Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2014, 6(1):18-23
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Isolation, identification, antioxidant, antibacterial and anticancer activity of new complex between isolated alkaloid from *Haloxylon. Sp and* soybean

Iqbal J. B. Alassadi

Department of Chemistry, College of Science, University of Basrah, Basrah, Iraq

---

ABSTRACT

Genistein, daidzein, glycitein and quercetin are flavonoids present in soybean and other vegetables in high amounts. These flavonoids can be metabolically converted to more active forms, which may react with guanine in the DNA to form complexes and can lead to DN depurination. New complex were synthesized between alkaloid isolated from Haloxylon,SP plant.& syobin. The complex &alkaloid was characterized by IR , Spectra ,& TLC. The compounds were screened in vitro for antibacterial activity by inhibition activity against Staphylococcus aureus &Escherichia coli bacteria . and the cytotoxicity assay against the human red blood cells. The result of in vitro study for Rhabdomyosarcoma cell growth assay has shown that the alkaloid isolated had more cytotoxic inhibition after 24,48 & 72 hours .

Key words: Haloxylon. Sp, Soybean complexes, Antimicrobial, Cytotoxicity, Anti oxidant & anticancer activity

---

INTRODUCTION

An antioxidant are compounds that stops an oxidation reactions from occurring such as vitamin E which prevents radical reaction that can cause cell damage by terminate radical chain mechanism [1,2]. Antioxidant which scavenge the free radicals and prevent the damage caused by them .They can greatly reduce the damage due to oxidants by neutralizing the free radicals before they can attack the cells and prevent damage to lipids ,proteins ,enzymes ,carbohydrates and DNA [3].

The endogenous antioxidant system is made up of various proteins ,enzymes and some cofactors that act synergistically to reduce the amount of free radicals in the organism as to reduce or prevent diseases associated with oxidative stress [4] Genistein, daidzein, glycitein and quercetin are flavonoids present in soybean[5]

The most common natural antioxidants are vitamin E (α-tocopherol), vitamin C and various phenol compounds .These natural antioxidants can be found in vegetables cereals grains fruits, tea, oils, and many spices [6-10]

The first isolations of alkaloids in the nineteenth century followed the reintroduction into medicine of a number of alkaloid-containing drugs and were coincidental with the advent of the percolation process for the extraction of drugs.

Alkaloids, taken in their broadest sense, may have a nitrogen atom which is primary (mescaline), secondary (ephedrine), tertiary (atropine) or quaternary (one of the N atoms of tubocurarine), and this factor affects the derivatives of the alkaloid which can be prepared and the isolation procedures. In the plant, alkaloids may exist in the Free State, as salts or as amine or alkaloid *N* -oxides.

Carcinogenesis is a complex pathological process, where normal cells become neoplastic. It is mainly the process associated with chemical modification of DNA. Chemical modification of DNA could be caused by viruses, photochemical reactions

Or reactive substances, called carcinogens [11–14]

Cancer is class of diseases characterized by uncontrolled division of cells and the ability of these cells to invade other tissues, either by direct invasion into adjacent tissue or by implantation into distant sites (metastasis) [15].

Cancer is classified as the second leading cause of death after cardiovascular disease in Europe and in United States (16) .Chemotherapy is one of the conventional cancer treatments in addition to surgery and radiotherapy .All these types of treatments are costly and carry a high risk of side effects (17).

Herbal medicine has a vital role in the prevention and treatment of cancer. Actually, more than 50% of drugs used during the last 20 years are directly, or chemically altered natural products (18). Different pattern of inhibition on tumor cell growth by green & black tea extract was demonstrated by (19)

(20) Demonstrated that *Saliva triloba* extract s have a direct cytotoxic effect on different malignant cell lines. (21) Found that *Artemisia herba Alba* extracts have a potent cytotoxc activity against tumor cell lines in a concentration and time-dependent manners. A number of epidemiological studies have suggested that consumption of soybeans and soy foods is associated with lowered risks for several cancers including breast, prostate, and colon, and cardiovascular diseases (22) (23); (24) (25) (26) and improves bone health (27). Furthermore, some studies have shown that a diet rich in soy can reduce breast cancer risk (28).

MATERIALS AND METHODS

1-Plant material

Leaves Haloxylon.Sp, was collected from Abu AL-khasseb region (South of Basrah in Iraq) in September 2011. The plant was botanically authenticated and voucher specimens were deposited in the Herbarium of Basrah (basrah , Iraq, college of science, university of Basrah).The leaves was dried at 30^0C then ground by blender (Rotel coffee grinder type 24) and kept in nylon bags until the day of used.

A-Preparation of Alcoholic extract of Haloxylon.Sp , Leaves

Twenty five gram of ground leaves powder were refluxed with 250 ml (70% EtOH-water)for 12hr,then cooled and filter by using Buchner funnel and filter paper .the solvent was dried and concentrated using Rotary evaporator .

B- Isolation of alkaloid compound

Fifty gram of dried leaves Haloxylon.Sp , were heated with 250 ml of (10% Acetic acid in EtOH) on water bath for 24 h. The residue was removed by filtration and the filtrate was concentrated under vacuum up to 15 ml ,and acidified with 2% Sulphuric acid .The acidic fraction then basified with ammonium hydroxide to P^H 9.and extracted with chloroform (3*25ml). the combined chloroform layers were evaporated under vacuum in rotary evaporated to afford 5.3 g (30) .The dried extract was dissolved in 80% methanol and run by thin layer chromatography (TLC) using silica gel in BAW (n-butanol :acetic acid :water,5:1:4) as eluent .

C-Preliminary qualitative Chemical Test for Soybean, Isolated alkaloid & complex

Some chemical tests were done on alcoholic extract and isolated alkaloid compound of Haloxylon.Sp, leaves to determine its active groups as following.

Phenolic compound ,Tanine(31) ,Flavonides(32) , Carbohydrates(33), proteins(34),free amino acid (35) Glycosides (34) ,Alkaloids (34) ,Saponine (34)

Reaction between soybean & isolated alkaloid. D-

20mg of isolated alkaloid and 20mg of soybean were heated with 100ml of EtOH on water bath for 24 h. The solvent was evaporated to afford 0.5 mg .The dried result was dissolved in EtOH and run by thin layer chromatography TLC using silica gel in

Identification of isolated alkaloid & complex E-

The IR-spectra were recorder on 9 SHMADZ 8400 FT-IR spectrophotometer. The melting points were measured by Galena point apparatus.

F- Antibacterial Screening

A filter disk assay was used to determine the antimicrobial activity of the soybean ,alkaloid &complex against types of strains gram positive and gram negative bacteria :which are (Staphylococcus auras and Escherichia coli) that were tested using plates of Muller –Hinton agar ,with the DMSO use as control .The anti activity was defined as the clear zone of growth inhibition .

G-Cytotoxicity assay:

The cytotoxicity activity of the isolated alkaloid & complex were determined against human red blood cells .Different concentration of the compound were prepared separately dissolved in DMSO solution ,then 100 of each concentration was add to 2ml .of blood .The turbidity of the mixture was examined after 30 10 and 60 min before the blood cells were heamolysate completely [35]

H- Determination of antioxidant activity

Antioxidant activity of complex was according to the β-carotene bleaching [37-38] with the following modification .A solution of β-carotene was prepared by dissolving 2mg of β-carotene in 10 ml of chloroform ,1ml of this solution was then pipit into a round –bottom rotary flask containing 20 mg of linoleic acid and 0.2g of Tween 20 .After removing the chloroform under vacuum using a rotary evaporator at 30C°,50 ml of aerated distilled water was added to the flask with manual shaking .Aliquots 5 ml of this prepared emulsion were transferred into tubes containing 0.2 ml of samples (complex ,α- tocopherol ,BHT ,prepared derivatives and control consisted of 0.2 ml of methanol instead of extract .As soon as the emulsion was added to each tube ,the zero time absorbance was read at 470 nm .The samples were then subjected to thermal aut oxidation at 50 C° in a water bath. Subsequent absorbance readings were recorded at 15 min intervals until the color of the β- carotene in the control sample had disappeared at 105 min .The extent of inhibition of the absorbance is related to the concentration of antioxidant compound .All samples were taken in triplicate .The degradation rate of extract was calculated according to zero order reaction kinetics .Antioxidant activity (AA)was calculated as percentage of inhibition relative to the control using the following equation :

$AA= [1-(^{Aj}A_t/A_j*-A_t*)] \times 100.$

Where A_j measured absorbance value of sample at zero time .A_t measured absorbance value of sample after incubation (105min) at 50 C° .A_j* measured absorbance value of control at zero time A_t *measured absorbance value of control after incubation (105min)at 50C°[37]

I- Cell Line -Rhabdomyosarcoma (RD)

Rhabdomyosarcoma (RD) was kindly provided by ICCMGR .The cells were taken from human rhabdomyosarcoma of the cervical girls with 7 aged, passage No.45 was used in the present study and the cells were maintained using MEM medium this assay preparation in biological department in college of Science University of Basrah they prepare 10000µgm\ml of complex between soybean& isolated alkaloid. The plates were incubated at 37 °C for the selected exposure times (24, 48, &72h) [38]

RESULTS AND DISCUSSION

Preliminary qualitative Chemical Test for Soybean, Isolated alkaloid & complex between them.

The result of qualitative chemical analysis of soybean ,isolated alkaloid & complex between them show that the soybean compound containing phenolic & flavoniod compounds ,alkaloid compound containing alkaloid compound ,complex containing alkaloid ,phenolic & flavoniod the test showed negative results of carbohydrate ,protein ,amino acid ,saponin ,glycoside,Tanins .The result of the thin layer chromatography of each Soybeann ,Isolated alkaloid & complex revealed the presence of one component of each Isolated alkaloid & complex development in different reagent such as Dragendroff ,Folin ,Ninhydrine &H2SO4,but Soybean presence four component of flavonoid development in Folin reagent different R_f Soybean give four components in R_f 0.210,0.550,0.914,0.777) , Alkaloid R_f (0.764) but the complex R_f (0.877)

Table (1) Thin layer chromatography and $R_{F\ value}$

Soybean	Alkaloid	Complex
0.210,0.550,0.914,0.777	0.764	0.877
Folin,5N KOH,1N FeCl3	Dragendroff	Dragendroff, Folin

Antioxidant activity

β- carotene bleaching activity which is used to assess the power of the new complex between soybean and alkaloid as antioxidant with the reference compounds ,gallic acid ,α- Tocopherol and BHT. The linolic acid free radical attack the highly unsaturated β-carotene .The presence of different antioxidant can hinder the extent of β- carotene bleaching by neutralizing the linoleat –free radical and other free radical formed in the system [37].According ,the absorbance decreased rapidly in sample without antioxidant (control)whereas in the presence of an antioxidant ,sample retained their color ,and thus absorbance ,for a longer time .The antioxidant activity of the new complex is shown in Table

Table (2) Antioxidant activity of complex between Soybean &isolated alkaloid , α-Tocopherol & BHT

Sample	AJ	At	A*J	A*t	AA
α-Tocopherol	0.507	0.462	0.187	0.115	37.500
BHT	0.667	0.387	o.600	0.178	33.00
Complex	0.383	0.351	0.187	0.115	55.600

Cytotoxicity assay: The result show that the cytotoxicity activity of the isolated alkaloid & complex were determined against human red blood cells .Different concentration of the compound they are no hemolytic effect on blood cell

FT-IR Spectral analysis

The FT-IR spectrum of, Alkaloid and complex between them were obtained and effective peaks .The FT-IR spectrum of the isolated alkaloid show in table (3)

Table (3) FT-IR show the locations of important bands in isolated alkaloid

Range(cm^{-1})& intensity	Bond shape	bond	Functional group
3402	St	O-Carboxylic acid & Alcohole	Hydrogen bonded O-H Stretching
2926		C-H of heterocyclic	C-H Stretching
2856		-CH_2inalphaticcompounds	C-H Stretching
1730		C=O Carboxylic acid	C=O Stretching
1629		C=C in conjugated system	C=C Stretching
1460		C-N in heterocyclic	C-N Stretching
1278		C-O in Carboxylic acid	C-O Stretching
1076		C-O in alcohol	C-O Stretching

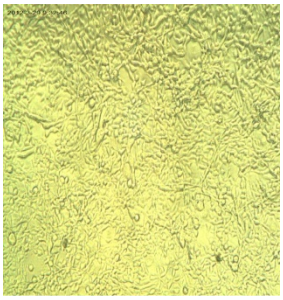
Table (4): FT-IR show the locations of important bands in complex between isolated alkaloid & soybean

Range(cm-1)& intensity	Bond shape	bond	Functional group
3413	St	O-H Carboxylic acid & Alcohol	Hydrogen bonded O-H Stretching
2929		C-H of heterocyclic	C-H Stretching
2858		-CH2inalphatic compounds	C-H Stretching
1734		C=O Carboxylic acid	C=O Stretching
1656		C=C in conjugated system	C=C Stretching
1458		C-N in heterocyclic	C-N Stretching
1178		C-O in alcohol	C-O Stretching

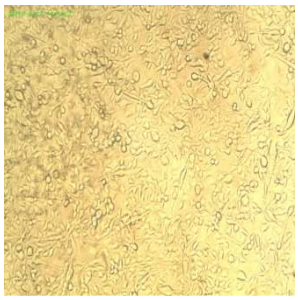
Cytotoxic effect of complex between soybean& isolated alkaloid on RD Tumor cell Lines in vitro

The result of the present study showed that the effect of complex between soybean& isolated alkaloid on RD tumor cell line have been shown in fig 1.2.3 .4 in all periods 24,48,&72h .of the treatment ,the interaction between complex and concentration was significant after 24,48,72h.

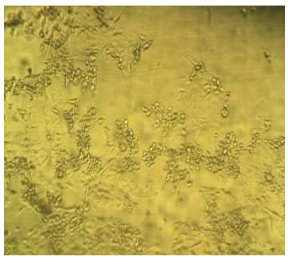
This study found the an inhibitory effect of the complex at high concentration after 72 h .the highly inhibition activity of complex between soybean& isolated alkaloid against the cell line may be explained by different mechanisms .Firstly ,the extract had different inhibitory properties on potassium fluxes ,that clearly inhibit the potassium intake of the cells by inhibit the Na-K ATP ase enzyme activity ,this lead to significant change in the permeability of the plasma membrane that allow the compounds enter to cell and makes disrupt the nitrogen base sequence of DNA [39];



B

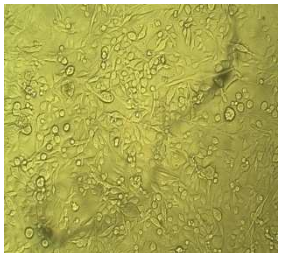


A



E

D



C

Figure (1) RD tumor cell line (A) & (B) Control confluent monolayer (C) Cells treated with 10000 µg\ml complex after 24h (D) Cells treated with 10000µg\ml complex after 48h (E) Cells treated with 10000 µg\ ml complex after 72h

Antibacterial Screening

All of Soybean ,Alkaloid and complex show an activity against the two selected bacteria as shown in Table(5) The compounds show activity against Staphylococcus auras and Escherichia coli ,It is well known from literatures that flavonoid &alkaloids compounds exhibit a wide range of biological activities .

Table (5) Antibacterial activity of the Soybean , isolated Alkaloid &complex

compounds	Gram positive bacteria zone of inhibition (mm)*Staphylococcus*	Gram negative bacteria zone of inhibition (mm)*Escherichia coli*
Soybean	26	26
Isolated Alkaloid	30	18
Complex	40	35

The result show that the complex inhibitors to *Staphylococcus*& *Escherichia coli* more than the isolated &Soybean

REFERENCES

[1] J Gorzynski, McGraw-Hill companies, Inc., **2006**.
[2] F Carey, third ed., McGraw-Hill companies, Inc., **1996**.
[3] Y Fang, S Yang and G Wu , *Nutrition*, **2002**, 18, 872: 879.
[4] T K Basu, cabin publishing, Canada, **1999**.
[5] O Potterat, *Curr.Org.Chem* ,**1997**, 1,415: 440.
[6] W Peschel, F Sanchez ,W Diekmann, A Plesher, D Jimenez, R Lamuela , S Buxaderas and C Codina ,*food Chem.*, **2006**,97,137.
[7] K Schwarz , G Berelsen , L Nissan, R. Gardner, P Heinonen, M Huynh-Ba, T P Lambelet, D McPhail , L H Skibsted and L Tijburg , *Euro .Food Res.Technol*, **2001**, 212, 319 :328.
[8] M MartinezTome, A M Jimenez, S Ruggieri, N Frega, R Strabbioli and M A Murcia, *.J. food Prot*, **2001**,64,1412 :1419.
[9] A Gadow, E Joubert and C Hansmann, *Agric .food Chem.*, **1997**,45,632 - 63
[10] J C E Underwood, 3rd ed.; Churchill Livingstone: Edinburgh, UK, **2000**, pp. 374 : 375.
[11] P Brookes and P.D. Lawley, *Nature* **1964**, *202*, 781:784.
[12] D E Volk, J S Rice, B A Luxon, H J C Yeh, C Liang, G Xie, J M Sayers, D M Jerina and D G Gorenstein, *Biochemistry* **2000**, 39, 14040 :14053.
[13] M E Smela, M L Hamm, P T Henderson, C M Harris, T M Harris and J M Essigmann, *Proc. Natl. Acad. Sci.* USA, 2002, 99, 6655

[14] N Mokhtar, The national cancer institute. Cairo University. **1998**.
[15] M Heron, D Hoyert, S Murphy, j Xu, K Kochanek, and B TejadaVera, National Vital Statistics Reports, **2009**, 57:1:134.
[16] V Devita, S Hellmany and Rosenberg's, principles and practice of oncology .5th ed .Lippincott -Raven publishers Phiadelphia,**1997**.
[17] D Newman, G Cragg and K Snader, , *J. Nat.Prod*., **2003**, 66:1022.
[18] P C Butler, J J Meier, A E Butler and A Bhushan, *Nature Clinical Practice Endocrinology and Metabolism*, Vol. 3, **2007**, pp. 758 -7.
[19] Sae ede, Thesis College of Pharmacy. Mosul University Mosul Iraq**, 2004**.
[20] A Ibrahim, PhD. Thesis University of Baghdad .Baghdad .Iraq, **2005**.
[21] A Al-Dabhawi, PhD. Thesis .Baghdad University. College of Science Al-Mustansiriya University, **2005**.
[22] J W Anderson, J E Blake, J Turner, and B M Smith, *American Journal of Clinical Nutrition*, **1998**, Vol. 68, 1347S :1353S
[23] M Messina, *Journal of Nutrition*, **1995**, Vol. 125, 567S: 569S68 trition, Vol. 68, 1347S: 1353.
[24] G Peterson and S Barnes, *Biochemical and Biophysical Research Communications*, **1991**,Vol. 179, 661: 667.
[25] G Peterson and S Barnes, *Prostate*, **1993**,Vol. 22, 335: 345S.
[26] S J Bhathena, and M T Velasquez, *American Journal of Clinical Nutrition*, **2002**,Vol. 76 ,1191: 1201.
[27] M Messina , *American Society for Clinical Nutrition*, **1999a**,Vol. 70, 439: 450.
[28] M Messina, *American Journal of Clinical Nutrition*, **1999b**, Vol. 70, 574: 575.
[29] K W Al-samarraie, MSc Thesis College of science, University of Baghdad, **1983**.
[30] P Gayon , 1st ed Oliver and Boye Edinburge, **1972** .
[31] N Alshahaat, Dar Al-Behaar. Beirut,**1986**.
[32] P Hawk, and H Sumer Son, Practical physiological chemistry.13th ed .,McGraw-Hill. Book Com, **1954**
[33] R Saadalla , Basrah University press.Basrah-Iraq,**1980**,Pp:54
[34] J Harborne, 2nd ed Chapman and Hall, London ,**1984**.
[35] M G Nair, A R Putnam, S K Mishra, M H Mulks, W H Taft and J R Miller, *J .Natural Product*, **1989**, 52,797: 809.
[36] G jayaprakasha, R Singh and Zachariah, *Food Chem*., **2001**,73 -285.
[37] G Marco, *Jam. oil Chem. Soc*., **1968**, 45, 594.
[38] M AL-Saikhan, L Hward and J Miller, *J .Food Sci*., **1995**, 60, 341.
[39] L Betancur – Galvis, J Granados , H Salazar, and J Ossa, *Mem. Inst*., **1999**, 94(4), 101:106.