.81
500	66.91	63.87	73.72	56.48	65.51	70.44	56.48
IC_{50} value	362	408	315	482	347	293	443

The ABTS scavenging activities of *A. hierochuntica* plant material's methanol and aqueous extract were determined and compared with ascorbic acid are shown in table 6. At the highest concentration of 500 µg/ml, only 62.86% and 51.34%

inhibition was observed for methanol and aqueous extract of seed while ascorbic acid inhibited 85.49%. As a consequence the IC_{50} value of standard was 205.07 µg/ml comparatively lower to seed extracts.

Table 6: Inhibition Percentage of ABTS radical scavenging activity for different plant parts *A. hierochuntica* extracts

Concentration (µg/ml)	Ascorbic acid %	Aqueous extracts			Methanol extracts		
		stem %	seed %	leaf %	stem %	seed %	leaf %
100	36.79	11.65	19.33	5.24	15.49	16.77	20.61
200	47.74	27.01	33.41	12.93	29.57	30.85	27.01
300	64.43	34.69	37.25	28.29	39.82	41.1	37.25
400	74.41	46.22	47.5	39.82	51.34	52.62	48.78
500	85.49	50.06	51.34	47.5	60.3	62.86	56.46
IC_{50} value	205.07	467.48	457.17	574	397.39	380.19	403.3

4. Discussion

Proximate and Mineral analysis are often necessary to be evaluated for plants as there may be changes in their nutritive components in respect to the period of harvest and environmental conditions [43-45]. In our study, precaution was taken to study these analyses after the completion of phenological stages in *A. hierochuntica* which are usually ended before April, as its most common for desert plants [46]. *A. hierochuntica* seeds have shown the high energy value which may be due to the presence of high fat content in the seeds [47]. Many studies have reported the significant importance of seeds in the medicinal plants [48-50]. Typically these high lipid content have been responsible for mechanism of desiccation tolerance and resurrection properties as witnessed in *A. hierochuntica* [51-53].

Elemental analysis results shows that leaves have reported the highest concentration for most of the micro and macro elements. Presence of high concentration of Ca, K and Na in the plant could have supported the generation of action potential and/or through excitation-contraction coupling in the uterine smooth muscle cell membrane of the expectant mother to stimulate uterine contraction during labour [54-57].

The rich Fe and Mn content may provide an extended hand to reduce the postpartum hemorrhage as they are involved in blood vessel functions including blood clotting and hemostasis[58-60]. Besides, the prevalence of Zn, Cu, Co and Ni possess the importance of the plant to mankind as the plant contains adequate concentrations of these essential trace element nutrients indispensable for proper physique growth and development [61-63].

However our results were not concordant with previous studies where *A. hierochuntica* plant parts were digested by ashing method [32, 64]. Sample digestion method plays a significant role for the proficient use any quantitative analytical techniques [65]. Microwave digestion has gained advantages over traditional methods as it digest and improve sample handling as well as decreased the loss of volatile compounds and contamination rate [66]. Therefore it has been widely postulated to be the appropriate digestion method for plant samples which ensured the complete destruction of plant matrix enabling the release of minerals [67-70].

Major secondary metabolites like Phenols, tannins, non-tannins and flavonoids were quantified in terms of their respective standard equivalent. Gallic Acid equivalent phenol concentration was reported higher in aqueous extracts of leaves compared to stems and seeds which was concordant with the previous study [32]. Quantity of phenols observed in the organic extract of the whole plant was similar to our results [29, 33]. Different phenolic compounds comprising of Dihydroxybenzoic acid hexoside, 3,4-Dihydroxybenzoic acid, 5-O-Caffeoylquinic acid, 3,4-O-Dicaffeoylquinic acid and 4,5-O-Dicaffeoylquinic acid have been identified in aqueous extracts of plant seeds were found to exhibit antioxidant properties [17].

The high tannins concentration was obtained in the aqueous extracts of the stems and in the methanol extracts of seeds and leaves. Mostly plants rich in tannins were used to treat intestinal disorders, thus the use of *A. hierochuntica* was a practice in ethonomedine [71, 72]. As well as tannins exist in hydrolysable and condensed form to quench free radicals, chelate transition metals, inhibit pro-oxidative enzymes and lipid peroxidation [73].

In our results, we observed that the amount of quercetin equivalent flavonoids was higher in the methanol extracts of leaves however other study reported high rutin equivalent flavanoids quantity in ethanol extracts of the whole plant [29]. This high flavonoids concentration would be possible due to the existence of wide range of flavanoids groups like flavones, flavonols, flavanones and flavonolignans present in the plant [17, 24, 26, 74]. These flavonoids have demonstrated antioxidant, anti-melanogensis and hepatoprotective properties. Apart from these properties many researches have supported the importance of the plants flavanoids in the activation of immune modulatory effects [31]. Previous research of *A. hierochuntica* reported that flavone glycosidic components are accountable for the stimulation of lipolysis via nucleic acids and protein metabolism and regeneration of pancreatic cells in alloxanand STZ-induced diabetic rats [21, 23].

According to both DPPH and ABTS assays, the free radicals accepts an electron to become a stable molecule and the colored oxidant reduced by the plant is being claimed as the antiradical capacity of the plant extract. For DPPH radical scavenging assay, we identified that at 500µg/ml, the methanol extract of seeds single-handedly exhibited 70.44% inhibition while earlier study reported that at 150 µg/ml the methanol extract of whole plant and aqueous extract of leaf exhibited 55.87% inhibition and 65% inhibition [32, 33]. However our results showed the lowest IC_{50} value for methanol extracts of seeds with 293µg/ml, which was similar to the ethanol extract of the whole plant at 150.85µg/ml [29].

In the case of ABTS free radical assay we observed that the methanol extract of seeds at 500µg/ml concentration presented 62% inhibition with an IC_{50} of 380.19 µg/ml. This was similar to the previous study where the prevailing phenolic and flavonoids compounds present in the aqueous extract of seeds displayed 56% and 41% inhibition [17]. Conversely, ethanolic extract of the whole plant exhibited 93.18% inhibition with an IC_{50} value of $3x10^{-13}$ which was completely conflicting to our results [29]. Thus significant antioxidant properties exhibited by the plant seeds was principally owed to the presence total phenolic content of the plant extracts [75, 76]. Even though DPPH and ABTS are widely accepted method for scavenging assays [76-78]. The chances of

reproducibility was higher in DPPH related to ABTS assays as reported in our experiments [79, 80].

5. Conclusion

Thus the present study report that *A. hierochuntica* seeds has showed to be rich grounds of energy value in their proximate composition while leaf has proclaimed to be the supremacy of the trace elements. The high amount of phenolic, flavonoid and tannin compounds was present in methanol and aqueous extract of both seed and leaves. Nevertheless, the methanol extracts of the seeds presented to be potential free radical scavenger. Therefore, additional researches are essential to elucidate the *in vivo* prospective of this plant in terms of the active compounds composition and biological activities for the treatment of human ailments as they may useful in nutraceutical formulations.

6. References

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