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Antioxidant Activity and Chemical Constituents of Essential Oil and Extracts of *Haplophyllum Tuberculatum* from Tunisia

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Abstract - The current study focuses on the evaluation of chemical composition and antioxidant activities of different solvent extracts of *Haplophyllum Tuberculatum* aerial parts. Thirty-two components of essential oils were identified, with *trans-p*-menth-2-ene-1-ol accounting for 18.25% of total volatiles, followed by β-phellandrene and piperitone, accounting for 14.46% and 14.33%, respectively. The results showed that antioxidant activities were significantly influenced by solvents polarities of *Haplophyllum Tuberculatum.* Acetone and methanol extracts of *Haplophyllum Tuberculatum* aerial parts showed better antioxidant activity than essential oils. DPPH scavenging ability of acetone extracts were higher than that of synthetic antioxidant BHT (IC_{50} = 9.98 µg/mL). The highest polyphenol contents were obtained by the acetone and methanol extracts. The aqueous and acetone extract showed the highest condensed tannin and according to 600 mg CE/g.

Keywords: *Haplophyllum Tuberculatum;* Essential oil; Solvent extraction; Antioxidant activities

1. Introduction

In general, the research focused on natural antioxidants, especially originating from plants. Among the various medicinal and culinary plants, some endemic species are of particular interest because they can inhibit the propagation of free radical reactions, protect the human body from diseases (Terao and Piskula, 1997) and retard lipid oxidative rancidity in foods (Duthie, 1993). Plant seeds are important sources of natural antioxidants of nutritional, industrial and pharmaceutical importance. Numerous isolated plant constituents as well as crude extracts of fruits and vegetables have been recognized as natural antioxidants that possess beneficial effects against free radicals in biological systems (Prasad et al. 2005). Actually, essential oils have been studied most from the viewpoint of their flavour and fragrance chemistry only for flavouring foods, drinks and other goods. However, essential oils are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multi-purpose functional use (Ormancey et al. 2001). Many authors, in fact, have reported antimicrobial, antifungal, antioxidant and radical-scavenging properties (Hirasa and Takemasa, 1998) by spices and essential oils and, in some cases, a direct food-related application has been tested (Madsen and Bertelsen, 1995).

Haplophyllum tuberculatum (Forsskal) A. Juss belonging to the family of Rutaceae, is a perennial herb occurring in "wadi" channels on silt deposit (Ghazanfar, 1992). This plant has been used as antispasmodic, to treat allergic rhinitis and gynecological disorders, asthma and breathing difficulties (Mohamed et al. 1996). On the other hand, H. tuberculatum has been used to treat malaria and rheumatoid arthritis (Al-Yahya et al. 1992). This plant showed a large degree of variability in its traditional uses as a function of geographic and ecological location. The essential oil of Haplophyllum tuberculatum is made up largely of monoterpene and sesquiterpene hydrocarbons and has a strong lime scent (Yari et al. 2000). The most abundant oil components identified in Oman are β-phellandrene (23.3%), limonene (12.6%), (Z)-β-ocimene (12.3%), β-caryophyllene (11.6%), myrcene (11.3%), and α-phellandrene (10.9%) (Al-Burtamani et al. 2005). While in Egypt, the major constituents are δ-3-carene (12.6%) cis-p-menth-2-en-1-ol (7%-12.3), trans-p-menth-2-ene-1-ol (9.7%-14.1) and cis-piperitol (5.9%-10.4%). The essential oil from Iran was reported to contain linalool (15.5%), α-pinene (7.9%) and limonene (5.3%) as main constituents. In the United Arab Emirates (Al-Yousuf et al. 2005), α-phellandrene (32.9%), β-caryophyllene (12.8%), β-pinene (7.6-8.0%), limonene (9.6%) and δ-3-carene (6.0%) are reported as the major components. In general, climatic conditions, geographic position of the growth region, agrotechnology of growing, as well as the vegetation stage of plants at the moment

of harvesting and the extraction technique applied, influence both the qualitative composition and contents of the individual components of the isolated essential oils (Soliman et al. 1994). The aim of our study was to investigate the essential oil composition and the antioxidant activity of extracts of different polarity from aerial parts of Haplophyllum Tuberculatum

2. Materials and methods

2.1. Preparation of plant extract

Fresh aerial parts of the *H. tuberculatum* plant were collected during the period of last June (2013) from Benguerdaine area (Sud Est of Tunisia, longitude: 33°07'59"N, latitude: 11°12'58"E, altitude: 57 m). Authentication was performed by Pr. A. Samoui, (Biotechnology Center at the Technopark of Borj-Cedria. The dried powdered aerial parts were successively extracted in a Soxhlet apparatus using solvents of increasing polarities: cyclohexane, chloroform, acetone, methanol and aqueous. Between each step, solvent extracts were filtrated with a Whatman filter paper (N°4) and concentrated under rotary vaccum evaporator at 40 °C. Finally, the crud extracts obtained were stored at 4 °C until further analysis.

2.2. Essential oil extraction

The haplophyllum aerial parts (300 g of dry matter) were hydrodistilled for 3 h using a Clevenger apparatus and the yield of volatile oil was calculated for meal sample. The obtained essential oil was dried over anhydrous sodium sulphate, then stored at 4 °C until it is tested and analysed

2.3. Gas chromatography–flame ionisation detector (GC–FID) analysis

Essential oils were analysed by gas chromatography (GC) using a Hewlett–Packard 6890 apparatus (Agilent Technologies, Palo Alto, CA, USA) equipped with a flame ionisation detector (FID) and an electronic pressure control (EPC) injector. A HP-Innowax capillary column (polyethylene glycol: 30 m x 0.25 mm i.d x·0.25 mm film thickness; Agilent Technologies, Hewlett–Packard, CA, USA) was used; the flow of the carrier gas (N_2, U) was 1.6 ml/min. Analyses were performed using the following temperature programme: oven isotherm at 35 °C during 10 min, from 35 to 205 °C at the rate of 3 °C/min and isotherm at 205 °C during 10 min. Injector and detector temperatures were held, respectively, at 250 and 300 °C. Diluted samples of 2.0 µl were injected in the split/ splitless mode (60:1 split) mode.

2.4. Gas chromatography–mass spectrometry (GC–MS)

The GC–MS analyses were performed on a gas chromatograph HP 5890 (II) interfaced with an HP 5973 mass spectrometer (Agilent Technologies, Palo Alto, California, USA) with electron impact ionisation (70 eV). A HP-5MS capillary column (60 m x 0.25 mm, 0.25 µm film thickness) was used. The column temperature was programmed to rise from 40 to 280 °C at a rate of 5 °C/min. The carrier gas was Helium with a flow rate of 1.2 ml/min. Scan time and mass range were 1 s and 50–550 m/z., respectively. The injected volume was 1 µl and the total run time was approximately 63 min.

2.5. Total phenolic content

Total phenolic contents were assayed using the Folin–Ciocalteu reagent, following Singleton's method slightly modified by Dewanto et al. (2002). The reaction mixture contained 125 µl of diluted extract solution, 125 µl of Folin–Ciocalteu reagent, 1250 µl of 7 % Na2CO3 solution and 1.5 ml of distilled water. After standing for 90 min, the absorbance of resulting bleu color was measured at 760 nm and reported as milligrams of gallic acid equivalents per gram of extract (mg Eq GA/g extract). Quantification of total phenolic was done on the basis of the standard curve of gallic acid (concentration range: 50-200 µg/ml). All samples were performed in triplicates

2.6. Total flavonoid content

Total flavonoid contents were measured according to the method of Dewanto et al. (2002). A total of 250 µl of different extract was mixed with 75 µl NaNO2 (5%). After 6 min, 150 µl of 10% AlCl3 and 500 µl of NaOH (1 M) were added to the mixture. Finally, the mixture was adjusted to 2.5 ml with distilled water. The absorbance versus prepared blank was read at 510 nm. Total flavonoid contents of cakes were expressed as mg catechin equivalents per gram (mg CE/g) through the calibration curve with catechin. The calibration curve range was 50–500 mg/ml. Triplicate measurements were taken for all samples.

2.7. Condensed tannin content

The total tannin content was measured using the modified vanillin assay described by Sun et al. (1998). A total of 3 ml of 4% methanol vanillin solution and 1.5 ml of concentrated H2SO4 were added to 50 µL of suitably diluted sample. The mixture was kept for 15 min, and the absorbance was measured at 500 nm against methanol as a blank. The amount of total condensed tannins was expressed as milligrams of (+)-catechin equivalent per gram of dry weight (mg of CE/g of DW) through the calibration curve with catechin. Triplicate measurements were taken for all samples.

2.8. DPPH assay

The donation capacity of the obtained extracts was measured by bleaching of the purple-coloured solution of the DPPH radical according to the method of Hanato et al. (1998). A total of 1 ml of different concentrations of extracts prepared in 80% acetone was added to 0.5 ml of a 0.2 mmol/l DPPH methanolic solution. The mixture was shaken vigorously and kept at room temperature for 30 min. The absorbance of the resulting solution was then measured at 517 nm after 30 min. The antiradical activity was expressed as IC50 (µg/ml), the concentration required to cause 50% DPPH inhibition. The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = [(A0 - A1) / A0] \times 100$$

where A0 is the absorbance of the control at 30 min, and A1 is the absorbance of the sample at 30 min. BHT was used as a positive control. Tests were carried out in triplicate.

2.9. Reducing power

The method of Oyaizu (1986) was used to assess the reducing power of different extracts. Of 1 ml different concentrations of extracts were mixed with 2.5 ml of a 0.2 M sodium phosphate buffer (pH 6.6, prepared from 62.5 ml of a 0.2 M Na_2HPO_4 and 37.5 ml of 0.2 M $NaH_2PO_4H_2O$) and 2.5 ml of 1% $K3Fe(CN)_6$ and incubated in a water bath at 50°C for 20 min. Then, 2.5 ml of 10% trichloroacetic acid were added to the mixture that was centrifuged at 650 g for 10 min. The supernatant (2.5 ml) was then mixed with 2.5 ml distilled water and 0.5 ml of 0.1% ferric chloride solution. The intensity of the blue–green colour was measured at 700 nm. The EC50 value (mg/ml) is the extract concentration at which the absorbance was 0.5 for the reducing power and was calculated from the graph of absorbance at 700 nm against extract concentration. Ascorbic acid was used as a positive control. Tests were carried out in triplicate.

2.10. Statistical analysis

All extractions and determinations were conducted in triplicate. Data are expressed as mean (standard deviation (SD). The means were compared by using the one way followed by Duncan's multiple range tests. Individual means were deemed to be significant at $p < 0.05$. A Cluster Analysis (CA) was performed in order to discriminate between different nozzle diameters on the basis of their essential oil composition. All analyses were performed by the 'Statistica v 5.1' software (Stasoft, 1998).

3. Results and discussion

3.1. Essential oil composition

The essential oil yield in haplophyllum aerial parts was 0.24% on the basis of their dry matter weight. Our results were thus in accordance with those of Al-Burtamani et al. (2005) who found that the essential oil yield of haplophyllum from Oman was 0.21%. Moreover, Yari and Masoudi (2000) reported that the essential oil content of Iran was 0.35%. In the same context, El-Naggar et al. (2014) studied the aerial parts essential oil of different locations grow of Egypt and found that the yield varied from 0.31% to 0.65%. This variation in essential oil yield can be explained by the major effects of geographical and ecological variations among habitats. Additionally, the variations in oil yield can be attributed to genetic, maturity stage and environmental factors, ontogeny and analytical methods.

The results obtained by GC–MS analysis showing the essential oil composition of the haplophyllum aerial parts were summarized in Table 1. Thirty-two compounds of the total essential oil constituents were identified. The major components of the essential oil of Haplophyllum tuberculatum are: trans-p-menth-2-ene-1-ol (18.23%), β-phellandrene (14.46%), piperitone (14.33%), cis-p-menth-2-ene-1-ol (13.66%), α-phellandrene (4.36%), β-myrcene (3.48%), n-octyl acetate (3.47%), trans-4-methoxy

thujane (3.98%), α-pinene (3.06%) and bornyl acetate (2.20%). A small amount of δ3-carene, α-terpinene, β-pinene and camphene were detected, with percentages of 1%. According to De Sousa et al. (2011), the trans-p-menth-2-ene-1-ol and cis-p-menth-2-ene-1-ol may be contributed to their potential insecticidal activities against three Anopheles gambiae spp of mosquitoes where inducing 100% of mortality to both sensitive and resistant Anopheles gambiae. Indeed, this synergistic effect has increased the medicinal importance of this group in controlling vectors of the malaria and may favors the medicinal use of this group as a source of natural biocides. In the other hand, β-phellandrene, myrcene and α-Phellandrene have shown in vitro activity against Bacillus sp, Candida albicans, Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus (Costa et al. 2000). The chemical composition of H. tuberculatum aerial parts showed the main differences where compared from different region and habitats. In Egypt, the essential oil composition showed the existence of two well defined groups (El-Naggar et al. 2014). The first one was represented by the cis-p-menth-2-en-1-ol (7%-12.3%), trans-p-menth-2-ene-1-ol (9.7%-14.1%) and cis-piperitol (5.9%-10.4%) as the major constituents. The second group was formed by the δ-3-carene (12.6%) which constitutes the main component of essential oil of H. Tuberculatum. However, Javidnia et al. (2006) reported that the major compounds in the essential oil of H. Tuberculatum in Iran were: linalool (15.5%), α-pinene (7.9%) and limonene (5.3%). While, in Oman some other considerable chemical variability of other β-phellandrene (23.3%), limonene (12.6%), (Z)-β-ocimene (12.3%), β-caryophyllene (11.6%) and α-phellandrene (10.9%) (Al-Burtamani et al. 2005). In the United Arab Emirates (Al-Yousuf et al. 2005), α -phellandrene (32.9%), β-caryophyllene (12.8%), β -pinene (7.6-8.0%), limonene (9.6%) and delta-3-carene (6.0%) are reported as the chief components. The oil of Haplophyllum tuberculatum is made up largely of oxygenated monoterpene (54.77%) and monoterpene hydrocarbons (33.25 %) and has a strong lime scent (Table 1). In general, oxygenated monoterpenes were identified as also the most abundant chemical of essential oil collected in Egypt (El-Naggar et al. 2014). The dominance of oxygenated monoterpene can be contributed to high percentage of trans-p-Menth-2-ene-1-ol (18.23%). According to Ruberto and Baratta (2000), monoterpenes hydrocarbons present antioxidant activity due to the presence of strongly activated methylene groups. However, Monoterpenes and sesquiterpenes have been shown to be the major components of the essential oil of Haplophyllum tuberculatum collected in Iran (Yari et al. 2000).

3.2. Antioxidant activities of Haplophyllum Tuberculatum aerial parts extracts

The stable DPPH radical is widely used to evaluate the free radical scavenging activity in many plant extracts (Williams-Brand et al. 1995). Solvent type had significant effect on the DPPH• scavenging activity of haplophyllum aerial parts (Table 2). Acetone and methanol extracts showed the highest antioxidant activity with IC50 values reaching 64 and 87 µg/ml, respectively. The lowest antiradical capacity was found in aqueous extracts and essential oil of H. tuberculatum aerial parts (IC50 values were 800 and 870 µg/ml, respectivly). Chloroform extract did not show DPPH radical scavenging activity. This variation in antioxidant potential according to the solvents used can be explained by the difference in polarity, and thus different extractability, of the antioxidative compounds (Maisuthisakul et al. 2007). Several studies showed that solvent polarity leads to significantly different extraction capacities for phenolic compounds in plants (Galvez et al. 2005). Wei and Shibamoto (2007) improved the presence of a significant antioxidant potential of essential oils rich in monoterpene hydrocarbons. In the case of Melissa officinalis L. essential oil, monoterpene aldehydes, monoterpene ketones and sesquiterpene hydrocarbons (were responsible of the DPPH neutralization (Mimica-Dukic et al. 2004). The effect of solvent on the antioxidant ability of H. Tubercalutum aerial parts was also assessed by the estimation of reducing powers (Table 2). Chloroform and acetone extracts of haplophyllum exhibited higher reducing power towards the Fe3+/ferricyanide complex (EC50 values ranged from 35 to 38 µg/ml). Methanol extracts of aerial parts showed also higher reducing capacity (EC50 = 68.33 µg/ml) compared to that of the essential oil (EC50 = 105 µg/ml). cyclohexane and aqueous extracts (EC50 = 121 and 170 µg/ml, respectively). The reducing power of a bioactive compound may also serve as a significant indicator of its potential antioxidant activity (Roginsky and Lissi, 2005). It is well known that the solvent polarity and the type of antioxidant substance influence the antioxidant capacity of the extract (Naczk and Shahidi, 2004). The reducing property is generally associated with the presence of reductones (Duh, 1998), such as ascorbic acid, which have been shown to exert antioxidant action by breaking the free radical chain (Gordon, 1990).

Table 1. Essential oil composition of *Haplophyllum Tuberculum* aerial parts.

Compound*	**IR[a]**	**IR[b]**	**%**	**Identification**
α-pinene	939	1032	3.06±0.03	GC–MS, Co-GC
Camphene	954	1076	1.01±0.01	GC–MS
Sabinene	976	1132	0.43±0.01	GC–MS
β-pinene	980	1118	1.33±0.00	GC–MS, Co-GC
β-Myrcene	991	1174	3.48±0.01	GC–MS
β-cis-Ocimene	995	1240	0.23±0.01	GC–MS
α-Phellandrene	999	1167	4.36±0.02	GC–MS
δ-^{3}-Carene	1011	1159	1.30±0.01	GC–MS
α-Terpinene	1018	1188	1.30±0.01	GC–MS, Co-GC
β-Phellandrene	1023	1218	14.46±1.21	GC–MS
p-Cymene	1026	1280	0.92±0.02	GC–MS, Co-GC
Butanoic acid 3-methyl, 3-methyl butyl ester	1048	1256	0.84±0.01	GC–MS
α-cis-Ocimene	1050	-	0.42±0.01	GC–MS
D-Limonene	1053	1209	0.39±0.01	GC–MS, Co-GC
cis-4-methoxy thujane	1088	-	1.26±0.02	GC–MS
Linalol	1098	1553	0.83±0.02	GC–MS, Co-GC
***cis*-p-Menth-2-ene-1-ol**	1108	1558	13.66±1.87	GC–MS
trans-4-methoxy thujane	1110	-	2.98±0.11	GC–MS
***trans*-p-Menth-2-ene-1-ol**	1126	1622	18.23±1.34	GC–MS
n-Octyl acetate	1149	1240	3.47±0.04	GC–MS
cis-Piperitol	1180	1674	0.08±0.01	GC–MS
trans-Piperitol	1191	1741	0.04±0.01	GC–MS
Piperitone	1216	1673	14.33±1.02	GC–MS
Bornyl acetate	1270	1571	2.20±0.02	GC–MS, Co-GC
n-Nonanyl acetate	1292	1581	0.23±0.01	GC–MS
Octyl isovalerate	1301	-	0.52±0.01	GC–MS
Neryl acetate	1362	1742	0.35±0.01	GC–MS, Co-GC
Valencene	1490	1726	0.33±0.01	GC–MS
α-Muurolene	1523	1714	0.21±0.01	GC–MS
Isovaleric acid	-	1660	0.48±0.01	GC–MS
Butanoic acid 2-methyl, 2-methyl butyl ester	-	1664	0.51±0.01	GC–MS
n-Amyl isovalerate	-	-	0.94±0.03	GC–MS
Monoterpene hydrocarbons (%)		33.25		
Oxygenated monoterpenes (%)		54.77		
Sesquiterpene hydrocarbons (%)		0.54		
Other (%)		6.18		
Total identified (%)		94.74		

** Order of elution in HP-5 column. a Apolar HP-5 column. b Polar HP Innowax column. GC-MS, gas chromatography-mass spectrometry.*

Table 2. Antioxidant activity of *Haplophyllum Tuberculatum* aerial parts.

Solvents extracts	DPPH (IC_{50}, µg/mL)	Reducing power (EC_{50}, µg/mL)
Chloroform	NA	35±1.96^{d}
Acetone	64±0.23^{d}	38.5±0.57^{d}
Methanol	87±0.54^{c}	68.33±2.61^{c}
Aqueous	800±2.21^{b}	170±2.33^{a}
Essential oil	870±1.77^{a}	105±0.77^{b}
Synthetic antioxidant		
BHT	9.98±0.33^{e}	-
Vitamine C	-	1.52±0.09^{e}

NA indicates no antioxidant activity. IC_{50} value: the effective concentration at which the antioxidant activity was 50%. The absorbance was 0.5 for reducing power, the EC_{50} value was obtained by interpolation from linear regression analysis. Each value is expressed as mean SD (n=3). Means with different capital letter within a row are significantly different (P< 0.05).

3.1. Total phenolic, total flavonoid and total tannin contents

Total phenol contents of H. Tuberculatum aerial parts using different extraction solvents, are presented in Table 3. The results showed that the acetone and methanol extracts have significantly higher contents of total polyphenol with 4.77 and 4.22 mg GAE/g extract, respectivly. However, the lowest contents were obtained in the cyclohexane extract. To our knowledge, total phenolic of haplophyllum aerial parts has not been reported previously. Our results were in agreement with previous studies and confirmed the effect of the used solvent on the total polyphenol contents (Edziri et al. 2012). In this context, El-Ghorab et al. (2010) reported that the highest total phenolic contents were found in the methanolic extract whereas the hexane extract showed the lowest one. Indeed, Liu et al (2007) have already mentioned the importance of the solvent type used in extraction. For example, methanol extract of buckwheat gave a higher yield than the ethanol extract although both extracts showed similar total phenol contents (Sun and Ho, 2005). So, because the various polarities characterizing the different phenolic components, no single solvent may be used to extract them quantitatively and qualitatively. It has been reported that the extraction solvents significantly affected the polyphenol content of the extract Sun and Ho. (2005). In this study, we evaluated the flavonoid contents of haplophyllum aerial parts, which constitute the one major group of polyphenolic compounds and occurs widely in plant foods, such as fruits, vegetables, cereals and beverages (Cook and Samman, 1996). The contents of total flavonoid in sample extracts exhibited also significant ($p<0.001$) variation depending on the used solvent (Table 3). The highest content of flavonoid was found with cylohexane extracts (10.98 mg CE/g extract), followed by acetone (7.90 mg CE/g extract), choloroform (6.54 mg CE/g extract) and aqueous extracts (4.28 mg CE/g extract). The lowest level of flavonoids was detected in the methanol extracts (2.76 mg CE/g extract). Our results were in contrast by Liu et al. (2007) who found that phenolic and flavonoid contents of endophytic Xylaria sp. were higher in methanol extracts than in hexane ones. Other authors have been reported that ethyl acetate is more efficient for recovering the higher amounts of flavonoids (Jayaprakasha et al. 2008).

Depending on the solvent used, the amount of condensed tannin extracted from haplophyllum aerial parts showed the highest level obtained by aqueous and acetone extracts (611.80 and 603.58 mg CE/g extract, respectively) (Table 3). As tannins can precipitate heavy metals and alkaloids (except morphine), they can be used in poisonings with these substances. It is also becoming clear that tannins often are the active principles of plant-based medicines. The lowest content of condensed tannins was enregistered in chloroform extract (145.08 mg CE/g extract). It is well known that an important function of tannins is their action in plant protection against infection, insects, or animal herbivory (Porter, 1989). Indeed, tannins have used as astringents, against diarrhoea (Yoshida et al. 1991), as diuretics (Hatano et al. 1991) against stomach and duodenal tumours (Saijo et al. 1989) and exhibiting antiinflammatory, antiseptic, and haemostatic pharmaceuticals. Therefore, it is assumed that the biological role in the plant

of many tannins is related to protection against infection, insects, or animal herbivory. The tannins appear as light yellow or white amorphous powders or shiny, nearly colourless, loose masses, with a characteristic strange smell and astringent taste. On the other hand, cluster analysis (CA) was carried out in order to determine the relationship between the different extracts (Figure 1). Obtained results showed the existence of two well-defined groups. Regarding the classification of the extracts, the methanol and chloroform, as a primary group, were discriminated in the acetone group were characterized by the highest antioxidant and related with their higher contents of total phenolic compounds and total flavonoid. (Figure 1). Cyclohexane and aqueous, which were found as second group, were characterized by the moderate polyphenols content, presented the highest contents of total flavonoid and condensed tannin.

Table 3. Total polyphenol, flavonoid, condensed and total tannin contents of different extraction solvents of *Haplophyllum Tuberculatum.*

Solvents	Total Polyphenols (mg GAE/g extract)	Flavonoïdes (mg CE/g extract)	Condensed tannin (mg CE/g extract)	Total tannin (mg GAE/g extract)
Cyclohexane	0.94±0.01^{d}	10.98±0.07^{a}	405.81±0.04^{b}	3.63±0.02^{d}
Chloroform	1.39±0.01^{c}	6.54±0.08^{b}	145.08±0.04^{d}	8.98±0.05^{c}
Acetone	4.77±0.01^{a}	7.90±0.03^{b}	603.58±0.09^{a}	16.16±0.02^{a}
Methanol	4.22±0.02^{a}	2.76±0.04^{d}	295.17±0.03^{c}	14.11±0.01^{b}
Aqueous	3.06±0.01^{b}	4.28±0.01^{c}	611.80±0.11^{a}	7.85±0.02^{c}

Total polyphenol and total tannin were expressed by mg GAE/g DW and total flavonoid, condensed total expressed as mg CE/g extract) were expressed by mg CE/g DW. Values are represented as mean ± standard deviation of triplicates.

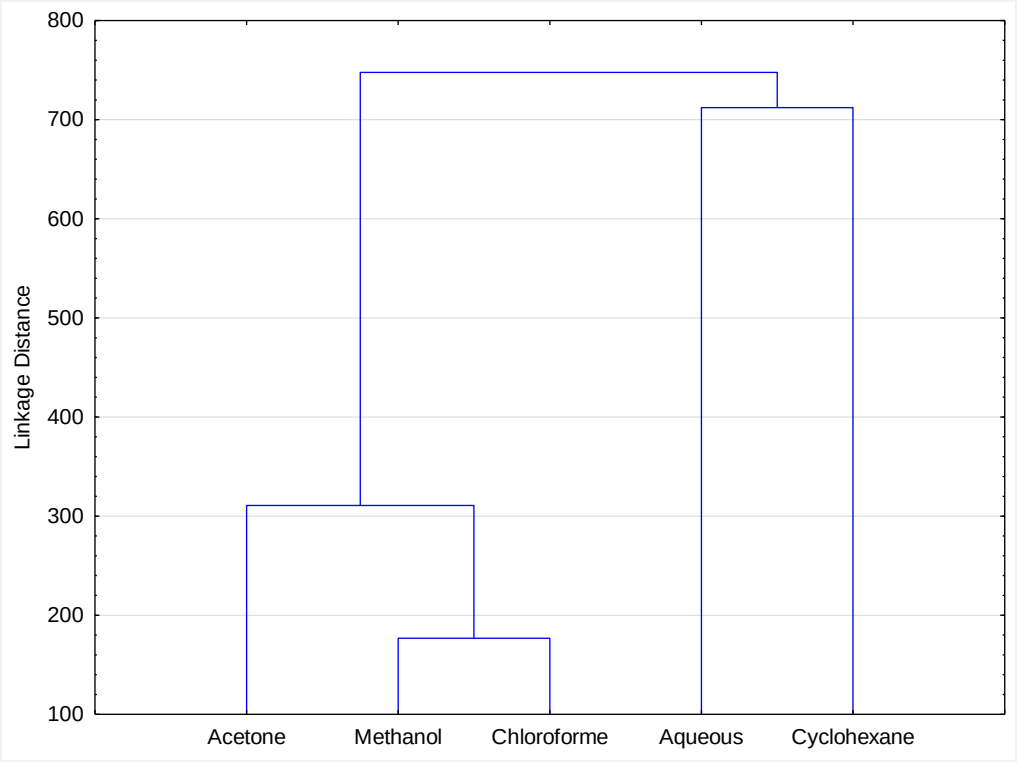

Figure 1. Dendrogram obtained with cluster analysis of extracting solvent used including cyclohexane, chloroform, acetone, methanol and aqueous

4. Conclusion

From the present study, it can be concluded that the solvents polarities affects significantly the phenolic content and the antioxidant activities. Statistical analysis showed that acetone and methanol extracts have significantly higher contents of total polyphenol.

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