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Antioxidant Potential and Phytochemical Profile of Althaea (Hatmi) and Hibiscus Flower Extracts: A Comprehensive Analysis

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Keywords
Althaea officinalis L., *Hibiscus sabdariffa*, Phytochemicals, Phenolic compound, Extraction, Chromatographic methods

Abstract: In this study, the bioactive components and antioxidant properties of Althaea (Hatmi) and Hibiscus plants were assessed using various methods. Both aqueous and ethanol extracts of these plants yielded distinct and effective results. Antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and Ferric Reducing Antioxidant Power (FRAP) assay at concentrations of 25, 50, 75, and 100 mg/mL. Hatmi extracts, both ethanol and aqueous, exhibited high DPPH activity, particularly at 75 and 100 mg/mL, while Hibiscus showed a linear increase in DPPH activity with concentration, reaching 2000 µM Trolox Equivalent (TE) /g dry weight (DW) at 100 mg/mL. In ABTS assays, lower concentrations of ethanol extracts were more effective, but higher aqueous concentrations showed greater activity. FRAP results indicated high antioxidant activity in Hatmi ethanol extracts, with activity reaching 2700 µM TE/g DW at higher concentrations. Phenolic analysis revealed high levels of apigenin 7-glucoside, hesperidin, and caffeic acid in Hatmi, while Hibiscus extracts contained significant amounts of chlorogenic acid and quercetin. Gas Chromatography-Mass Spectrometry (GC-MS) analysis showed that Hatmi had a higher abundance of volatile organic compounds compared to Hibiscus.

Althaea (Hatmi) ve Hibiscus Çiçek Ekstrelerinin Antioksidan Potansiyeli ve Fitokimyasal Profili: Kapsamlı Bir Analiz

Anahtar Kelimeler
Althaea officinalis L., *Hibiscus sabdariffa*, Fitokimyasal bileşenler, Fenolik bileşik, Ekstraksiyon, Kromatografik yöntemler

Öz: Bu çalışmada, Althaea (Hatmi) ve Hibiscus bitkilerinin biyoaktif bileşenleri ile antioksidan özellikleri çeşitli yöntemlerle değerlendirildi. Her iki bitkinin sulu ve etanol ekstrelerinden farklı ve etkili sonuçlar elde edildi. Antioksidan aktiviteler, 25, 50, 75 ve 100 mg/mL konsantrasyonlarında 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) ve Ferric Reducing Antioxidant Power (FRAP) testleri kullanılarak ölçüldü. Hatmi ekstreleri, hem etanol hem de sulu ekstraktlarda, özellikle 75 ve 100 mg/mL konsantrasyonlarında yüksek DPPH aktivitesi gösterdi. Hibiscus ekstrelerinde ise DPPH aktivitesi, konsantrasyon arttıkça doğrusal bir şekilde yükseldi ve 100 mg/mL'de 2000 µM Trolox Eşdeğeri (TE) /g kuru ağırlık (DW) seviyesine ulaştı. ABTS testlerinde, düşük konsantrasyonlardaki etanol düşük konsantrasyonlarda bile etki gösterirken (25, 50 mg/mL), sulu ekstraksiyonlarının yüksek konsantrasyonları (75, 100 mg/mL) daha fazla aktivite gösterdi. FRAP testlerinde, Hatmi etanol ekstrelerinde yüksek antioksidan aktivite gözlemlendi ve yüksek konsantrasyonlarda 2700 µM TE/g DW seviyelerine ulaşıldı. Hatmi çiçeklerinde yüksek miktarda apigenin 7-glukozid, hesperidin ve kafeik asit gibi fenolikler elde edilirken, Hibiscus ekstrelerinde ise önemli miktarda klorojenik asit ve kuersetin fenolik bileşenleri elde edildi. Gaz Kromatografisi-Kütle Spektrometrisi (GC-MS) analizi, Hatmi çiçeklerinin uçucu organik bileşen çeşitliliği ve miktarı açısından Hibiscus çiçeklerinden daha zengin olduğunu göstermiştir.

1. INTRODUCTION

Consumers are becoming increasingly health-conscious and prefer foods with high nutritional value [1,2]. Many consumers prefer products derived from natural sources over those containing synthetic chemicals due to potential negative health impacts [2,3]. Therefore, many researchers have focused on the potential benefits and importance of wild medicinal plants for food and human health, showing growing interest in this field [2–5]. Some studies have indicated that a quarter of the world's medicines and drugs are produced from medicinal plants [6,7]. Research on the chemical and pharmacological aspects of wild plants has played a significant role in increasing the use of medicinal plants by revealing the presence of bioactive compounds and their beneficial effects on human and animal health systems[8–10].

The Malvaceae family is represented worldwide by over 80 genera and more than 1000 species. Most commonly found in South America, members of this family are present nearly everywhere except for the coldest regions of the world [11,12]. The plants of this family are herbs or shrubs, usually with stellate hairs. The Malvaceae family has medicinal uses thanks to mucilage, fixed oils and essential oils. Some of the most commonly used species in folk medicine are as follows: Althaea officinalis, Malva sylvestris, Alcea biennis, Abelmoschus esculentus, Hibiscus [13–17].

Figure 1. *Althaea officinalis L.*

Althaea officinalis Linn (AO), known as marshmallow (Hatmi in Türkiye), is a hairy herb, annual and perennial plant belonging to the family Malvaceae (Figure 1) [18]. AO parts have been traditionally utilized in the treatment of various ailments such as coughs, colds, stomach ulcers, kidney stones, enteritis, and mucous membrane irritation [19,20]. Multiple studies have indicated the diverse therapeutic properties of AO extracts, including antitussive, anti-inflammatory, anti-estrogenic, antimicrobial, immunomodulatory, and antioxidant effects [19–22] Analytical investigations have revealed the predominant composition of AO, which comprises starch (25%-35%), pectin (11%), sucrose (10%), mucilage (5%), and saccharides [18–21,23]. Despite its extensive traditional use and therapeutic potential, the chemical profile of AO remains relatively understudied, with only 46 compounds identified thus far, encompassing 17 flavonoids, 3 coumarins, 1 steroid, 1 triterpenoid, and 24 other miscellaneous compounds [24].

Figure 2. *Hibiscus sabdariffa*

Hibiscus sabdariffa (HS) is a tropical shrub belonging to the Malvaceae family with red or green edible calyxes (Figure 2). These parts are rich in protein, calcium, niacin, riboflavin, iron, phenols, amino acids, carotene, and vitamin C [25]. Due to its high content of polyphenolic acid, triterpenoids, polysaccharides, organic acids (citric, malic acids, etc. , and flavonoids, HS is widely known as a medicinal plant in tropical countries [26]. The calyces contain high levels of anthocyanins, such as delphinidin-3-sambubioside, cyanidin-3-sambubioside, cyanidin-3-glucoside, and delphinidin-3-glucoside, which make them a promising natural colorant for various food industrial purposes, including the production of juices, wines, and carbonated soft drinks [27]. Additionally, studies have shown that beers supplemented with Hibiscus sabdariffa or sea buckthorn have higher levels of bioactive compounds and antioxidant activity compared to control samples [28,29].

The purpose of this study is to compare the phytochemical compositions and antioxidant properties of Hibiscus sabdariffa (HS) and Althaea officinalis Linn (Hatmi), emphasizing their medicinal and industrial potentials. Both plants have significant potential in these areas, but they exhibit important differences. HS is notable for its nutrient-rich calyces and potential as a natural colorant, while AO is recognized for its versatile therapeutic properties. This review underscores the necessity for further research into the chemical constituents and pharmacological effects of HS and AO, offering insights that could lead to expanding applications in both medicine and industry.

2. MATERIAL and METHOD

2.1. Extraction methods

Dried *Hibiscus sabdariffa* (Hibiscus) and *Althaea officinalis L.* (Hatmi) flowers were obtained from a local market in Manisa, Türkiye (**Figure 3**). A sample of 1 gram was taken, and 40 mL of ethanol (100%) was added. Extraction was carried out using Ultra-turrax (IKA T25, Staufen, Germany) at 5000×g for 3 min at room temperature for 30 min. The resulting extract solution was filtered and stored in amber glass bottles at +4 ^{0}C until further analysis. In the experiments, the abbreviations used are as follows: HA: Althaea ethanol extract, HA AQ: Althaea aqueous extract, HIBIS ETOH: Hibiscus ethanol extract, and HIBIS AQ: Hibiscus aqueous extract.

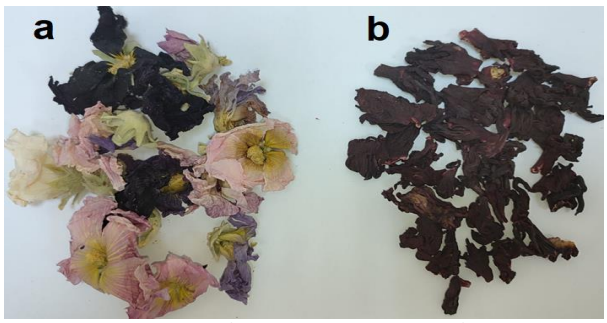

Figure 3. a) Dried Hatmi flowers, b) Dried Hibiscus flowers.

2.2. Antioxidant activity assays

The FRAP analysis was performed according to the following procedure with some modifications [30]. The stock solutions included 300 mM acetate buffer (3.1 g C2H3NaO2.3H2O and 16 mL C2H4O2), pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM FeCl3.6H2O solution. The fresh working solution mix was prepared as follows: 25 mL acetate buffer, 2.5 mL TPTZ solution, and 2.5 mL FeCl3.6H2O solution and then warmed at 37 0C before use. Leaves extracts (150 µL) were allowed to react with 2850 µL of the FRAP solution for 30 min in a dark condition. Then, absorbance was taken at 593 nm using the spectrophotometer (TECAN, Männedorf, Switzerland). The standard curve was linear between 25 and 600 mM Trolox. Results were expressed in mM Trolox equivalents (TE)/g dry mass (DM).

The DPPH analysis was performed according to the following procedure with minor modifications [31]. The stock solution was freshly prepared by dissolving 24 mg of DPPH in 100 mL of methanol, and then 10 mL of this solution was taken and diluted with 45 mL of methanol. Leaves extracts (150 µL) were allowed to react with 2850 µL of the DPPH solution for 2 h in a dark condition. Then, absorbance was taken at 515 nm using the spectrophotometer (TECAN, Männedorf, Switzerland). The standard curve was linear between 25 and 800 mM Trolox. Results are expressed in mM Trolox equivalents (TE)/g dry mass. In all measurements, additional dilution was needed if the analysis value measured was over the linear range of the standard curve.

For ABTS assay of leaf extracts was performed according to the following method with some modifications [32]. A stock solution containing 7.4 mM ABTS and 2.6 mM potassium persulfate was prepared. The prepared stock solution was kept at room temperature for 12 h and then 1 mL was taken and diluted with 60 mL of methanol before the analysis. Leaves extracts (150 µL) were allowed to react with 2850 µL of the ABTS solution for 2 h in a dark condition. Then, absorbance was taken at 734 nm using the spectrophotometer (TECAN, Männedorf, Switzerland). The standard curve was linear between 25 and 600 mM Trolox. Results were expressed in mM Trolox equivalents (TE)/g dry mass).

2.3. Determination of phenolic compounds by LC-MS/MS

Determination of phenolic profiles of leaves extracts, high-performance liquid chromatography-mass spectrometer - mass spectrometer (Agilent 1260 Triple Quadrupole MS/MS) were used. Each analysis was performed with three replications. HPLC column C18 ODS used in the analyses (25x4.6 mmx5 µm) was used. Injection volume for analysis: 2 µL. Water/0.1% formic acid (A), and methyl alcohol (99.9%) (B) were used as a carrier phase. The gradient method is as follows: 3 min 2% B, 6 min 25% B, 10 min 50% B, 14 min 95% B, 17.5 min 2% B. Flow rate: 0.4 mL/min. The identification of compounds was performed in positive and negative modes [33].

2.4. Determination of volatile organic molecules by GC-MS

Volatile molecules in the extract were qualitatively analyzed in electron ionization (EI) mode with Agilent Technology 7890A Gas Chromatography (GC) Mass spectrometer (MS). Chromatographic column Agilent HP-5 MS, capillary column (30 m x 0.25 mm, the film thickness of 0.25 mm). The furnace temperature was started at 40°C, followed by standing for 5 min, then at 5°C min-1 at 280°C and held for 5 min. Helium gas (99.999%) was used as the carrier gas. The constant flow rate is 1.5 mL min-1 and the injector temperature is 250°C. The extract was injected in splitless mode with 1.0 mL. Interpretation of the mass spectrum was performed according to the National Institute of Standards and Technology (NIST) database.

3. RESULTS and DISCUSSION

3.1. Antioxidant activity results

Antioxidant activity was evaluated using DPPH, ABTS, and FRAP parameters. The ethanol and aqueous extracts of Hatmi and Hibiscus plants were compared at concentrations of 25, 50, 75, and 100 mg/mL. In the DPPH activity assays, both the ethanol and aqueous extracts of Hatmi flowers exhibited high levels of activity, particularly at concentrations of 75 and 100 mg/mL. In contrast, for the Hibiscus flowers, a linear increase in DPPH activity was observed with increasing concentrations in both extracts. At a concentration of 100 mg/mL, the activity reached up to 2000 µM TE/g DW. Consequently, it was determined that Hibiscus flowers provided a more significant response in DPPH activity (Figure 4a). In ABTS assays, low-dose ethanol extractions of Hatmi and Hibiscus plants have proven more effective (25, 50 mg/mL). Antioxidants are essential for maintaining overall health because they protect the body from oxidative stress, which is caused by free radicals. Free radicals are unstable molecules that can damage cells, proteins, and DNA, potentially leading to aging and various diseases, including cancer, heart disease, and neurodegenerative disorders [34-36].

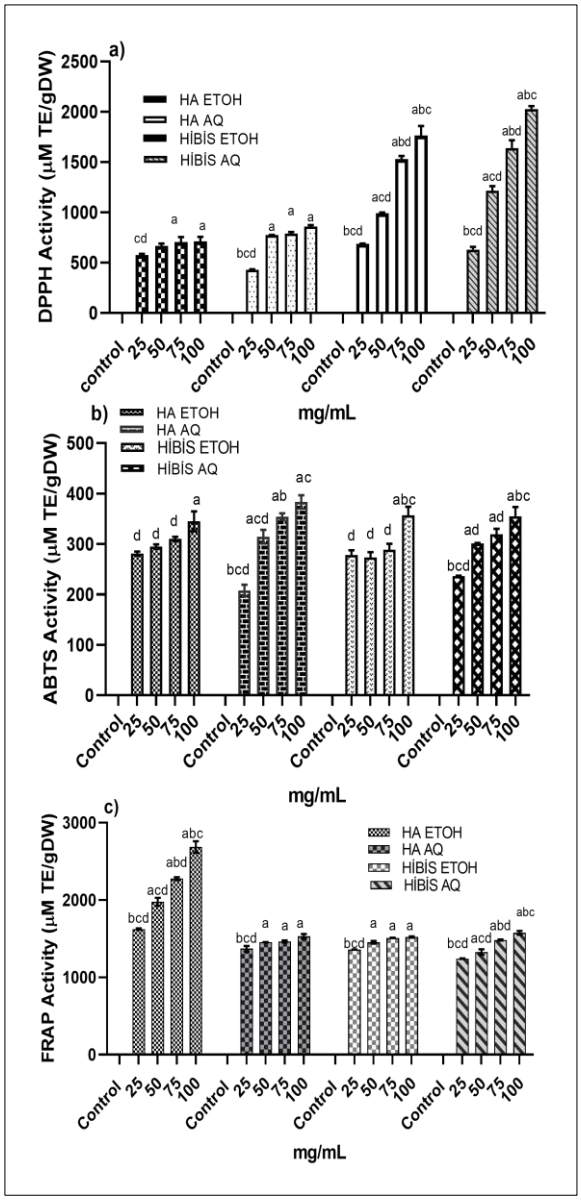

Figure 4. a) DPPH activity, b) ABTS activity, c) FRAP activity results of extracts. a: compared to 25 mg/mL, b: compared to 50 mg/mL, c: compared to 75 mg/mL, d: compared to 100 mg/mL. P<0.05.

The ABTS activity results at these concentrations range between 220 and 280 µM TE/g DW. However, in the aqueous extractions of both plants, higher concentrations demonstrated even greater activity (75, 100 mg/mL). At these concentrations, the ABTS activity levels reach the range of 350-400 µM TE/g DW (Figure 4b). According to the FRAP results, the ethanol extract of Hatmi flower exhibited high antioxidant activity. Specifically, at concentrations of 75 and 100 mg/mL, activity results of 2400 and 2700 µM TE/g DW were obtained, respectively. Additionally, the aqueous extract of the Hibiscus flower also demonstrated significant antioxidant activity. It was observed that as the concentration increased, the FRAP activity also showed a linear and significant increase (Figure 4c). Farhat et al. found that the capacity of Althaea officinalis water extract to scavenge DPPH free radicals increased with higher concentrations, achieving a strong antioxidant activity of 75% at 6.0 mg/mL [37]. The findings of Farhat et al. show a significant similarity to our study. Their research indicates that the capacity of plant extracts to scavenge free radicals increases with higher concentrations. This aligns with our results, which also demonstrate that higher concentrations tend to enhance antioxidant activity. In particular, the ethanol extract of the Hatmi flower exhibits strong antioxidant activity, paralleling the findings of Farhat et al. Similarly, the increase in activity observed in the aqueous extract of the Hibiscus flower highlights the critical impact of concentration on antioxidant effectiveness. These findings suggest that concentration plays a crucial role in enhancing the antioxidant potential of plant sources [37] .

3.2. Phenolic compounds results by LCMSMS

Table 1. Phenolic compounds of Hatmi plant extracts.

Compound Name	HA ETOH (µg/g)	HA AQ (µg/g)
(-)-Epicatechin	0.680±0.03	0.658±0.08
(+)-Catechin	ns	0.549±0.054
2.5-Dihydroxybenzoic acid	1.535±0.10	4.309±.005
3,4-Dihydroxyphenylacetic acid	0.194±0.01	0.237±0.163
3-Hydroxybenzoic acid	9.420±0.28	29.490±1.07
3-hydroxytyrosol	0.708±0.01	1.409±0.037
4-Hydroxybenzoic acid	8.729±0.21	25.995±0.12
Apigenin 7-glucoside	884.195±2.8	30.005±1.53
Apigenin	53.522±0.11	75.292±1.81
Caffeic acid	34.211±0.02	85.764±4.38
Chlorogenic acid	0.912±0.07	11.935±0.10
Eriodictyol	0.671±0.06	3.207±0.04
Ferulic acid	16.254±0.32	80.174±1.34
Gallic acid	1.144±0.01	6.242±0.39
Hesperidin	200.592±5.04	199.320±6.51
Hyperoside	0.402±2.544	113.594±1.27
Kaempferol	29.354±0.05	26.310±1.04
Luteolin	1.635±0.202	2.837±0.134
oleuropein	0.043±0.002	0.032±0.001
p-Coumaric acid	14.983±0.280	61.565±0.54
Pinoresinol	0.375±0.100	131.416±12.6
Protocatechuic acid	1.502±0.045	4.211±0.02
Pyrocatechol	0.611±1.31	0.168±0.05
Quercetin	2.90±0.10	7.849±0.52
Rosmarinic acid	0.073±0.008	0.010±0.001
Sinapic acid	0.438±0.04	2.387±0.16
Syringic acid	4.804±0.14	13.709±0.93
Taxifolin	12.417±0.07	264.049±15.92
Verbascoside	0.077±0.07	0.820±0.155

According to the results obtained from the analysis of phenolic components, different compounds were obtained from the ethanol and aqueous extractions of Hatmi flowers. Specifically, apigenin 7-glucoside was found in high amounts in the ethanol extract at 884.19 µg/g.

Subsequently, hesperidin (200.592 µg/g), apigenin (53,52 µg/g), and caffeic acid (34.21 µg/g) were determined to be present in high amounts. In the aqueous extracts of Hatmi flowers, phenolic components such as hesperidin (199.32 µg/g), pinoresinol (131.41 µg/g), and taxifolin (264.04 µg/g) were found in high amounts (Table 1). Farhat et al. identified the following phytochemicals in the extraction of the Athena plant using LC-MS analysis: 5 phenolic acids (syringic acid, gallic acid, caffeic acid, p-coumaric acid, and trans-ferulic acid) and 8 flavonoids (catechin, apigenin, chrysin, quercetin, kaempferol, genistein, rutin trihydrate, and galangin) [37]. The anti-cancer effects observed in the water extract can be attributed to its major constituents, including polysaccharides, flavonoids, phenolic acids, and coumarins [38,39]. Especially, Quercetin exhibits strong antioxidant and anti-inflammatory properties that are closely related to the prevention and treatment of cardiovascular diseases and cancer [40].

Table 2. Phenolic compounds of Hibiscus plant extracts.

Compound Name	HİB ETOH (µg/g)	HİB AQ (µg/g)
2,5-Dihydroxybenzoic acid	8.01±0.31	27.39±0.71
3,4-Dihydroxyphenylacetic acid	0.11±0.03	0.01±0.002
3-Hydroxybenzoic acid	1.20±0.16	3.54±0.21
3-hydroxytyrosol	0.07±0.001	0.11±0.01
4-Hydroxybenzoic acid	0.94±0.01	2.80±0.26
Apigenin 7-glucoside	0.32±0.016	ns
Apigenin	1.49±0.012	ns
Caffeic acid	18.64±0.08	28.41±0.30
Chlorogenic acid	452.50±4.14	942.34±6.06
Ferulic acid	2.77±0.10	3.75±0.16
Gallic acid	13.29±0.12	203.45±3.12
Hesperidin	32.18±0.31	8.09±0.30
Hyperoside	24.53±0.45	56.96±0.04
Kaempferol	6.51±0.19	1.94±0.36
p-Coumaric acid	1.92±0.13	3.51±0.18
Pinoresinol	0.80±0.02	24.18±0.38
Protocatechuic acid	8.12±0.08	28.63±0.06
Pyrocatechol	5.81±0.27	25.53±0.22
Quercetin	69.72±0.06	49.69±0.15
Sinapic acid	3.52±0.08	4.79±0.11
Syringic acid	25.11±0.53	38.09±0.65
Taxifolin	0.55±0.07	0.74±0.02
Verbascoside	0.01±0.001	0.23±0.01

In the ethanol and aqueous extracts of hibiscus flowers, common phenolic compounds such as chlorogenic acid, quercetin, syringic acid, caffeic acid, and hyperoside were obtained in high amounts. However, some were found predominantly in ethanol extracts, while others were more abundant in aqueous extracts. For instance, chlorogenic acid (942.34 µg/g), hyperoside (56.96 µg/g), and caffeic (28.41 µg/g) acid were found in higher quantities in the aqueous extracts, whereas quercetin (69.72 µg/g) and hesperidin (32.18 µg/g) were obtained in higher amounts in the ethanol extract (Table 2). Plant phenolic compounds possess strong free radical scavenging activity and high antioxidant capacities, making them more antimicrobial and effective against various diseases and infections [41]. Therefore, the high phenolic content found in this study suggests that consuming H. sabdariffa plants and their products could enhance human health by neutralizing free radicals, which may help prevent neurodegenerative diseases and cancer development [9].
The findings regarding the phenolic compounds in both ethanol and aqueous extracts of hibiscus flowers highlight the complexity and variability of these bioactive compounds. The presence of significant amounts of chlorogenic acid, hyperoside, and caffeic acid in the aqueous extracts suggests that these compounds may be particularly effective in enhancing the antioxidant properties of the plant when extracted with water. Conversely, the higher levels of quercetin and hesperidin in the ethanol extracts indicate that certain phenolic compounds are more soluble in organic solvents, potentially enhancing their bioavailability.

Given that plant phenolic compounds are known for their strong free radical scavenging activity and high antioxidant capacities, the findings imply that the consumption of H. sabdariffa and its products could provide substantial health benefits. The ability of these compounds to neutralize free radicals may contribute to the prevention of oxidative stress-related conditions, such as neurodegenerative diseases and cancer.

3.3. Volatile organic molecules result by GC-MS

In GC-MS analysis of volatile organic compounds from extracts of Hatmi and Hibiscus flowers, it was found that Hatmi flowers contained a greater abundance of organic compounds than Hibiscus. The results were determined by scanning against the device library, accepting matches above 80%. Tricosane, linoleic acid ethyl ester, ethyl oleate, nonanoic acid, p-vinylguaiacol, 9,12-octadecadienoic acid (Z, Z), cis-vaccenic acid, and other organic volatile compounds were obtained from the extract of Hatmi flowers (Table 3). In a study, it was demonstrated that the hexane extract of A. officinalis flowers is rich in both saturated fatty acids (including palmitic acid, nonacosane, heptacosane, and pentacosane) and unsaturated fatty acids (such as omega-3 α-linolenic acid and omega-6 linoleic acid) [42]. Flavonoids are a class of heterocyclic natural compounds that are widely distributed in plants, occurring as glycosides and free aglycones. Consistent with the authors' findings, A. officinalis was found to be rich in quercetin, rutin, apigenin, coumarins, and kaempferol [43]. These compounds exhibited cytotoxic activities against cancer cells by interacting with various molecules involved in apoptosis and proliferation pathways [44,45].

Table 3. Volatile organic molecules of Hatmi plant extracts.

CAS	Label	Mass (DB)	Formula (DB)	m/z	Library	Score (Lib)
5906-76-3	Dimethylsilanol; Silanol, dimethyl	76	C2H8OSi	45.1	Wiley7Nist05.L	84.12
1464-53-5	2,2-Bioxirane	86	C4H6O2	55.1	Wiley7Nist05.L	84.58
600-22-6	Propanoic acid, 2-oxo, methyl ester	102	C4H6O3	43.1	Wiley7Nist05.L	85.68
42403-25-8	Pyrrolidine- Alpha, Alpha, Alpha, Alpha- D4	75.1	C4H5D4N	43.1	Wiley7Nist05.L	85.38
98-00-0	2-furanmethanol	98.1	C5H6O2	81.1	NIST11.L	93.6
10230-62-3	2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	144	C6H8O4	101	NIST11.L	88.74
33325-40-5	Propanoic acid,3- (acetylthio)-2-methyl	162	C6H10O3S	43.1	Wiley7Nist05.L	80.81
65-71-4	Thymine	126	C5H6N2O2	43.1	Wiley7Nist05.L	83.47
30533-08-5	2-propanamine, N-methyl-N-nitroso	102	C4H10N2O	43.1	Wiley7Nist05.L	80.15
28564-83-2	4H-pyran-4-one, 2,3-dihdyro-3,5-dihydroxy-6-methyl	144	C6H8O4	43.1	NIST11.L	95.68
25395-31-7	1,2,3-propanetriol, diacetate	143.9	C7H12O5	43.1	Wiley7Nist05.L	82.1
112-05-0	Nonanoic acid	158	C9H18O2	43.1	Wiley7Nist05.L	88.75
7786-61-0	p-vinylguaiacol	150.1	C9H10O2	135	Wiley7Nist05.L	93.36
1000130-99-3	Z-10-Tetradecen-1-ol acetate	254	C16H30O2	43.1	NIST11.L	84.77
1000130-14-3	d-glycero-d-ido-heptose	210	C7H14O7	57.1	NIST11.L	82.02
0-00-0	3-deoxy-d-mannoic acid lactone	141.9	C6H10O5	57.1	Wiley7Nist05.L	83.39
502-69-2	2-pentadecanone,6,10,14-trimethyl	268	C18H36O	43.1	NIST11.L	87.98
57-10-3	Hexadecanoic acid	256.2	C16H32O2	73	Wiley7Nist05.L	96.06
628-97-7	Hexadecanoic acid, ethyl ester	284.3	C18H36O2	88.1	Wiley7Nist05.L	96.13
60-33-3	9,12-Octadecadienoic acid (Z,Z)	280.2	C18H32O2	67.1	NIST11.L	85.07
506-17-2	Cis-Vaccenic acid	281.1	C18H34O2	55.1	NIST11.L	87.99
544-35-4	Linoleic acid ethyl ester	308.3	C20H36O2	55.1	NIST11.L	82.69
111-62-6	Ethyl oleate	310.3	C20H38O2	55.1	Wiley7Nist05.L	88.75
111-61-5	Octadecanoic acid, ethyl ester	312.3	C20H40O2	88.1	NIST11.L	87.35
638-67-5	Tricosane	324.4	C23H48	57.1	NIST11.L	95.95
301-02-0	9-Octadecenamide, (Z)	281.1	C18H35NO	59.1	NIST11.L	81.72
629-99-2	Pentacosane	352.4	C25H52	57.1	NIST11.L	90.91
593-49-7	Heptacosane	380.4	C27H56	57.1	NIST11.L	90.75
621-61-4	Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	358.2	C21H42O4	98.1	NIST11.L	89.89

Table 4. Volatile organic molecules of Hibiscus plant extracts.

CAS	Label	Mass (DB)	Formula (DB)	m/z	Library	Score (Lib)
1112-39-6	Silane, dimethoxydimethyl	120.1	C4H12O2Si	105	Wiley7Nist05.L	81,23
497-23-4	2 (5H)- Furanone	84	C4H4O2	55.1	NIST11.L	90.22
98-01-1	Furfural	96	C5H4O2	39.1	NIST11.L	99.15
8.03.2170	2,5 - Furandione, d	112	C5H4O3	68.1	NIST11.L	93.53
620-02-0	2-Furancarboxaldehyde, 5-methyl	110	C6H6O2	53.1	NIST11.L	97.76
161500-43-2	Oxazolidine, 2,2-diethyl-3-methyl	140.2	C8H17NO	114	NIST11.L	83.96
932-85-4	2 (3H)- Furanone, 5-ethoxydihydro	129.1	C6H10O3	85	NIST11.L	91.29
28564-83-2	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl	144	C6H8O4	43.1	NIST11.L	95.21
67-47-0	5-hydroxymethylfurfural	126	C6H6O3	97	NIST11.L	97.92
498-07-7	beta-D-Glucopyranose ,1,6,anhydro	161.8	C6H10O5	60.1	NIST11.L	91.96
80286-58-4	Arteannuic acid	234.2	C15H22O2	121.1	NIST11.L	82.4
112-39-0	Hexadecanoic acid, methyl ester	270.2	C17H34O2	74.1	NIST11.L	86.12
57-10-3	Hexadecanoic acid	256.2	C16H32O2	73.1	Wiley7Nist05.L	94.89
60-33-3	9,12-Octadecadienoic acid (Z,Z)	280.2	C18H32O2	67.1	NIST11.L	93
301-02-0	9-Octadecenamide, (Z)	281.1	C18H35NO	59.1	Wiley7Nist05.L	87.08

Volatile organic molecules such as furfural, arteannuic acid, hexadecanoic acid methyl ester, hexadecanoic acid, and 9-octadecenamide (Z) were obtained in extracts of Hibiscus flowers. The gas chromatography-mass chromatography (GC–MS) analysis of oil from H. sabdariffa flower obtained from Nigeria showed the presence of linoleic acid (22.7%) and hexadecenoic acids of 64.3% [46]; the seed oil from Austria showed oleic acid (24.7%), linoleic acid (43.2%) and palmitic acid (17.3%) as the major chemical compositions [6]. However, α-terpineol and linalool dominated the seed oil from Cuba [47]. It can be observed that there is no uniformity in the chemical compositions of the oil; it varies based on geographical locations [48].

These findings suggest that environmental factors, such as soil composition, climate, and agricultural practices, play a crucial role in determining the chemical profiles of Hibiscus oils. This lack of uniformity highlights the importance of considering geographic variations when evaluating the potential applications of these oils in food, cosmetics, or medicinal uses. Further studies could explore the implications of these differences for the antioxidant and therapeutic properties of Hibiscus extracts, potentially leading to more targeted applications based on specific regional profiles.

4. CONCLUSION

The findings of this study indicate that aqueous and ethanol extractions produce different effects on the bioactive components and antioxidant properties of the plants. Notably, the aqueous extract of the Hibiscus flower exhibited high DPPH radical scavenging activity, while the ethanol extract of the Hatmi flower was found to be more effective in FRAP assays. This suggests that the choice of solvent significantly influences the profile of active compounds and their corresponding activities. Aqueous extractions typically tend to extract polar compounds, particularly phenolic compounds and antioxidants that are soluble in water. The richness of such compounds in the Hibiscus flower may be one of the key reasons for its high antioxidant activity. In contrast, ethanol and other less polar solvents are more effective at extracting phenolic compounds and fatty acids.

The high activity observed in the ethanol extract of the Hatmi flower may therefore result from ethanol's ability to solubilize these compounds more effectively. These differences highlight the critical role that the extraction methods play in determining the active components obtained and, ultimately, their potential health benefits. Thus, further investigation into these plants, particularly using various extraction methods, is essential to better understand their health benefits and enhance their potential in pharmaceutical applications.

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