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INVESTIGATION OF BIOACTIVE CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF DIFFERENT FRACTIONS FROM *HERNIARIA HEMISTEMON* J.GAY

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Abstract

This study was carried out the phytochemical screening and evaluate antibacterial, antifungal and antioxidants effects of petroleum ether (40-60 °C), diethyl ether, chloroform, ethyl acetate, ethanol 96% and ethanol 70% fractions obtained from the aerial part of Herniaria hemostimon J. Gay. Phytochemical analysis showed the presence of terpenoids, steroids, flavonoids, alkaloids, tannins and saponins in different fractions the plant. Quantitative concentration of phenols, flavonoids, alkaloids, tannins and saponins were detected. HPLC analysis of H. hemostimon identified 21 flavonoids and 21 phenolic compounds. Successive fractionation has been carried out where Di-(2-ethylhexyl) phthalate (DEHP and protocatechuic acid were isolated and identified using FT-IR, ^{1}H-NMR and MS spectroscopic. Furthermore, the antimicrobial activity of successive extract of H. hemistemon against 5 bacterial strains and 2 fungal and yeast strains carried out by the disc diffusion method. The diethyl ether and ethyl acetate fraction showed the highest activity against all the tested bacterial and fungal strains. The ethyl acetate fraction showed the highest antioxidant activity. This study suggested that the bioactivities of H. hemistemona can be used as a source of medicinal compounds due to there's significant antioxidant and antimicrobial activities.

Keywords: *Herniaria hemistemon*; antimicrobial; antioxidants; HPLC.

1. INTRODUCTION

Medicinal and aromatic plants are considered the most important natural products which represent the main source of novel drugs used in treatment of many diseases that infect human [1]. These plants contain highly bioactive components. They also have great importance in folk medicine in treatment of diseases such as, diabetes, ulcers, cancer, skin inflammation, intestinal disorders and hypertension. The drugs isolated from natural sources are safer and with fewer side effects than chemically synthesized [2]. *H. hemistemon* is a genus of *Herniaria* belonging to family Caryophyllacae (pink family), which is one of the largest families in the plant kingdom known to be rich in medicinal plants. It consists of about 89 genera and 2070 species [3]. *Herniaria* genus represented in Egypt by five species [4]. The genus name derived from the Latin word hernia because it was believe that the herb could cure hernia. *H. hemistemon* had significant antioxidant activity [5]. *H. hemistemon* is grazing and medicinal plant [6], where the whole plant used in Europe as a treatment for hernias [7]. Phytochemical analysis of *H. hemistemon* revealed that five flavonoids compounds were isolated and identified as vitexin, kaempferol, quercetin-3-*O*-glucoside-7-*O*-rhamnoside, kaempferol-7-*O*-rhamnoglucoside, and kaempferol 4'-methyl ether and showed high antioxidant activity against (DPPH). GLC analysis of the fatty acid revealed the presence of the 14 fatty acids in which palmitic acid (21.62%) represented the major constituent, the fatty acids are exhibit antibacterial and antifungal properties [5]. In the present study, the phytochemical screening, antibacterial, antifungal and antioxidant activities of different fractions were evaluated. Secondary metabolites were identified in order to understand the compounds involved in each individual fraction bioactivity.

2. MATERIAL AND METHODS

2.1. Plant Material

Collection of *Herniaria hemistemon* J.Gay aerial parts occurred during May 2015 in the

flowering stage from Siwa region Matrouh governorate, northwest coast, Egypt. Dr Atia Eisa, lecturer of plant taxonomy, Faculty of Science, Damanhur University recognized the plant species a voucher specimen is deposited in the herbarium, Desert Research Center (DRC), Cairo, Egypt. Synonyms*: Herniaria fruticosa* Delile and *Herniaria sphacelata* hochst.

2.2. Equipment, Materials and Chemicals

Organic solvents of different polarities were purchased from El-Naser pharmaceutical chemicals Co. (ADWIC) Egypt, Sigma and Merck Co. The Successive extraction of *Herniaria hemistemon* was performed using a Soxhlet apparatus, HPLC, Agilent 1100 series quaternary pump (Waldborn, Germany), connected to a photodiode array detector with variable wavelengths λ max 340 and 280 nm, (Agilent Technologies, USA). Column used is Zorbax 300 SB C18 (150 mm, 4.6 mm, 5 µm). ^{1}HNMR (Bruker 400 MHz) Micro analytical Unit, Faculty of Pharmacy Cairo University. Mass spectrum (Thermo Scientific; GC/MS model ISQ LT) using Thermo X-Calibur software at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Nasr City, Cairo. PerkinElmer Spectrum 100 FT-IR using KBr method Spectrophotometer. 1, 1-diphenyl-2-picrylhydrazyl radical ((DPPH) Sigma-Aldrich).

2.3. Preparation of the Plant Extracts

Polarity is a very important factor when considering the choice of solvent for extraction and fractionation processes in testing the extracts of different organic solvents for detecting the plant constituents. 100 gm of the air dried powder of *H. hemistemon* were subjected to successive extraction using different organic solvents using soxhlet apparatus, Petroleum ether (40-60°C), diethyl ether, chloroform, ethyl acetate, ethanol 96% and ethanol 70%, solvents were used in the order of increasing polarity and each extract was concentrated using rotary vacuum evaporator (Buchi, G. Switzerland) at 40- 50°C.

2.4. Phytochemical Screening

2.4.1. Preliminary Phytochemical Screening

Testing for tannins, saponins, Glycosides and /or carbohydrates according to Balbaa [8], testing for sterols and terpenes according to Brieskorn et al [9], testing of alkaloids according to Woo et al [10] and testing for flavonoids and phenolic compounds according to Edeoga et al [11].

2.4.2. Investigation of total active Constituents:

The total active constituents content were determined in May 2015, this may be due to high water resources (rainfall) leading to high metabolic rates in accordance to our results. Total flavonoids were determined spectrophotometrically and calculated as rutin according to Samatha et al [12]. Total phenolic according to Makkar et al [13], Total tannins according to Ali et al [14]. Finally saponin and alkaloid content were determined according to Honerlagen and Tretter [15].

2.4.3. Identification of Phenolic and flavonoid compounds using HPLC.

Quantitative and qualitative estimation for the phenolic and flavonoid compounds of *H. hemostimon* methanol extract were achieved by HPLC, where each compound was separated and identification was done using authentic pattern [16].

3. ANTIMICROBIAL ASSAY:

3.1. Microbial strains:

The effect of successive extracts using organic solvents of petroleum ether (40-60 °C), diethyl ether, chloroform, ethyl acetate ethanol 96% and ethanol 70% fractions on some pollutant micro-organisms were achieved. The bacterial strains were obtained from Plant Protection Department, Desert Research Center (DRC), Cairo, Egypt. While fungal and yeast strains were obtained from nosocomial infections. The bacterial, fungal and yeast strains checked for purity, identity and regenerated to obtain active microorganisms. Gram positive species (*Staphylococcus aureus, Bacillus subtilis* and *Enterococcus faecalis*), gram negative species (*Escherichia coli* and *Pseudomonas aeruginosa*) and fungal species *Aspergillus niger* and *Candida albicans* were tested. The successive extract was dissolved in Dimethylformamide (DMF) for antimicrobial investigation at the final concentration of (10

mg/ml). The antimicrobial activities of successive fractions were carried out by the disc diffusion method. For preparation of microbial inocula, the bacterial density was adjusted with sterile saline to approximately 108 colony forming units (CFU) per ml (optical density was adjusted at 0.5 McFarland turbidity) and the fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (v/v). The spores suspension were adjusted with sterile saline to a concentration of approximately 1.0x105 in a final volume of 100 µl per ml [26].Yeast inoculum suspension was adjusted with sterile saline to approximately 1×106 to 5× 106 yeast cell per ml (optical density was adjusted at 0.5 McFarland turbidity [17].

3.2 Minimum inhibitory concentrations (MIC's) determination of the effective plants extract

To determination of MIC of The most effective plant extracts which exhibiting a strong antibacterial and antifungal by using disk diffusion method and evaluate their efficiency against test bacterial and fungal strains. Different concentrations of the effective plant extract (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 ug/ml) were prepared separately and dissolved in DMSO, and requisite amount over sterilized filter paper discs (8 mm in diameter). Sterilized Petri dishes containing Mueller-Hilton agar and potato dextrose agar media were seeded with bacterial and fungal strains. The loaded filter paper discs with different concentrations of the effective plant extract were placed on the top of media plates and kept in the fridge at5°C for 2 h. then incubated at suitable temperature for 24 h. The inhibition zones of effective plant extract were recorded.

4. Determination of antioxidant activity

Free radical scavenging assay using 1, 1-diphenyl-2- picrylhydrazyl radical (DPPH) Sigma-Aldrich) was carried out according to Yildirim et al [24], concentrations of the tested extract and ascorbic acid as a reference antioxidant control (125, 250, 500 and 1000 µg/ml), (%) Scavenging DPPH calculated as following:

$$(\%)\ \text{Scavenged DPPH} = \frac{\text{Absorbance of control} - \text{Absorbance of extract}}{\text{Absorbance of control}} X100$$

5. RESULTS AND DISCUSSION:

5.1. Phytochemical Screening:

The results of phytochemical screening of the successive extracts of *H. hemistemon* plant showed that, it contains flavonoids, coumarins, alkaloids, tannins, sterols and/or terpenes, glycosides and/or carbohydrates and saponins, the resins not detected as represented in table (1).

5.2. Investigation of total active constituents

Quantitative analysis of phenols, flavonoids, tannins, alkaloids and saponins were recorded in table (2) which showed as 312±0.8 mg/g (as gallic acid equivalent), 238±0.6 mg/g (as rutin equivalent), 3.67±0.2 mg/g, 0.83±0.2 mg/g and 3.24±0.6 mg/g respectively.

Table (2): Total active materials of the aerial parts of *Herniaria hemistemon.*

Secondary Metabolites	Dry Weigh(mg/g)

Table (1). Phytochemical analysis of successive extracts of *Herniaria hemistemon* Aerial Parts.

Test	Petroleum Ether (40-60 °C)	diethyl ether	Chloroform	Ethyl acetate	Ethanol 96%	Ethanol 70%
Flavonoids	-	-	+	+	+	+
Coumarins	-	-	+	-	-	-
Alkaloids	-	-	+	-	-	-
Tannins	-	-	-	+	+	+
Glycosides and / or carbohydrates	-	-	-	-	+	+
Sterols and / or terpenens	+	+	+	-	-	-
Saponins	-	-	-	+	+	+
Resins	-	-	-	-	-	-

(+) mean present, (-) mean absent.

Total phenolic (Gallic acid)	312±0.8
Total flavonoids (rutin)	238±0.6
Total tannins	3.67±0.2
Total alkaloids	0.83±0.2
Total Saponins	3.24±0.6

5.3. Determination of percentage yield of successive extraction

Percentage yield obtained from each solvent using a Soxhlet apparatus[18] was concentrated, dried and weighed (2.02%, 1.88%, 0.76%, 9.01%, 12.99% and 14.43 %) of petroleum ether (40-60°C), diethyl ether, chloroform, ethyl acetate, ethanol 96% and ethanol 70%, respectively. The obtained percentage of total residues was (41.09%). Successive fractionation showed that ethanol 70% fraction has the highest percentage amounted to 14.43% while the lowest one was that of chloroform extract as shown in table (3).

Table (3). Determination of percentage yield of successive extraction of *Herniaria hemistemon* using soxhlet apparatus

Solvent extract	Percentage (%)
Petroleum ether(40-60°C)	2.02
Diethyl ether	1.88
Chloroform	0.76
Ethyl acetate	9.01
Ethanol 96%	12.99
Ethanol 70 %	14.43
Total %	41.09

5.4. Identification of Phenolic and flavonoid compounds using HPLC.

Quantitative and qualitative estimation for the phenolic and flavonoid compounds of the methanol extract of *H. hemostimon* were achieved by using HPLC, where each compound was separated and identified using

Table (4). HPLC analysis for Phenolic compounds of *Herniaria hemistemon*

No.	Phenolic Compound	RT	Result (ppm)
1	Pyrogallol	6.99	103.89
2	Gallic	7.07	5.35
3	4-Amino-benzoic	8.39	1.52
4	Protocatechuic	8.44	9.27
5	Catechin	8.62	11.43
6	Catechol	9.04	25.51
7	Epicatechin	8.62	4.73
8	4 hydroxybenzoic acid	9.66	30.11
9	Chlorogenic	10.06	8.50
10	Vanillic	10.16	16.56
11	Caffeic	10.29	3.97
12	P-coumaric	11.58	4.83
13	Ferulic	11.9	11.90
14	Iso- ferrulic	12.12	3.00
15	E-vanillic	12.24	35.17
16	Benzoic	13.3	26.64
17	Ellagic	13.43	252.92
18	3,4,5- methoxycinnamic	14.12	13.56
19	Coumarin	14.47	3.48
20	Cinnamic	15.26	0.30
21	Salicylic	16.31	3.30

authentic pattern. It found that twenty one phenolic compounds according to their retention time, the maximum value reviled methanolic extract of *H. hemostimon* Ellagic acid 252.92 ppm as table (4) and fig. (1) While twenty one flavonoid compounds were identified and the maximum value reveled methanolic extract of *H. hemostimon* luteolin-6-arabinose-8- glucoside 174.06 ppm as table (5) and fig. (2).

5.5. Extraction and isolation

Ethyl acetate fraction (7.4 g) was applied on the top of silica gel column chromatography. Elution started with diethyl ether gradual increasing of ethyl acetate (5%, 10%, 15%,

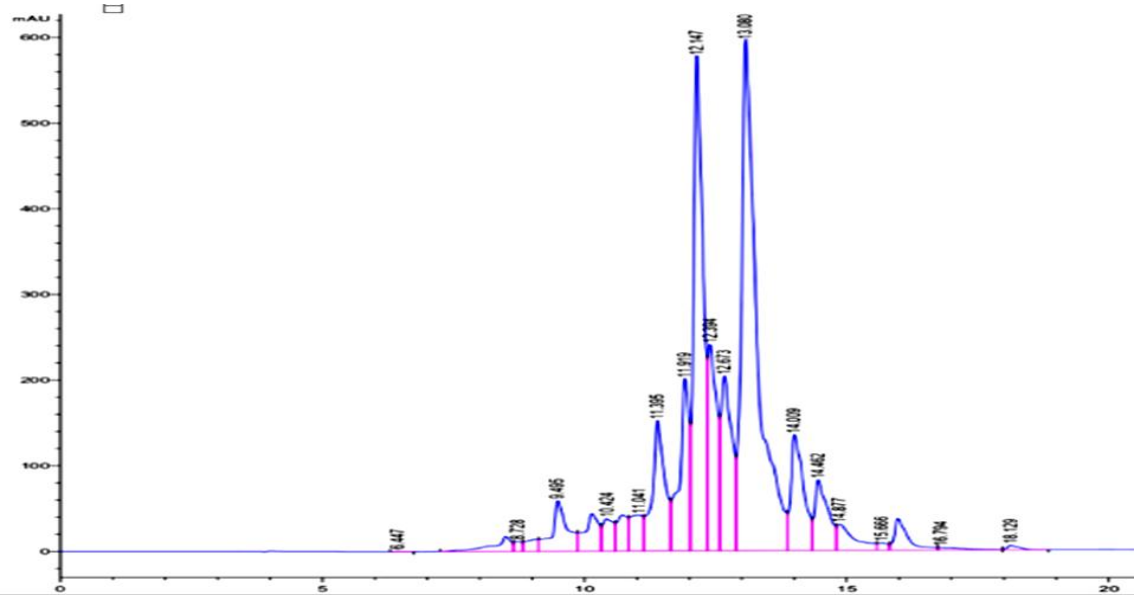

Fig. (1). HPLC of phenolic compounds for *Herniaria hemistemon*.

Table (5). HPLC of flavonoid compound for Herniaria hemistemon

No.	Flavonoid Compound	RT	Results (ppm)
1	luteolin-6-arabinose-8- glucoside	9.55	174.06
2	luteolin-6-glucosyl-8-arabinoside	10.75	3.03
3	apigenin-6-arabinose-8- glactose	11.69	6.64
4	Apigenin-6-rhamnosyl-8-glucoside	12.08	9.87
5	Apigenin-6-glucosyl-8- Rhamnoside	12.14	3.96
6	Naringin	12.33	8.40
7	Hesperidin	12.44	39.64
8	Quercetin-3-*O*- glucoside	12.54	4.06
9	Rutin	12.60	6.36
10	Apigenin-7-*O*-neohesperidoside	13.05	3.96
11	Kaempferol 3,7-dirhamnoside	13.24	4.04
12	Quercetrin	13.44	16.07
13	Quercetin	14.99	1.22
14	Naringenin	15.07	1.07
15	Kaempferol-3-glucoside-2"-pcoumaroyl	15.16	5.92
16	Hesperetin	15.36	1.08
17	Kaempferol	16.38	0.92
18	Rhamnetin	16.44	0.45
19	Apigenin	16.58	1.3
20	Apigenin -7-*O*-glucoside	17.25	0.43
21	Acacetin	18.84	7.75

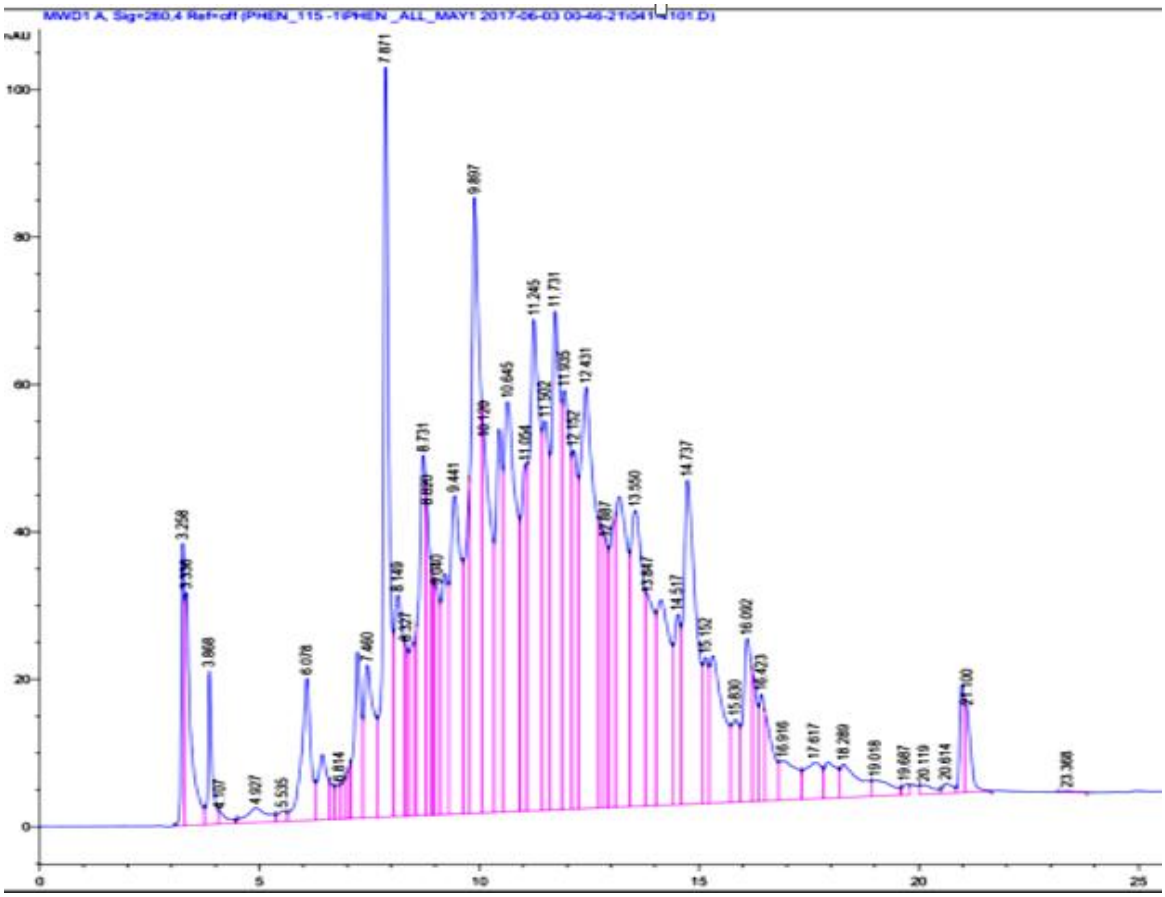

Fig. (2). HPLC of flavonoid compound for *Herniaria hemistemon*

20%, till pure ethyl acetate finally. About 134 fractions were collected, evaporated and concentrated under reduced pressure, collective fractions were obtained according to paper chromatography manner using system (*n*-Butanol: acetic acid: water (BAW) 4: 1: 5 v/v/v) upper phase and TLC using different solvent systems (ethyl acetate: methanol: water 30: 5: 4) and (chloroform: methanol 95: 5), examined under UV light. One collective fraction (F1) was subjected to preparative paper were obtained containing two band. Then each band was purified on column sephadex LH-20 and the eluting system was ethanol (compound 1, 2).

5.5.1. Identification of Compound 1

Colorless in visible, oily liquid, Rf value in solvent systems (acetic acid 15% 0.90, B.A.W. (4:1:5) 0.94). ^{1}H-NMR in $CDCL_3$ δ (ppm) 7.72 (2H, *dd*, *J* = 5.6, 3.2 $H_{Z,}$ H-2, 5), 7.53 (2H, *dd*, *J* = 5.6, 3.2 Hz H-3, 4), 4.19 - 4.27 (4H, *m*, - O-CH_2), 1.67 - 1.71 (2H, *m*, -CH at 4`, 4``), 1.23 - 1.39 (16H, *m*, -$CH_2$ at 5`, 5``, 6`, 6``, 7`, 7``9`, 9``) 0.89 - 0.95 (m, 12H, -methyl groups). IR (KBr cm-1); 1600 (C=C), 1727 (C=O), 2931 (C-H aliphatic) and 2960 (O-H aromatic). Mass spectrum: M. wt = 390 m/z, molecular formula C_{24} $H_{38}O_4$ $[M]^+$ m/z (391), m/z (331), m/z (286), m/z (244), m/z 89, m/z (100 base peak).

From the previously mentioned data and by comparing with those published before [19], compound 1 was identified as di-(2-ethylhexyl) phthalate (DEHP).

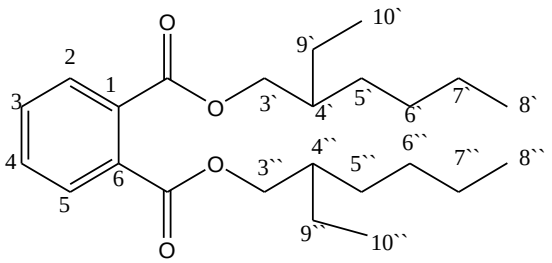

Fig. (3): Di-(2-ethylhexyl) phthalate (DEHP)

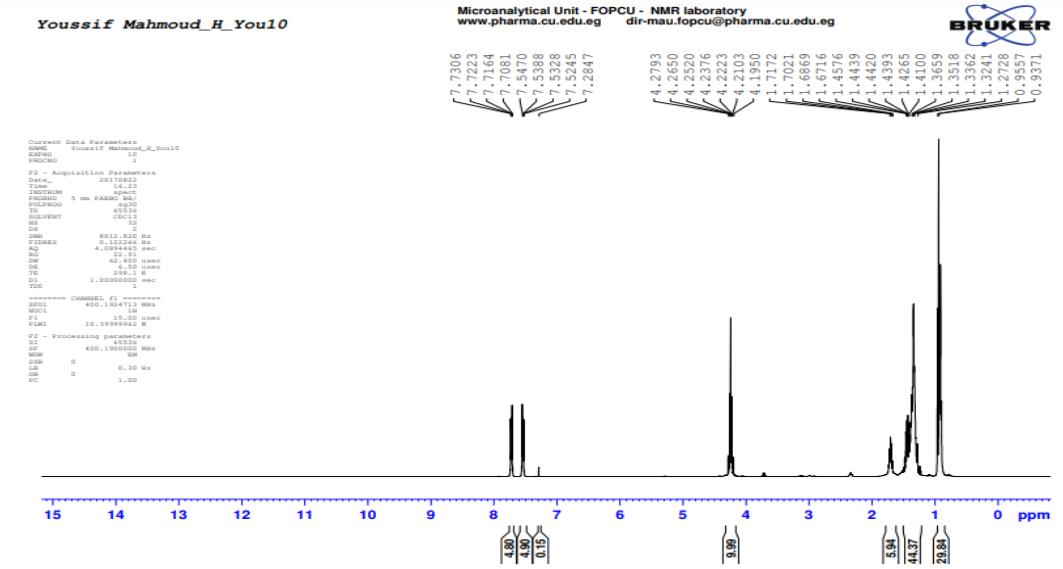

Fig. (4): ^{1}H NMR spectrum of compound 1

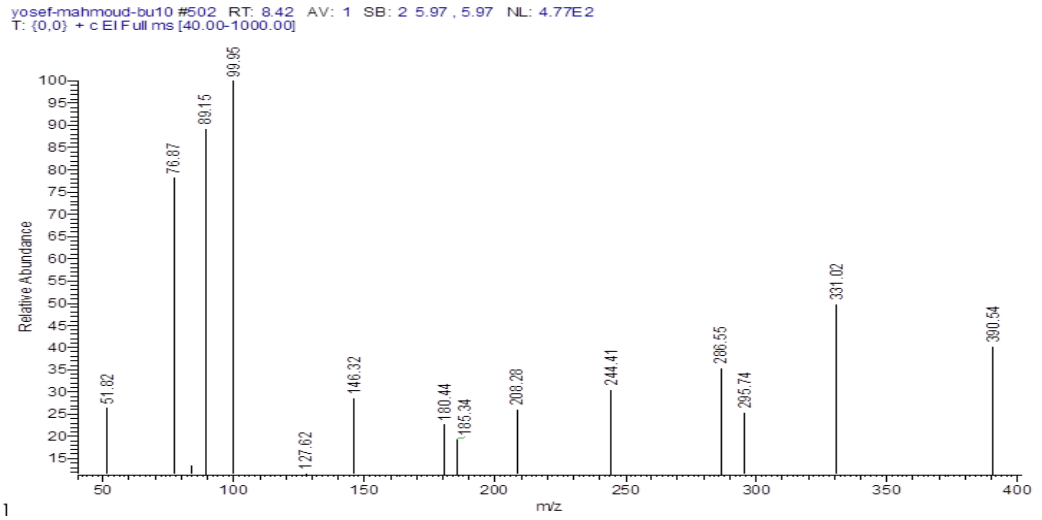

Fig. (5): Mass spectrum of compound 1

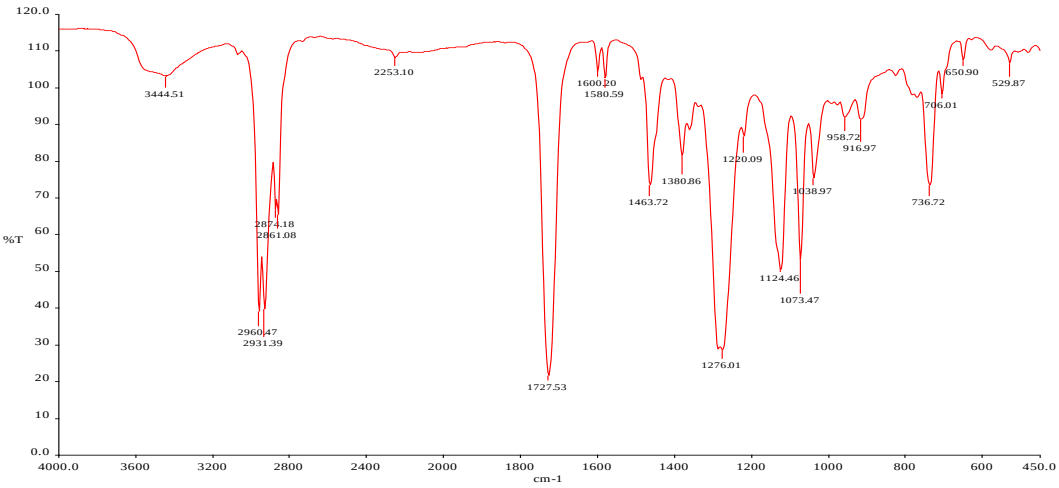

Fig. (6) IR spectroscopy of compound 1

5.5.2. Identification of compound 2

Colorless, gum and insoluble in water. Rf value in solvent systems (acetic acid 15% 0, B.A.W. (4:1:5) 0.49). ^{1}H-NMR in CD_3OD: δ (ppm) 7.3 (1H, *d*, *J* = 2.8 Hz, H-2), 6.8 (1H, *dd*, *J* = 2.8, *J* = 8.4 Hz, H-6), 6.6 (1H, *d*, *J* = 8.8 Hz, H-5). IR (KBr, cm-1): 1580 (C=C), 1680 (C=O), 2925 (C-H), 3448 (O-H). Mass spectroscopy: M wt = 154 m/z, molecular formula $C_7H_6O_4$, m/z (154), m/z (138), m/z (535 base peak), m/z (110), m/z (94), m/z (58) m/z (53) base peak.

From the previously mentioned data and by comparing with those published before [20], compound 2 was identified as protocatechuic acid

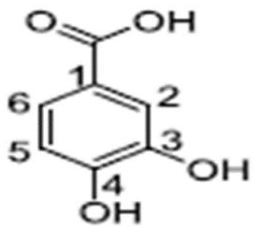

Fig. (7): Protocatechuic acid

5.6. Antibacterial activity

The different organic extracts of the aerial part of *H.hemostimon* J.Gay were screened for antibacterial activity against five bacterial species in a disc-diffusion assay. The results showed the effect of all extract except ethanol 70% extract, which had no effect against tested bacteria fig. (11). It also observed that the diethyl ether and ethyl acetate fractions were displayed broad spectrum among the remaining five fractions, whereas it showed a zone of inhibition ranged between 12 to 17 mm against all tested gram positive bacteria (*Bacillus subtilus, Enterococcus faecalis* and *Staphylococcus aureu*) and gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*). Chloroform and ethanol 96% fraction were less activity from the diethyl ether and ethyl acetate fractions, whereas they showed antibacterial activity against tested bacteria except *E. faecalis*. Less antibacterial effect on gram positive tested bacteria was observed by petroleum ether fraction, whereas there is no inhibition activity against *B. subtilus* and *E. faecalis*, on the other hand the same fraction showed inhibition zone against two gram negative tested bacteria ranged 16 mm and 13 mm in diameter on *E. coli* and *P. aerugino sa* table (6).

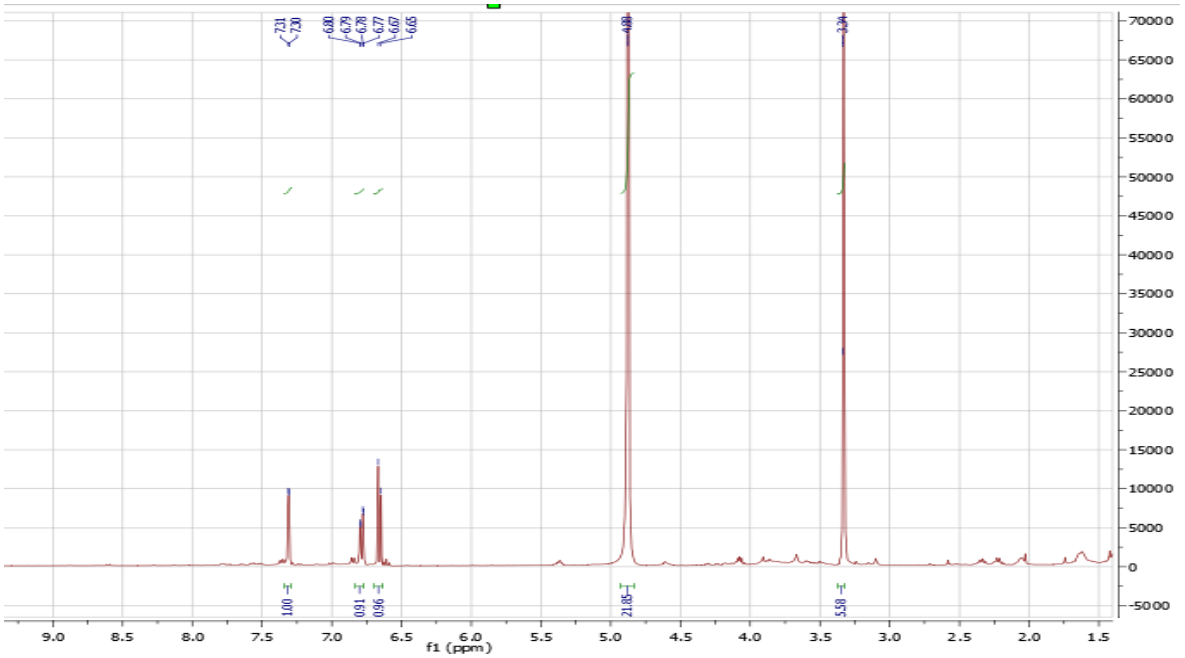

Fig. (8): 1H-NMR spectrum of compound 2

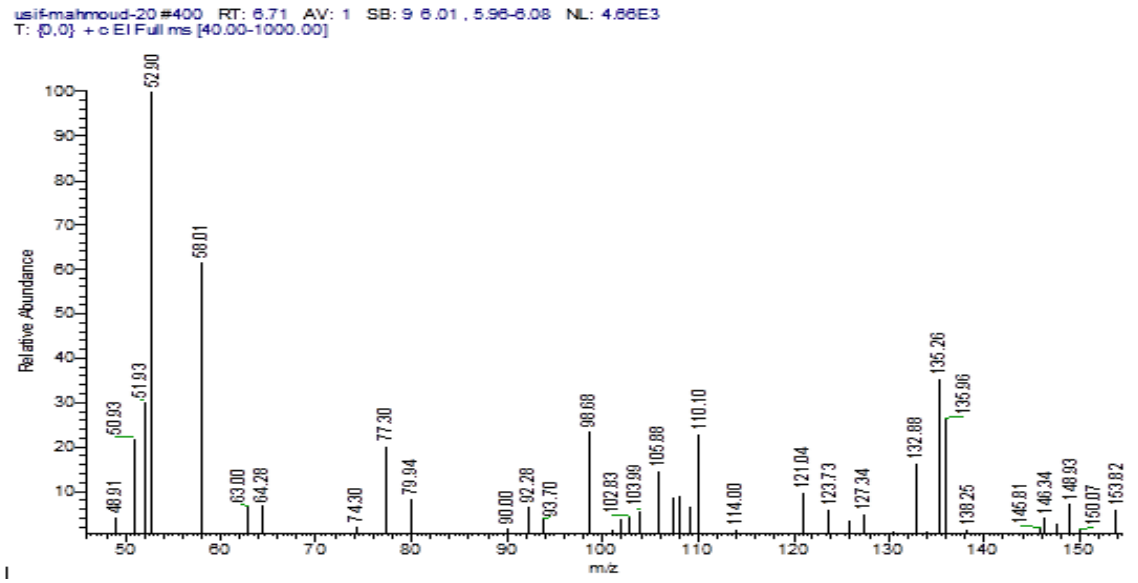

Fig. (9): Mass spectrum of compound 2

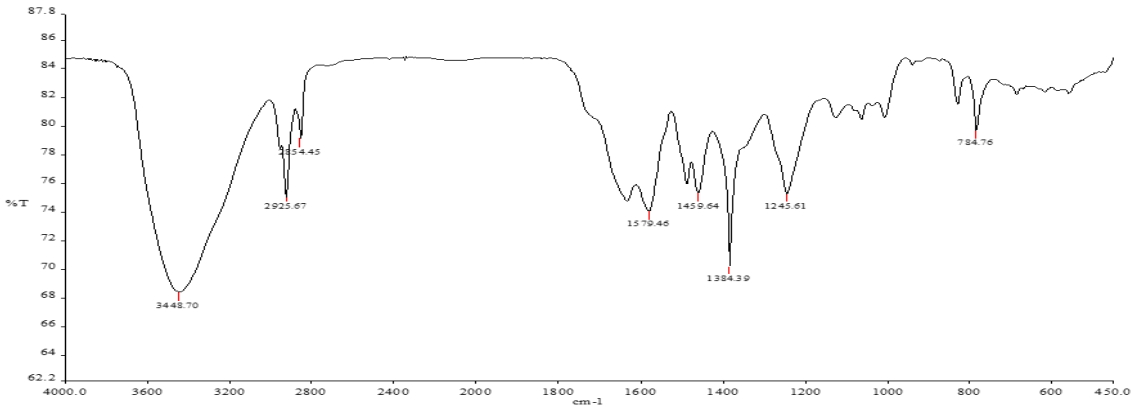

Fig. (10): IR spectroscopy of compound 2

Table (6). Antibacterial activity of successive fractions of *Herniaria hemistemon*

Bacteria	Diameter of inhibition zones (mm)					
	Petroleum ether	Diethyl ether	Chloroform	Ethyl acetate	Ethanol 96%	Ethanol 70%
Bacillus subtilus	0	15	13	15	12	0
Enterococcus faecalis	0	16	0	12	0	0
Staphylococcus aureus	13	16	10	16	12	0
Escherichia coli	16	17	12	15	10	0
Pseudomonas aeruginosa	13	16	12	14	10	0

5.7. Antifungal activity

Antifungal activity results were summarized in table (7) on the same trend of the antibacterial activities, all of organic solvent extracts showed antifungal activity except the ethanol 70%. Both of the diethyl ether and ethyl acetate fractions showed good inhibition activity on *aspregillus niger* and *candida albican*, by disc diffusion method whereas the other fractions showed antifungal activity on one tested fungi without another as shown in fig. (12).

5.8. The minimal inhibition concentration (MIC

The minimal inhibition concentration (MIC) values of the most effective extracts diethyl ether and ethyl acetate fractions *Herniaria hemistemon* were determined for *B. subtilis, S. aureus, P. aeruginosa, E. coli, Enterococcus faecalis, A. niger* and *C. albicans*. The results showed that the levels of MIC of diethyl ether fractions range observed from 10 to 30 mg/ml and 50 to 80 mg/ml for ethyl acetate extracts respectively as showed in Table (8).

The antimicrobial effect of diethyl ether and ethyl acetate fractions of *H. hemistemon* against these organisms may be due to the ability of these solvents to extract some of bioactive compounds like phenolic, saponins and other secondary metabolites which are reported to be antimicrobial effects [21, 22]. The obtained results revealed that diethyl ether and ethyl acetate fractions showed the best antibacterial and antifungal activity, these results may be contributed to the presence of flavonoids which were isolated from the plant such as, vitexin, kaempferol, quercetin-3-*O*-glucoside-7-*O*-rhamnoside, kaempferol-7-*O*-rhamnoglucoside, and kaempferol-4'-methyl ether [5], Ellagic acid which have biological activities including antioxidant, antimicrobial and anticancer activity [23, 24, 25]. Plant possessed coumarins which can have influence in its higher antibacterial effect [32]26, the fatty acids exhibited antibacterial and antifungal properties [27, 28], where Ellagic acid, Coumarin, kaempferol, quercetin, and rutin, were identfided using HPLC, Vitexin (5,7,4-trihydroxyflavone-8-glucoside) has wide pharmacological effects, including anti-oxidant, anti-cancer and anti-inflammatory effects [29], Quercetin and Quercetin-3-*O*-rhamnoside have a significant antioxidant activity [30].

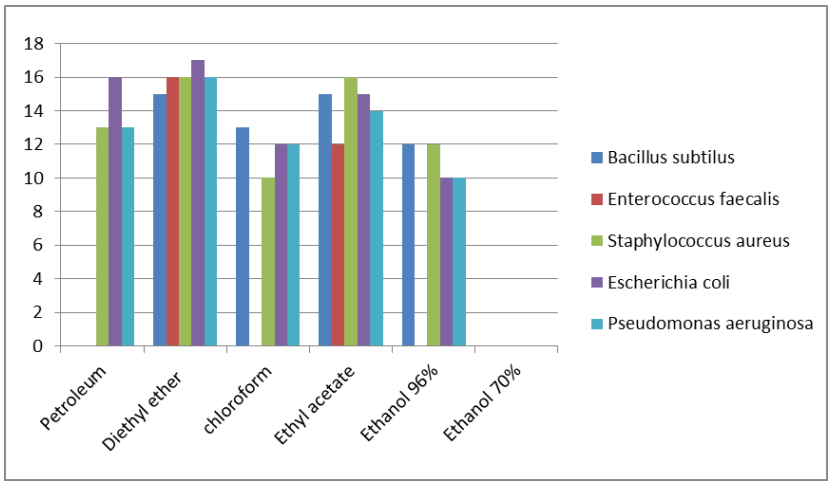

Fig. (11): Effect of successive extracts of *Herniaria hemistemon* on the tested strains of bacteria.

Table (7). Antifungal activity of different fractions of *Herniaria hemistemon*a erial parts

Fungi	Petroleum ether	Diethyl ether	Chloroform	Ethyl acetate	Ethanol 96%	Ethanol 70%
Aspergillus niger	0	17	14	15	14	0
Candida albicans	15	15	0	15	0	0

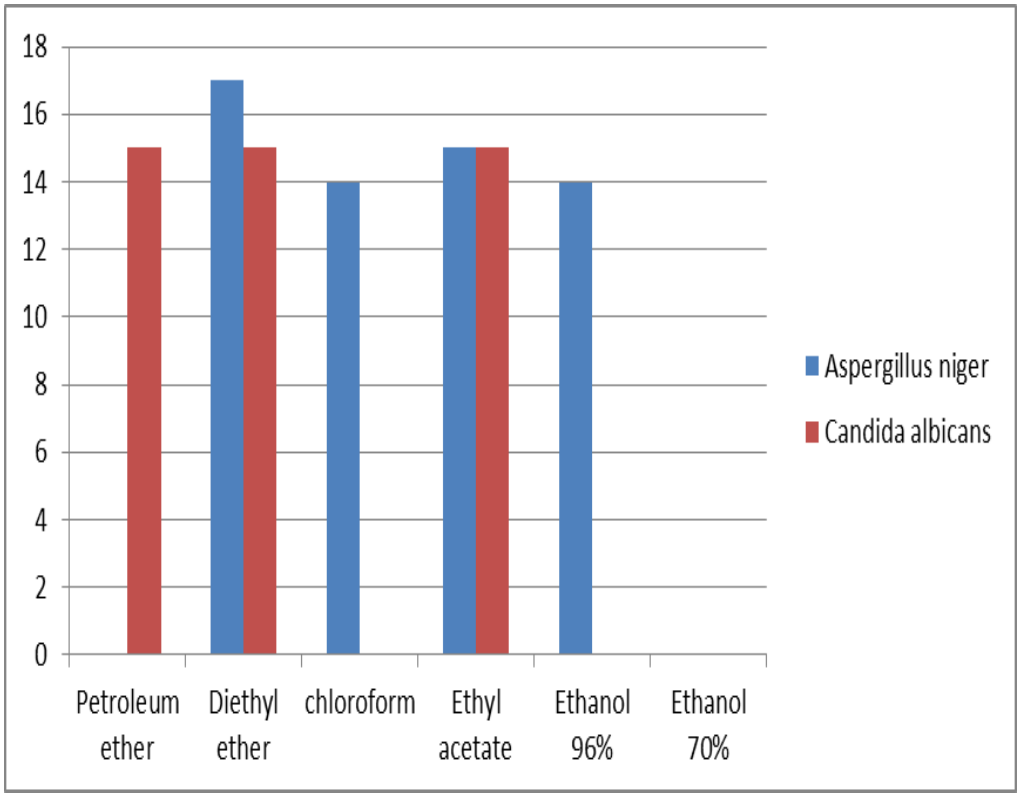

Fig. (12): Effect of successive extracts of *Herniaria hemistemon* on the tested strains of fungi.

Table (8). MIC (µg/ml) of different plant fractions on tested Bacteria and fungi.

Extracts	MIC (mg/ml)						
	Gram +ve bacteria			Gram -ve bacteria		Fungi	Yeast
	E. Faecalis	*B. subtilis*	*S. aureus*	*P. aerugiosa*	*E. Coli*	*A. niger*	*C. albicans*
Diethyl ether	30	20	10	10	30	30	20
Ethyl acetate	50	80	70	70	50	80	80

5.9. Antioxidant activity

Antioxidant activity using DPPH on the successive extracts of *H. hemistemon* with ascorbic acid (natural antioxidant) was carried out, ascorbic acid showed the highest radical scavenging activity attaining aplate aupattern of activity (93.92 %) of concentration (1000 µg/ml), results of antioxidant activity revealed that the scavenging activity of diethyl ether, ethyl acetate and ethanol extracts *H. hemistemon* were dose dependent as shown in Table (9) and fig.(13). The highest percent of DPPH scavenging activity was obtained by ethyl acetate extract (92.17 %) at concentration (1000 µg/ml), (91.07%) at concentration (500 µg/ml) and (90.91 %) at concentration (250 µg/ml), and the lowest effect was obtained (28.88%) in pet.ether extract of the plant. Percentage of DPPH scavenging effect increased dramatically with increasing concentration. From the above result, it was observed that ethyl acetate extract at concentration (1000 µg/ml) showed aplateau scavenging potency presuming radical scavenging activity, as result of presence of phenolic compounds as flavonoids and coumarins which regarded as a hydrogen donors having antioxidant activity [31].

Table (9): Scavenging activity of successive extracts of *Herniaria hemistemon* J.Gay against (DPPH.).

Conc. (µg/ml)	% Scavenging						
	petroleum ether	diethyl ether	chloroform	ethyl acetate	Ethanol 96%	70% ethanol	ascorbic acid
125	28.66	70.01	30.91	87.88	59.65	31.29	85.67
250	36.96	77.40	32.5	90.91	66.22	37.01	88.00
500	51.46	82.42	59.32	91.07	87.57	60.00	92.30
1000	71.05	88.10	70.10	92.17	90.17	63.72	93.92

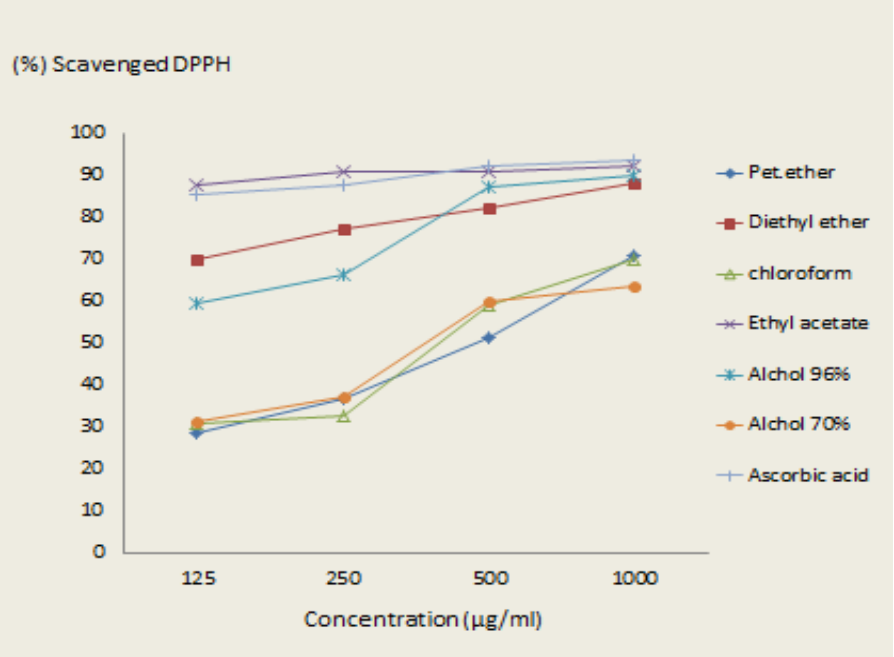

Fig. (13). Scavenging activity of successive extracts of *Herniaria hemistemon* J.Gay against (DPPH.).

CONCLUSION:

The current results highlighted the chemical constituents and biological effects of *Herniaria hemistimon* as a promising sources of future bioactive compounds..

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الملخص العربي:

أجريت هذه الدراسة لتقييم التأثيرات الكيميائية النباتية والميكروبية ومضادات الأكسدة على المستخلصات الجزئية للأثير البترولي (٤٠-٦٠ درجة مئوية) و الإيثيل إيثر والكلوروفورم وإيثيل الأسيتات والإيثانول ٩٦٪ والإيثانول ٧٠٪ التي تم الحصول عليها من الأجزاء الهوائية لنبات هرنيريا هيستيمون . أظهر التحليل الكيميائي للنبات وجود terpenoids والاستيرولات والفلافونويدات والقلويدات و الصابونين. تم تقدير المحتوي الكمي للفينولات والفلافونويد والقلويدات والصابونين. اظهرتحليل HPLC للأجزاء الهوائية لـ Herniaria hemostimon J.Gay وجود ٢١ مركب فلافونويد بجانب ٢١ مركبات فينولية. تم إجراء تجزئة متتالية القطبية وأظهر أن نسبة ٧٠٪ من الإيثانول لديها أعلى نسبة بلغت ١٤.٤٣٪. تم فصل و تعريف مركب ثنائي (إيثيل هكسيل) فثالات DEHP وحمض البروتوكاتيشويك وحُددا باستخدام FT-IR و ^{1}H-NMR و MS الطيفي . علاوة على النشاطات المضادة للميكروبات ومضادات الأكسدة للمستخلص المتتالية القطبية من Hemistemon Herniaria ضد ٥ سلالات بكتيرية و سلالة فطر وخميرة حيث أظهر كل من ثنائي إيثيل أيثر و الإيثيل أسيتات أعلى نشاط ضد جميع السلالات البكتيرية والفطرية المختبرة بالاضافة الي أسيتات الإيثيل أعلى نشاط مضاد للأكسدة. من خلال هذه الدراسة يمكن استخدام الأجزاء الهوائية لـ نبات Herniaria hemistemona كمصدر للمركبات الطبية بسبب الأنشطة المضادة للأكسدة والمضادات البكتيرية و الفطرية.