Volume : 4 | Issue : 10 | October 2015 • ISSN No 2277 - 8179

Research Paper

Chemical Composition and Bioactivity of Isolated Components of Echinophora Platyloba, a Medicinal Plants Find in Kurdistan Region of Iraq, Using Gc-Ms, Nmr and Mtt Bioassay Method.

Chemistry

KEYWORDS : Echinophora Platyloba, Kushndar, Kurdistan Medcinal plant, Antioxidant, Bioactivity.

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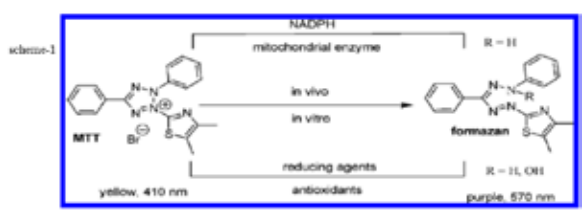
ABSTRACT

In this study, a useful medicinal plant, Echinophora Platyloba (known as Kushndar) find in Our region and used daily by many people as disordering, preserver and anti-lipid absorbent , has been studied. Extractions by different solvents have been carried out (using Methanol, Ethyl acetate and Hexan) it found that MeOH is best solvent for extraction (7.38%). More than 25 compounds determined by Gc-Mass-Mass technique and Elemental analysis shows high ratio of some useful elements (Fe, Mn, Cu and Zn) with (63150 ppb, 8756 ppb,1617 ppb and 1264 ppb respectively) in addition to Chromium(Cr 367.1 ppb) know as diabetic regulator element. Bioactivity (using ,MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) for each extract shown that all of them has high activities (MeOH extracts 0.2635, Ethyl acetate 0.3720 and hexane 0.526), Hence they need to be studied in order to find all active components for each parts by different separation methods such as (mplc, GC-Mass and TLC preparative) and finally identifying by NMR spectrophotometer.

Introduction:

Plants contain hundreds of phytochemicals such as flavonoids and phenolic acids (Fukumoto and Mazza, 2000)[1]. Research indicates phytochemicals such as polyphenols have high antioxidant activity (Stojicevic et al., 2008)[2], Free Radical Scavenging Activity and Phenolic and Flavonoid Contents of Echinophora Platyloba DC has been studied previously which showed that this plants has a good activities against free radicals and it contains a sufficient amount of polyphenolics and flavonoids that exhibit also very good antioxidante activity[3], this determined by DPPH of methanol extracts (30.93±1.58 (EC50)), which let us to investigate more and more about this plant find in our region of Kurdistan.

MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, assay is a well known method for determining bioactivity in vitro and it has been applied, by Nair et all[4], as in scheme-1,



A local name of Echinophora Platyloba in Kurdish is (Kushndar). The methanol extract of E. platyloba showed the highest AA by DPPH assays, contained the highest concentration of flavonoids. Stems of E. platyloba have been used traditionally as flavoring and preservative in cucumber pickles by indigenous people of Iraq and Iran[5], Effects of the flavonoid and phenolic cotenants has been studies[3], the free radicals produced the cell are responsible for oxidative stress and Antioxidants scavenge free radicals, singlet oxygen, and electrons in cellular oxidation reduction reactions which is thought to contribute in the damage of cells for that we must use different antioxidant to kill or stop actions of these free radicals. A class of molecules called antioxidants inhibits the formation of these free radicals by removing their intermediates by (Radimerk,2004), while there are many foods contribute the overall health and gloving wellness, plants that contain antioxidants have a special ability to actually protect the body from the formation of these free radicals.

Fig(1) Echinophora Platyloba officinal plant with flour

Fig(2) Echinophora Platyloba

Experimental procedure

1-Preparation of the plant Extracts:

Echinophora platyloba plants were collected from the Hawraman - Kurdistan (East-north of Iraq) during May 2013. E . platyloba is a perennial plant, distributed only in the Mediterranean region and some central and provinces Eastern of Iraq and western of Iran. A voucher specimen was deposited in the herbarium of the university of Salahaddin (7110).

The aerial parts of the plant were separated, air-dried, and finely powdered. Extraction was carried out by macerating 508 g of powdered dry plant in 800 ml of hexane for 8hs at room

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temperature (r.t.) all three extract were collected together, then dried, bioactivity has been calculated by MTT methods, residue treated with 800ml of ethyl acetate also for 8hs at (r.t.), three times then collected, dried, weighted and Bioassay by MTT, finally the residue putted in methanol 1000ml two times for 8hrs but the last time for 24hs at room temperature, collected dried, its bioactivity determined by the same method.

2-Determination of total phenols and flavonoids :

The contents of total phenolic compounds and flavonoids in methanolic, hydroalcoholic and water extracts of Echinophora platyloba previously studied and they are presented in Table1. The obtained results showed that the contents of total phenolic compounds and flavonoids in methanolic extracts of E. platyloba were significantly higher than in the corresponding water extracts)[3].

3-Measurement of free radical-scavenging activity (DPPH assay):

The free radical scavenging activity of the plant extracts was evaluated using the stable radical DPPH. A series of extracts with different concentration (1.00-0.01 mg/ml-1) were prepared. Then 2.5 ml of the extract and 1.0 ml of a 3.0×10-4 M DPPH solution in methanol were mixed and placed in the dark at the room temperature for 30 min. the absorbance of each sample of the plant extract containing DPPH (As) was measured at 517 nm using UV–VIS lambda spectrophotometer, Radical scavenging capacity was determined according to the technique reported by[6] **Blois (1958).** An aliquot of 1.5 ml of 0.25 mM DPPH solution in ethanol and 1.5 ml of extract at various concentrations were mixed. The mixture was shaken vigorously and allowed to reach a steady state at room temperature for 30 min. Decolonization of DPPH was determined by measuring the absorbance at 517 nm with a Varian spectrophotometer. The DPPH radicals scavenging activity was calculated according to the following equation[3]:

Scavenging activity= $[(A^0 - A^1/A^0) \times 100]$

Where A^0 is the absorbance of the control (blank, without extract) and A1 is the absorbance in the presence of the extract or standard sample

4-Bioactivity By MTT

The extracts were tested individually against MTT, Vitamin C and tert-butyl hydroquinone (BHQT) (25ppm) used as positive control[4] , see table-2 and Figure-3, the results show that all extracts were bioactive and has good activities in which determined by Elisa instrument ELx800 at 570nm, by taking 10ml of stock solution (1000ppm) mixed (190ml) of MTT solution, mixed vigorously by Vortex mixer then incubated for 24hs, after that 200ml of DMSO (Dimethylsulfoxide) has been added, mixed for 30 sec by Vortex then transferred to 96wells and data were recorded at 570nm, for this good results one should try to studied, investigate and identified the composition of each one.

5- Determination of elements:

Elemental analysis have been done on Optical Emission spectrophotometer OES-ICP Perkin Elmer optima 2100 DV, by using 10gm of Echinophora Platyloba in to a porcelain crucibles, then it placed at 900^{0}C in a muffle furnace for more than 5hours, the sample was removed, cooled and weighted, the determine percentage of ash for sample was (6.86gm)% . The residual ash was dissolved in 2ml of nitric acid filtered and the volume was completed to (50ml) distilled water, the solution was injected the instrument and several elements (Cr, Co, Cu, Zn, Ni, V, Se, Mn, Pb and Fe) have been determined.

6- Medium pressure liquid chromatography (mplc) , TLC and GC-Mass:

Each extract have been tested by TLC analysis which they showed existent of many different components for that best solvent has been chosen to separate them by mplc (medium pressure liquid chromatography), shown in Figure-4, after collecting fragments some of them contains 2 or 3 compounds have been separated by TLC preparative method and they were identified using NMR, Figure-5 and GC-Mass spectroscopy Figure-6, in which triglyceride(TGA) hydrolyzed then methylated by CH_2N_2 for indicating all type of fatty acids composing that TGA.

Results and discussion

1-Total phenolic and flavonoid contents

Khazai et all calculated the contents of total phenolic compounds and flavonoids in methanolic, hydroalcoholic and water extracts of Echinophora platyloba, the result quote, they obtained also that the contents of total phenolic compounds and flavonoids in methanolic extracts of E. platyloba were significantly higher than in the corresponding water extracts[3], see Table 1.

2-IC_{50} value in DPPH

To calculate the concentration of the extract necessary to decrease DPPH radical concentration by 50% (called EC50). The EC50 value was used to measure the antiradical activity of the extract: the lower EC50, the higher is the value of the anti-radical activity. In Table 1, the extract obtained by Methanolic extract of E. platyloba showed the highest antioxidant activity.

The result of IC50 value of E.Platyloba in different extracts has been presented in Table -1. Among all the extracts a chloroform extract showed the IC50 value is significantly higher than other extracts which means the inhibition rate is lowest. It means that chloroform extract of each plant showed the high antioxidant activity against DPPH scavenging assay, reducing power assay[3].

3- Bioactivity assay

The result of bioactivities of all extracts from (Methanol BH/115/3C, Ethyl acetate BH/115/3B, and Hexane BH/115/3A) extracts of Echinophora Platyloba herb was presented in Table-1- and Table-2- and Figuer-3, which showed that Hexane extracts higher bioactivity using MTT method and Vitamin C(VC) and tert-butyl hydroquinone (TBHQ) as a control by using Elisa instrument ELx800 at 570nm.

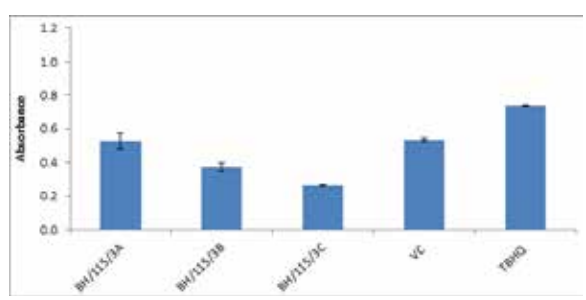


Figure-3- Bioactivity of methanol , ethyl acetate and hexane extracts of E.Platyloba Using MTT methods with controls, such as vitamin C and TBHQ

Table (1): Total phenol and antioxidant activity of *Echinophora platyloba* *in Hawraman-Kurdistan region of Iraq*

Extracts	DPPH[3]	MTT[a]	Total Flavonoids (mg/g)[3]	Total phenol compounds (mg/g)[3]
	IC50 µg/mL		µmol Torolox /g extract	mg Gallic acid/g extract

Methanol	30.93±1.583	0.2635	3.15±04	8.15±22
Ethyl acetate	73.78±1.363	0.3720	1.18±07	3.16±14
hydroalcoholic	57.20±4.363	---	3.9±09	1.78±03
Hexane	------	0.5260	------	------
Vit C (fresh)	------	0.5345	-----	-------
TBHQ (fresh)	-------	0.7360	------	------

a: MTT bioassay has been done on Elisa spectrometer using wells 96, in the Michigan State University, September 2014 USA.

Table (2): Bioactivity / bioassay of E.Platyloba extracts using (50ppm) concentration and determined on 511nm on Elisa instrument.

Extracts	Samples	Elisa Reading 1	Elisa Reading 1	Average	Subtracts MTT reading
Hexane ext.	BH/115/3A	0.575	0.64	0.6075	0.5260
EtOAc ext.	BH/115/3B	0.471	0.436	0.4535	0.3720
MeOH ext.	BH/115/3C	0.347	0.343	0.345	0.2635
Vit C (fresh)	VC	0.609	0.623	0.616	0.5345
TBHQ (fresh)	TBHQ	0.82	0.815	0.8175	0.736
MTT (fresh)	MTT	0.08	0.083	0.0815	0.0

Table (3): Percentage of methanol, ethyl acetate and hexane extracts of E.Platyloba 508g (3times x 8hrs) but methanol 24hrs.

Extracts	Percentage %
Methanol	7.38
Ethyl acetate	0.499
Hexane	0.2828

4-Elemental analysis

The result of elemental analysis of E.Platyloba officinal plant in parts per billion (ppb) showed in (Table-4) showed that contained higher amount of Iron (63150ppb= 63.150ppm) , Mn (8756.5ppm), Cu (1617ppb) and zinc (1264.5ppb), which all of them are essentials for human body and red blood components like hemoglobin. Some other elements also have been determined such as ; Cr 367.1 ppb, Ni 279.9 ppb, V 238.3 and Co 48.5, these elements play a good physiological role in human body especially Cr in the case diabetes which regulating diabetes and play as glucose tolerance factor[7]. Lead (Pb) ratio, hopefully, was low 7.6PPb in the other hand, unfortunately, Selenium (Se) element doesn't detected in this plant which has a big importance[8], (Carvalho,2008).

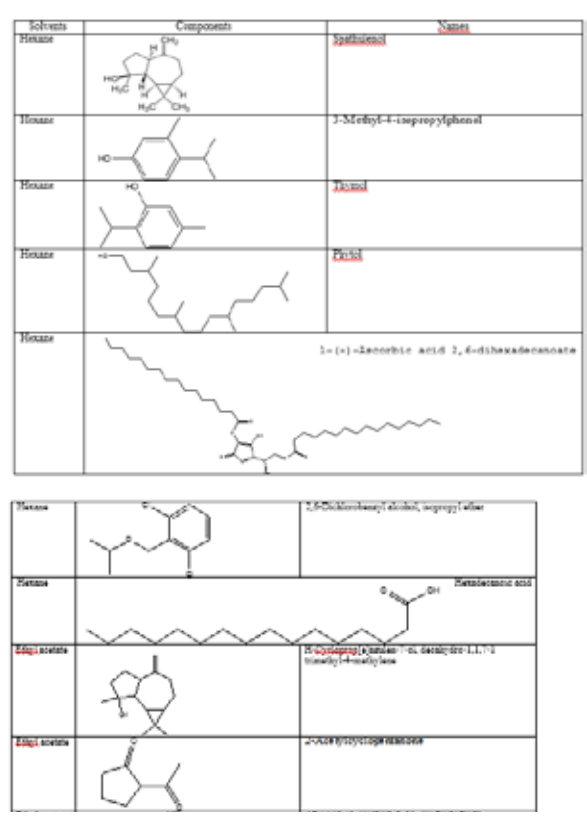
Table(4) Elemental analysis of Echinophora Platyloba in PPb done by ICP Optima 2100

Elements	Concentration
Cr	367.1
Ni	279.9
Pb	7.6
Fe	63150
Mn	8756
Co	48.5
Cu	1617
Zn	1264
Se	Nil
V	238.3

5- Separation results by mplc, TLC and Gc-Mass:

Gokbulut et al, in their research, found in the essential oil extracted δ-3-carene (17.93%), p-cymene (8.99%), methyleugenol (16.41%), and α-phellandrene (9.33%)[9] and another group, Mazloomifar et al , from Iran studied also the composition of the essential oil from aerial parts of *Echinophora platyloba* DC., which was native plant of Iran, (E)-β-ocimene (49.9%) was the main constituent of the oil, followed by γ-decalactone (8.4%), α-pinene (6.0%) and linalool (5.6%)[10], but we have found more than 7 components in each extracts of the dried aerial part, not only the above components of oil but the compounds listed in table-5- by GC-Mass-Mass (Agilent PAL 500), GC- of the Hexane extract shows more than 10 peaks and that of ethyl acetate 11 peaks and for methanol extracts 12 peaks in different retention times.

Table-5: major components found in three extracts of E.Platyloba by GC-Mass-Mass



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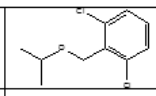
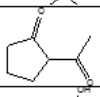
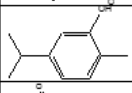
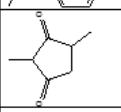
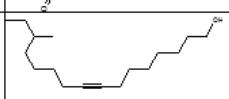
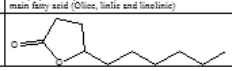
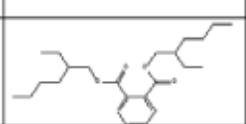
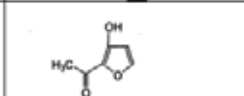
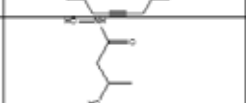
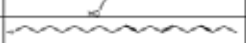
Hexane		2,6-Dichlorobenzyl alcohol, isopropyl ether
Hexane		Hexadecanoic acid
Ethyl acetate		H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-1 trimethyl-4-methylene
Ethyl acetate		2-Acetylcyclopentanone
Ethyl acetate		Phenol, 2-methyl-5-(1-methylethyl)-
Ethyl acetate		2,4-dimethy-1,3- Cyclopentanedions-
Ethyl acetate		14-Methyl-8-hexadecyn-1-ol
Ethyl acetate	Esters of Oliec acid and Linolic and Linolinc acid	
Ethyl acetate	A high percentage of Trielyceride also found with three main fatty acid (Oliec, linlic and linelinic)	
Methanol		2(3H)-Furanone, 5-hexyldihydro-

Methanol		2-Methyl-2,3-3,13-octadecadienol
Methanol		Bis(2-ethylhexyl) phthalate
Methanol		asenolin
Methanol		Octadecyne-9
Methanol		3,N-Dihydroxy-butyramide
Methanol		Octadecatrien-1-ol-9,12,15
Methanol	TOA, Oliec, linelic and linolinc acid esters are also exist	

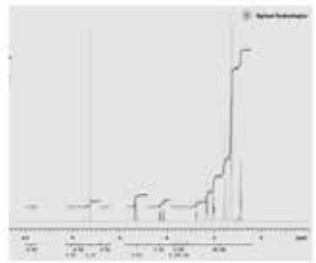


Figure-4- mplc instruments for fragmentation of ethyl acetate and hexane extracts of E.Platyloba

Figure-5- NMR of tryclyceride found by purification by mplc and TLC in extract s of E.Platyloba

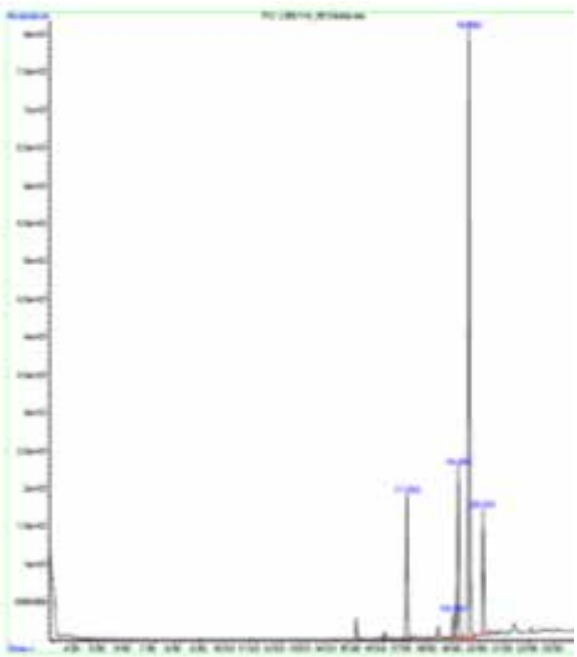


Figure-6- NMR of GC-Mass of Hydrolyzed Triglyceride after methylation by CH_2N_2 to found types of Fatty acids

Acknowledgement

I am so indebted to the Department of chemistry, faculty of science and science education, University of Sulaimani and CRDF Global organization through ISFP program, for providing me, encouragement and for their financial support during my sabbatical research in USA .Also thanks due to Dr.M.G.Nair, Dr.Scott at Mass spectrophotometer service who trained me and let me do all samples by myself and to our colleagues in Michigan state university for their kind contribution and opening all the laboratories for me during June 2014 till October 2015, finally thanks is due to M.Dler M.Salih from our department for the analysis of elements by ICP.

REFERENCE

1- Fukumoto, L. And Mazza, G. (2000). Assessing antioxidant and prooxidant activities of phenolic compounds. Journal of Agricultural and Food Chemistry. 48: 3597-3604 | 2- Stojicevic, S.S, Stanisiavljevic, I.T, Velickovic, D.T, Veljkovic, V.B and Lazic M.L. (2008). Comparative screening of the anti-oxidant and antimicrobial activities of Sempervivum marmoreum L. extracts obtained by various extraction techniques. J. Serb. Chem. Soc. 73(6): 597-60. | 3- V. Khazai , KH. Piri , S. Nazeri , R. Karamian , N. Zamani , Free Radical Scavenging Activity and Phenolic and Flavonoid Contents of Echinophora Platyloba, Asian Journal of Medical and Pharmaceutical Researches, Asian J. Med. Pharm. Res. 1(1): 09-11, 2011 A | 4- Yunbao Liu and Muraleedharan G. Nair, An Efficient and Economical MTT Assay., J. Nat. Prod. 2010, 73, 1193–1195 | 5- ABDOLLAH GHASEMI PIRBALOUTI, herba polonica, Vol. 59 No. 3 2013. | 6- Blois MS. 1958. Antioxidant determination by the use of a stable free radical.Nature.181: 1199–1200. | 7- Bayzid Hassan, Ph.D.thesis, 2004, and Dyari Ibrahim Tofiq, Ph.D.Thesis 2009, university of Sulaimani, Kurdistan, Iraq and William T. Cefalu, MD1 and Frank B. Hu, MD, PHD2, diabetes care, vol.27, no.11. November 2004, page 2741-2751. | 8- Carvalho, CML; Chew, Hashemy SI; Hashemy, J; Lu, A.; Holmgren, A (2008). "Inhibition of the human thioredoxin system: A molecular mechanism of mercury toxicity". Journal of Biological Chemistry 283 (18): 11913–11923 | 9- Incilay Gokbuluta, Tugca Bilenler and Ihsan Karabulut, Determination of Chemical Composition, Total Phenolic, Antimicrobial, and Antioxidant Activities of Echinophora tenuifolia Essential Oil, International Journal of Food Properties, Volume 16, Issue 7, 2013 | 10- Hefzollah Mazloomifar, Mohammad Saber-Tehrani, Abdolhossein Rustaiyan and Shiva Masoudi, Constituents of the Essential Oil ofEchinophora platyloba DC. Growing Wild in Iran, Journal of Essential Oil Research Volume 16, Issue 4, 2004. | | http://worldwidejournals.com/ijsr/contact.php