Contribution to the valorization of *Herniaria fontanesii* J. Gay (*Caryophyllaceae*) grown in Septentrional Sahara and its antioxidant activity

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Abstract
This study aims to study a wild plant Herniaria fontanesii J. Gay which grows in Septentrional Sahara in El Oued Algeria. The evaluation of total phenolic and flavonoids content has been estimated in both aqueous and butanolic fractions of 80% methanolic extracts obtained from the plant's aerial part. Antioxidant activity was evaluated in vitro using scavenging assays of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radicals, ferric reducing power FRAP and molybdenum reducing power methods.

The extracts showed that the plant has a good antioxidant activity reaching to 1.525± 0.010 mg/ml for IC_{50} in DPPH assay and 0.011± 2.16610^{-5} mg/ml for IC_{50} in ABTS assay. A comparison has been done between these results and other results for the same plant harvested in Morocco, Tunisia and Laghouat, Algeria.

Keywords: Herniaria fontanesii, DPPH, ABTS, FRAP.

Introduction

The medicinal plants had been used for ages as treatments for human diseases. The derived compounds from them are getting more interest because of their adaptable applications in many fields[2]. An increasing number of reports dealing with the assessment of the effects of different medicinal plants extracts on many disorders come of oxidative stress[1,5,15,19,21,22]. Oxidative stress is a state where oxidative forces exceed the antioxidant systems due to loss of the balance between them[26]. It plays a central role in neuronal injury and cell death in acute and chronic pathological conditions. The cellular responses to oxidative stress embrace changes in mitochondria and other organelles[10] leading to damage in cellular, tissue and organ systems. Therefore, many diseases will result including cancer, cardiovascular diseases, neurodegenerative diseases like Parkinson's disease and Alzheimer's dementias, diabetes, ischemia/reperfusion injuries, rheumatoid arthritis, Inflammations, malignancies and even the process of aging[1,15,18,19,21,26].

Nowadays medicine still faces more difficulties to cure these diseases. This leads to the necessity of folk medicine to search in, for plants that may be rich in antioxidants. Where they can neutralize the effects of reactive oxygen spaces and thus help in preventing diseases[20]. Caryophyllaceae is one of the large dicotyledonous families, comprising of about 80 genera and 2100 species, known for ornamental plants, generally rich in triterpenoids and saponins, which are very often responsible for a wide range of medicinal uses due to their anti-inflammatory, antispasmodic, antidiabetic and anticarcinogenic properties[9]. The *Herniaria* L. genus (Rupturewort) includes well-known traditional medicinal plants used in different regions of Europe, Asia and Africa. The ethnomedicinal significance of some species had been confirmed by their presence in Pharmacopoeias of many countries, Ruptureworts are mainly recommended to alleviate disorders related to the urinary system including kidney and bladder stones as well as bladder infections[12]. Many studies explored this genus that includes plants such as *Herniaria hirsuta*, *Herniarnia inaca* etc.[3,12,14,24,25]

The *Herniaria fontanesii J. Gay.* or Camouna (traditional name) is a spontaneous perennial plant; it has several woody stems at the base with fragile branches with discarded internodes. The more or less hairy leaves are linear to lanceolate with small stipules. Flowers are mostly green in axillary few-flowered glomeruli; triangular haired bracts. Its calyx tube is pubescent with hooked hairs. Outer sepals are slightly fleshy and bristly. Forth Stamens have large anthers (up to 5 mm)[12] It is widely distributed plant in the Mediterranean area. Its aerial part is used in folk medicine as a diuretic and for the treatment of lithiasis[7,14].

In this study we are going to evaluate the phytochemical and antioxidant activity of butanolic and aqueous fractions of methanolic extract of the plant collected from EL Oued in Algeria (Septentrional Sahara) by estimating total phenolics and total flavonoids content and by testing its antioxidant activity using DPPH, ABTS, FRAP and molybdenum methods.

Material and Methods

Reagents and Materials: Absolute methanol was purchased from Merck (Darmstadt, Germany), n-butanol 99% was purchased from Sigma Aldrich (HPLC grade), all other organic and non-organic reagents were purchased from Sigma Aldrich.

Plant material: The aerial part of *Herniaria fontanesii* J. Gay (*Caryophyllaceae*) was grown wild and collected during the flowering phase in March 2017 in the village of

Debila in El Oued Algeria. GPS-Coordinates are 33◦28′20.2116″N, 6◦56′40.4916″E. The plant was identified by Mr Youcef Halis in Scientific and Technical Research Center for Arid Areas, Biskra, Algeria. Harvested plant material had naturally dried in a well-ventilated space with no direct sunlight. Then, dried material was pulverized and transferred into tightly closed containers and kept in dark cold place until the extraction.

Figure 1: Herniaria fontanesii J. Gay

Solid-liquid extraction: 30g of pulverized dried sample was put in a clean and a dry glass and extracted three times with MeOH/water 80/20 (v/v) system at room temperature for 24 hours in dry and dark place with continues shaking. Then the mixture was filtered with Whatmann paper no. 1. The methanol crude extract was completely evaporated under reduced pressure at 40 ºC using a rotary evaporator.

Liquid-liquid extraction: Petroleum ether was used to extract chlorophyll from aqueous residue three times in a row, n-butanol was the organic solvent used to extract polyphenols from the rest dechlorophylled aqueous phase. This liquid - liquid extraction was repeated three times for maximum yield. The obtained butanolic fraction was dried with $MgSO_4$ to eliminate residual water and then filtered with Whatmann paper no.1. Butanolic and the rest aqueous fractions were evaporated under reduced pressure at 40 ºC using a rotary evaporator. The two dried fractions were saved after they have been lyophilized for 72 hours (P= 0.03, T= -90°C) and then kept in a dark and cool area.

Total phenolic content (TPC): Total phenolic content (TPC) of the extracts was carried out by the colorimetric method of Singleton-Rossi using Folin-Ciocaltu reagent as described by Al-Owaisi et al[4] with slight modifications. 100µL of gallic acid standard was mixed with 0.5 mL of Folin-Ciocalteu (diluted 1:10 with distilled water) and 2 mL of sodium carbonate solution (20 %, w/v). The reaction mixture was incubated in the dark at room temperature for 30 min. The absorbance was measured at 765 nm. The TPC was determined from standard gallic acid curve and expressed by milligrams of gallic acid equivalents per 100 grams of dry weight (mg GAE/100g DW).

Total flavonoid content (TFC): Total flavonoids content of the extracts was determined by the colorimetric method described by Djeridane et al[8] using aluminum chloride reagent and querctin as standard. 1ml of 2% $AlCl_3$ ethanol solution was added to 1ml of extract. The absorbance was determined at 430nm. The calibration curve was prepared with querctin and the results were expressed by milligrams of querctin equivalents per 100 grams of dry weight (mg QE/100g DW).

DPPH radical scavenging assay: Free radical scavenging activity of extracts was determined as described by Himaja et al[11] using 1,1-diphenyl-2-picrylhydrazyl (DPPH). Different dilutions of extracts were prepared from each extract. About 1 mL of 0.1mmol/L DPPH solution in ethanol was added to 1mL of sample solution and incubated for 30 min in the dark at room temperature and the absorbance recorded at 517 nm. Each test was done in triplicate and the mean values were calculated. The inhibition efficiency was calculated using the following equation:

$$I\% = 100 \times (Abs0 - Abs)/Abs0$$

where *Abs0* is absorbance of control and *Abs* is absorbance of sample.

ABTS assay: ABTS assay was measured as described by Re et al[17]. The ABTS radical cation $ABTS^{\bullet+}$ was produced by the reaction of 7 Mm ABTS and 2.45 mM potassium persulfate in the dark at 25 ºC for 14-16 h. The $ABTS^{\bullet+}$ solution was diluted with ethanol (50%) to absorbance of 0.800- 0.700 at 734 nm. Different dilutions of extracts were prepared from each extract. 2.5ml diluted $ABTS^{\bullet+}$ solution was added to 0.3µL of sample or methanol for blank. The absorbance was recorded at 734 nm. The inhibition percentage was calculated using the following equation:

$$\mathrm{I\%} = 100 \times (\mathrm{Abs0} - \mathrm{Abs})/\mathrm{Abs0}$$

where *Abs0* is absorbance of control and *Abs* is absorbance of sample.

Ferric reducing power assay: The reducing power of extracts was estimated according to the reported method of Kumaran et al[13] with slight modifications. 1mL of each extract was mixed with 2.5 mL of phosphate buffer (0.2 mol/L, pH 6.6) and 2.5mL of potassium ferrocyanide $[K_3Fe(CN)_6]$ (1 %). The mixture obtained was incubated at 50 ºC for 20 min.

Then, 2.5mL of 10% trichloroacetic acid was added. 2.5 mL was taken from the mixture and mixed with 2.5 mL of deionized water followed by the addition of 0.5 mL of 0.1 % $FeCl_3$. The absorbance was measured at 700 nm. Reducing power was expressed in (mM) as ascorbic acid equivalent antioxidant capacity (AEAC).

Phosphomolybdenum reducing assay: The phosphormolybdenum reducing power of extracts was estimated according to the method by Prieto et al[16]. The technique is based on the reduction of molybdenum Mo (V) to Mo (IV) by the extracts and subsequent formation of a green phosphate/Mo (V) complex at acid pH. 0.3 mL of the extract was mixed with 3 mL of the reagent solution (0.6 M sulfuric acid, 28 mM sodium and 4 mM of ammonium molybdate). The tubes were incubated at 95C° for 90 min. After cooling, the absorbance of the solutions was measured at 695 nm against the blank. Total antioxidant capacity TAC was expressed in milligrams of ascorbic acid per gram of dry matter (mg AA/g DW).

Results and Discussion

Total phenolic content (TPC): Acid gallic standard curve was drawn (figure 2). Moreover, the total phenolic content assay (Table 1) showed differences between butanolic and aqueous fractions of the methanolic extract. Maximum value was for butanolic fraction which is 31.368±0.115 mg GAE/100g DW and the minimum value was for aqueous fraction 17.088 ± 0.257 mg GAE/100g DW (figure 3). The result of TPC is different from the study of Hammami et al[9] on the same plant collected in South East of Tunisia where they found TPC equal to 92.27 ± 6.15 mg GAE/g DW on the methanolic extract.

Moreover, Tlili et al[23] found 27.23 ± 0.012 mg GAE/g DW for extracts of methanol 70%; but Benalia et al[6] found a value of 4,926±0,030 mg GAE/g DW in methanol 50% extract and 0,968±0,042 mg GAE/g DW in water extract for the same plant harvested in the Saharian Atlas at 40km north of Laghouat (Algeria). This discrepancy in values was caused by the difference in the nature of the areas from which the plant was collected (soil and climatic conditions) as well as the method of extraction.

Total flavonoid content (TFC): Quercetin standard curve was drawn (figure 4). Moreover, the results obtained from TFC (Table-1) show that the butanolic fraction has 1.77± 0.003 mg QE/100g DW and aqueous fraction has 0.383± 0.013 mg QE/100g DW. That may refer to the climatic conditions on El Oued region and to the poorness of soil.

DPPH radical scavenging assay: The results of DPPH radical scavenging activity of extracts have been shown in table 1.

Table 1
Test results for two phase's methanolic extract

		Methanolic extract	
Test		**Butanolic fraction**	**Aqueous fraction**
TPC	mg GAE/100g DW	31.368±0.115	17.088 ± 0.257
TFC	mg CE/100g DW	1.77± 0.003	0.383± 0.013
DPPH	IC_{50} µg/ml	7.886± 0.036	0.406± 0.002
ABTS	IC_{50} µg/ml	0.992± 0.001	6.831± 0.055
P. Molybdenium test	TAC mM	3.760±0.120	2.897±0.070
FRAP	AEAC mM	62.582±3.383	7.490±0.045

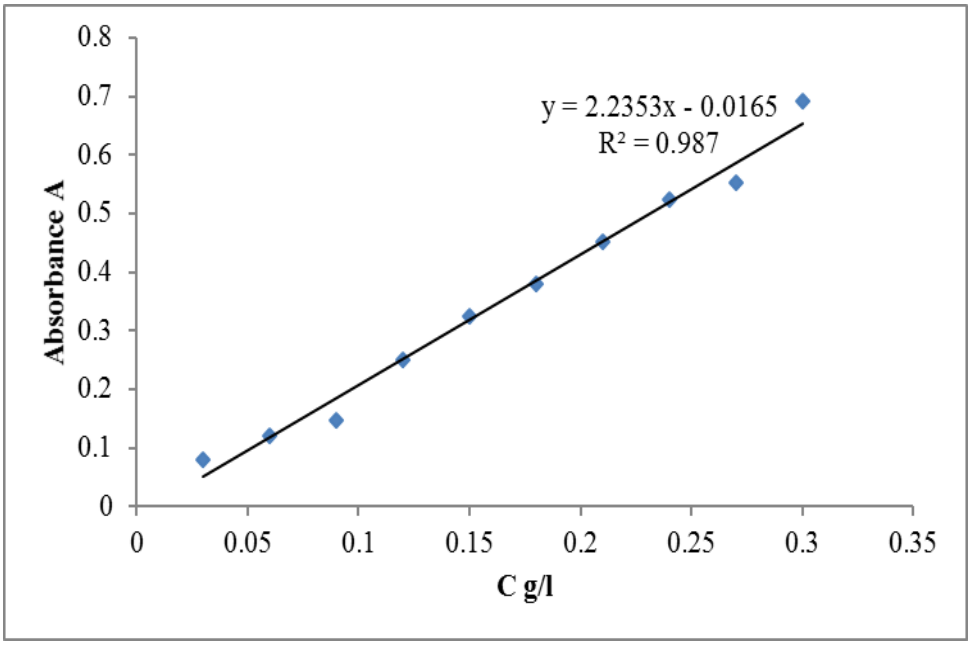

Figure 2: Standard curve of Gallic Acid

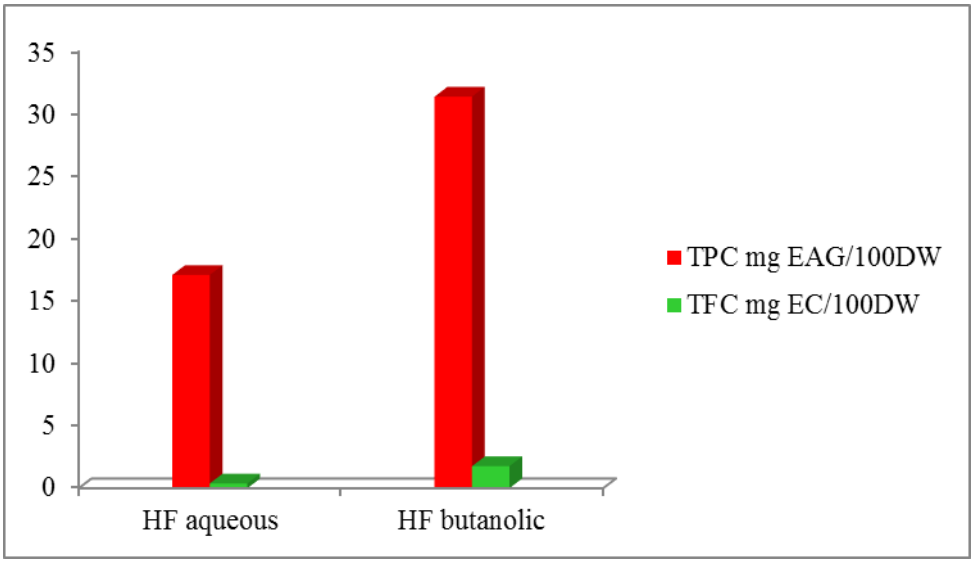

Figure 3: Polyphenols and flavonoids in methnolic extract fractions

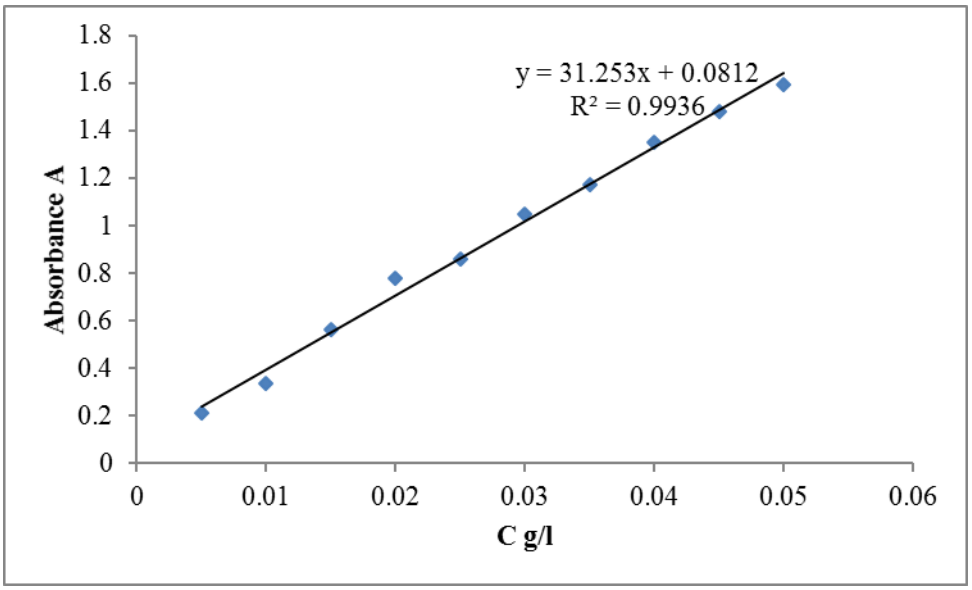

Figure 4: Standard curve of Quercitin

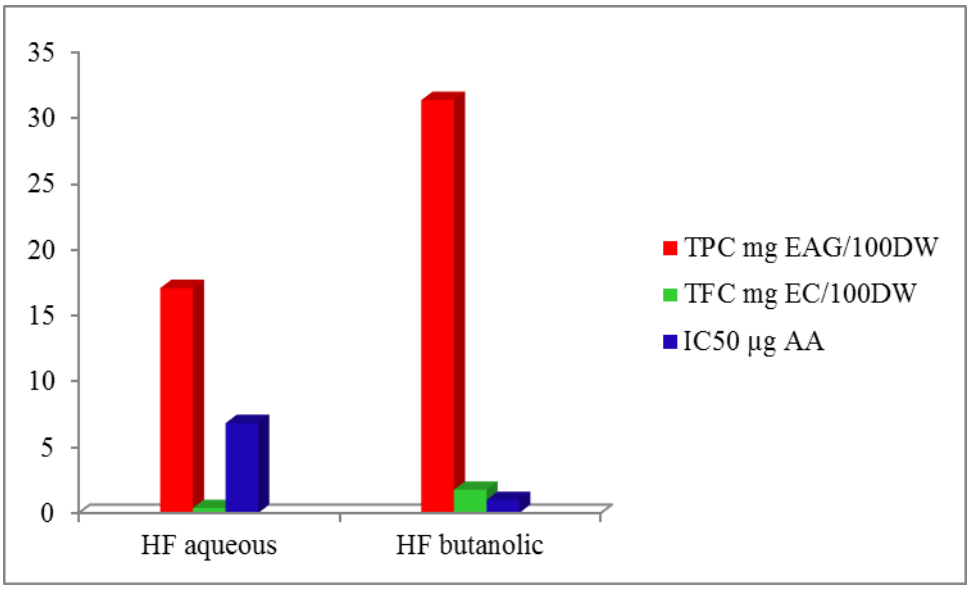

Figure 5: Relationship between ABTS, TPC and TFC

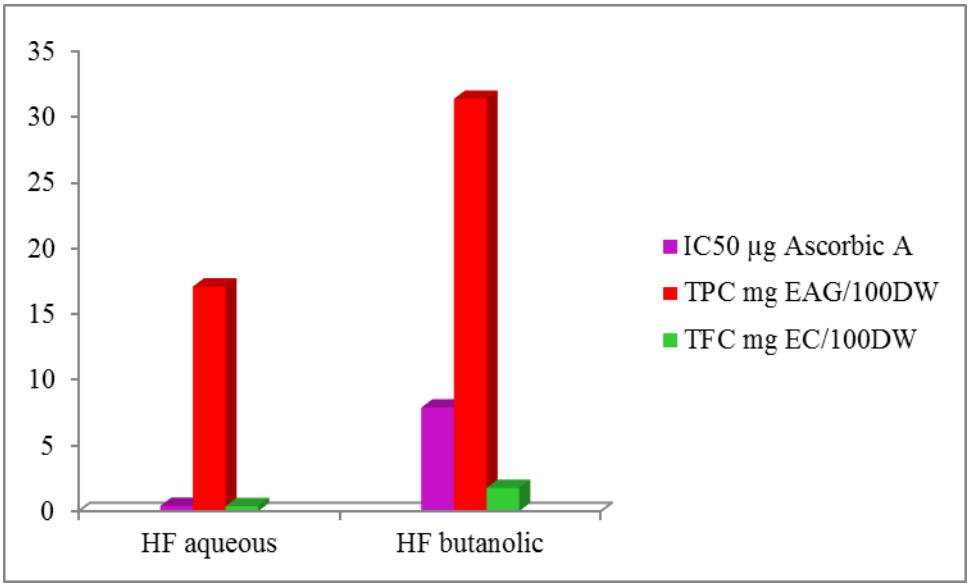

Figure 6: Relationship between DPPH, TPC and TFC

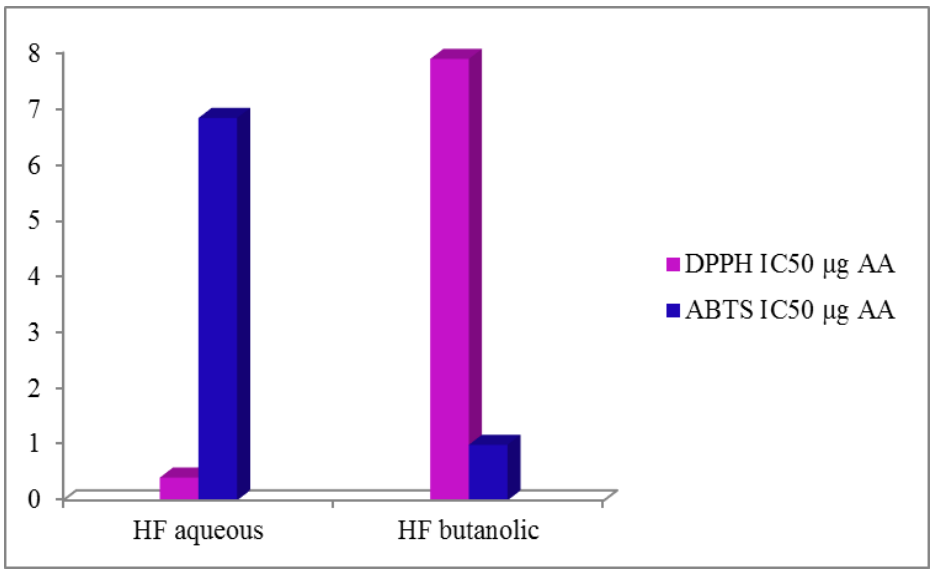

Figure 7: Relationship between ABTS and DPPH

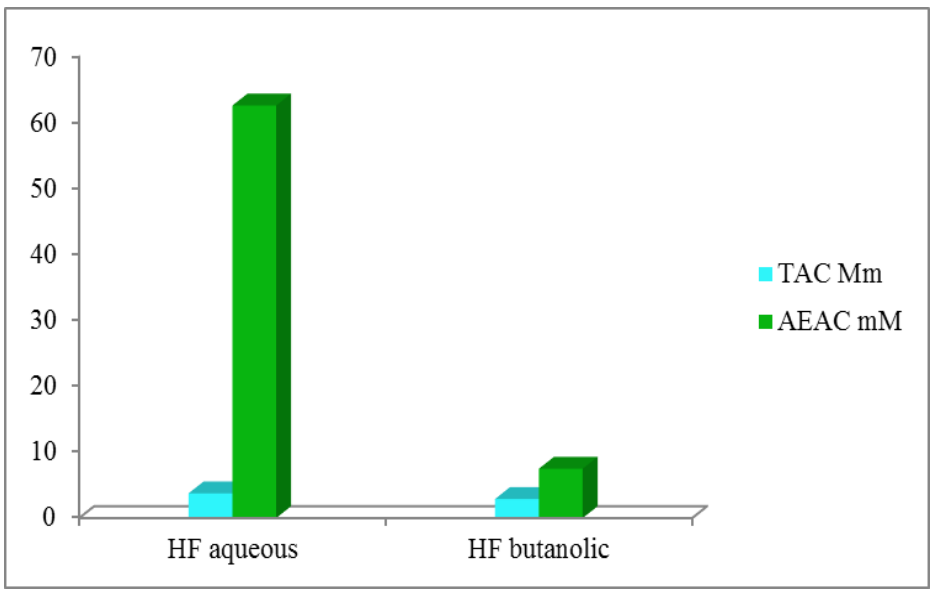

Figure 8: Relationship between FRAP and Phosphomolybdenum test

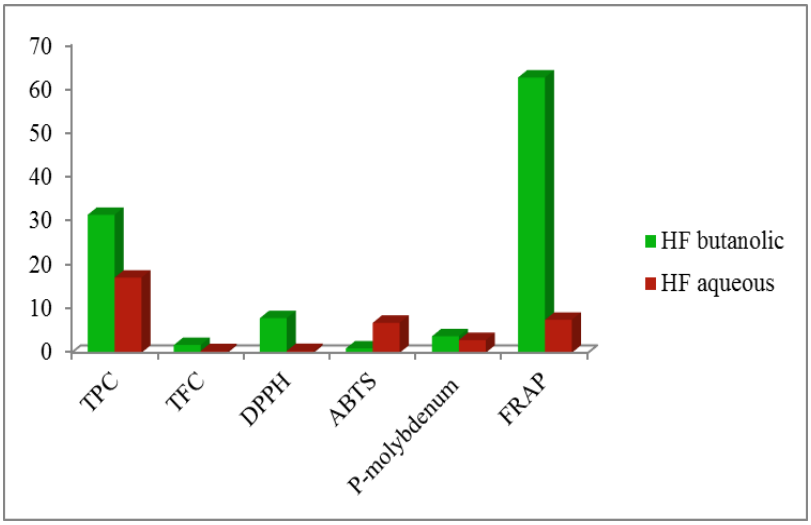

Figure 9: Relationship between antioxidant assays and between TPC and TFC in two fraction

For aqueous fraction, which was the strongest, IC_{50} was 0.406± 0.002 µg/ml, then IC_{50} was 7.886± 0.036µg/ml for butanolic fraction. The results were opposite with the results of TPC and TFC, where the higher value of inhibition capacity is less for phenolic content (figure 6).

This may relate to the hydroxyl groups acting as protons donating and could be considered as the first responsible chemicals of the scavenging activity in other components where they exist in aqueous fraction more than butanolic fraction. Tlili et al[23] found IC_{50} equal to 1.30 ± 0.023 mg/ml in their methanolic extract, which means that the two studies are similar in this point.

ABTS assay: The results of ABTS radical scavenging activity of extracts have been shown in table 1. For butanolic fraction, which was, the strongest, IC_{50} was 0.992± 0.001µg/ml and then IC_{50} was 6.831± 0.055µg/ml for aqueous fraction (figure 7). The results are compatible with TPC and TFC values. where the higher value of TPC and TFC the less value of IC_{50} then the strongest radical scavenging activity. These results are opposite to DPPH results where we can attribute that components reacted differently with different radicals involved in different antioxidant testes.

Ferric reducing power assay: AEAC values (Table 1) varied from 62.582±3.383mM for the butnolic fraction to 7.490±0.045mM for aqueous fraction. As seen, these results are opposite to ABTS results and compatible with DPPH results (figure 7). This caused by antioxidant agents reacted with Fe (III) to reduce it to Fe (II), that means the reacted agents in this case are electron givers.

Phosphomolybdenum reducing assay: TAC values shown in table 1 varied from 3.760±0.120mM for butanolic fraction to 2.897±0.070mM for aqueous fraction. This assay is based on reduction of Mo (V) to Mo (IV) (reaction with electron givers) that justifies the similarity with FRAP and the compatibility with DPPH results (figure 8).

Conclusion

The obtained results in this study (figure 9) showed that the plant *H.fontanesii gay* has different values in total phenolic content and total flavonoid content for butanolic and aqueous fractions. where butanolic fraction contained important amounts of them. The values of antioxidant assays gave us a good idea about the nature of the methanolic extract fractions, where we found that butanolic fraction contains a high content of electron givers more than aqueous fraction, that appeared by the high and compatible values of AEAC and TAC in FRAP and Phosphomolybdenum assays respectively In front of low values of IC_{50} in DPPH assay. ABTS assay gave us opposite results with DPPH, which attributed that components react differently with different radicals involved in different antioxidant testes.

Comparison with previous studies from different regions showed differences in results that refer to difference in soil and climatic conditions. This plant has a good antioxidant activity in its methanolic extract and further studies must be carried out for better valorization.

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