

PROTOSCOLICIDAL ACTIVITY OF AQUEOUS AND ALCOHOLIC EXTRACTS OF *JUNCUS RIGIDUS* (JUNCACEAE) *IN VITRO*

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ABSTRACT

The aim of the present study was to evaluate the protoscolicidal activity of aqueous and 70 % alcoholic extracts of the seed of the plant (Semmar) *Juncus rigidus* with different concentrations (5,10,15,20) % .

The results showed that all concentrations have the ability to kill the protoscolices at 4-8 days after treatment .

The aqueous extract of the concentration (20)% showed the ability to kill all the protoscolices at the fourth day after treatment more than any concentration according to R.L.S.D. test.

The aqueous extract in all concentrations have more effect than the alcoholic extract on killing the protoscolices at the same concentrations.

Hank's reservative solution has been proved to keep the control group alive up to (20) days.

INTRODUCTION

Juncus rigidus (Juncaceae), the common name is samara and assla. It is grown native in Iraq specially in Basrah.

In germination and propagation testing, *J. rigidus* has a wide ecological tolerance.[1] (Figer-1-) . *In vitro* studies have shown that phototoxins from *J. effusus* enhanced antimicrobial activities in light and have antifungal activity to *Candida albicans*. [2] *In vivo* the alcoholic extract of *J. acutus* showed significant anti-eczematic activity.

The isolation of five phenolic glycosides which responsible for this activity were isolated and identified as oxyresveratrol 2-OB-D-glucopyranoside 1 resveratrol 3, 4-O, 0-di-B-D, canthoside B4, and caffeic acid glucopyranoside 5. glycopyranoside 2 markhamioside F3 compound 3 and 4 were found to have the highest activity; they cured eczema by 90 and 100 % respectively .[3]

Cystic echinococcosis is an important parasitic disease caused by a small taeniid-type tape worm *Echinococcus granulosus*.

Hydatidosis refers to the existence of larval cystic stages called hydatid cyst and/or rotoscolices in normal intermediate hosts, generally herbivorous as well as in human accidentally infected with this parasite .[4]

General ;

Juncus rigidus (family Juncaceae); description: a tufted , perennial rhizomatous herb with inflorescences to 60 cm tall; of salt-marsh and brackish pools in Saharan oases and coastal sand-dunes. The common names in West African : GHANA AKAN-FANTE Kokyi; ARABIC semmar , sumir, uses in general Agri-horticulture : ornamental, cultivated or partially tended. Products : household, domestic and personal items. Root; Medicines : dropsy, swellings, oedema, gout.[5]

The *Juncus rigidus* represented wild plants from seven species *Atriplex portulacoides* , *Zygophyllo domingensis* , *Cyperus leavigatus*, *Arthrocnemum macn* , *Phragmites australis* ; utilize from the akesl's vegetation , as biomonitors in comparison with monitor for heavy metal pollution as biofilters. [6]

The aim of this study is to determine the efficiency of the different concentration of aqueous and alcoholic extracts of *J. rigidus* on killing the protoscolices of *Echinococcus granulosus* in serial period times.

Sampling and extraction;

Juncus rigidus was collected from area between closely watery region in Garmat Ali (north Basrah). The studied part of the plant included the seeds, extracted according to [7] method with some modifications:-

25 gm of dried plant mixed with 100 ml of distilled water, left for 24 hr. on magnetic stirrer, then filtration of the extract was done with the helping of Vacuum pump using filter paper Whatman NO-1 . the filtrate was concentrated using Rotary vacuum evaporator . The extract put in clean sterilized bottle after drying until using . Four concentrations were made from the extract (5,10,15,20) % .

The same previous method used in alcoholic extraction using 70% ethanol as solvent instead of distilled water, and the concentrations (5,10,15,20) % was made.

Hydatid cyst : The livers of liver sheep were collected from the central slaughter of Basrah and the protoscolices serial step of covering and sterilized methods:

The liquid of the cyst was has been withdrawn by 10 ml cyringes after sterilization of the external surface of the cyst with alcohole. The liquid was put in steril glass beaker, also the whole germinal layer was isolated as a whole pecice put it in sterile Petri dish. Then cut it in to small pieces , washed with Hank's solution containing Penicillin and Streptomycin, then filtered to get the protoscolices only , collected in steriled glass flask, then washed with Hank's solution 3-5 times after little shaking then allowed 15 minutes to precipitate , and the filterate wasa collected finally [8].

Hank's solution (pH= 7.2)

0.800 gm Nacl
0.400 gm Kcl
0.100 gm MgSO₄.7H₂O
0.048 gm Na₂HPO₄
0.018 gm Cacl₂.2H₂O
0.350 gm NaHC0₃
0.060 gm KH₂PO₄
0.100 gm Mgcl₂.6H₂O
0.100 gm C₆H₁₂O₆

The volume was completed to 1 liter of disttiled water using filter paper 0.45 μ

Examination of the viability of protoscolices using an aqueous Eosin dye 1% :

A little pit of the liquid of hydatid cyst with scolices put on slide adding one or two drops of aqueous, then examined microscopically . The a live protoscolices took green color while the dead once took the red color (Fig -1-) because of the permeability of the dye through its membrane [9].

Calculation of protoscolices :

The protoscolices were calculated using the method of fixed volume transfaring with micropipette 10 μl , the whole number of protoscolices was calculated in volume using Dissecting microscope for three times.

The number of protoscolices calculated in one milliliter as follows :

Number of protoscolices in 1 ml = their number in 10 μl X 100

Experimental designing of protoscolicidal activity of extracts :-

The activity and effect of an aqueous and alcoholic extract of *Juncus rigidus* on killing the protoscolices *in vitro* .

After the collection of the protoscolices and their activity calculation, 1 ml of the scolices suspension containing 1500 scolices washed with Hank's solution was moved and treated with different concentrations of aqueous and alcoholic extracts of *Juncus rigidus* (5,10,15,20) % for three times , and one tube as control . The percentage average of protoscolices killing in serial period times was calculated .

RESULTS

Table -1- shows the results of effects of the seed extract of the plant on protoscolices killed in serial period times (days). In control group the Hank's solution keeps protoscolices a live up to 21 days.

In general the concentration 20 % of the seed extract has a great effect on protoscolices killing, while 5 % has the lowest effect.

The concentration 10 % , 15 % killed all protoscolices in the 8th days and 7th day respectively.

Table (1) : Effect of various concentrations of extract of *Juncus rigidus* on killing of protoscolices in serial period times.

Average of protoscolices killing / time after treatment								
Time after treatment Conc. gm / ml	1	2	3	4	5	6	7	8
5 %	15	20	38	50	78	91	94	100
10 %	24	34	44	57	86	95	95	100
15 %	27	45	56	59	88	96	100	100
20 %	16	57	78	100	100	100	100	100
Control	0	0	9	15	20	22	21	25

R. L. S. D. = 7.14



B

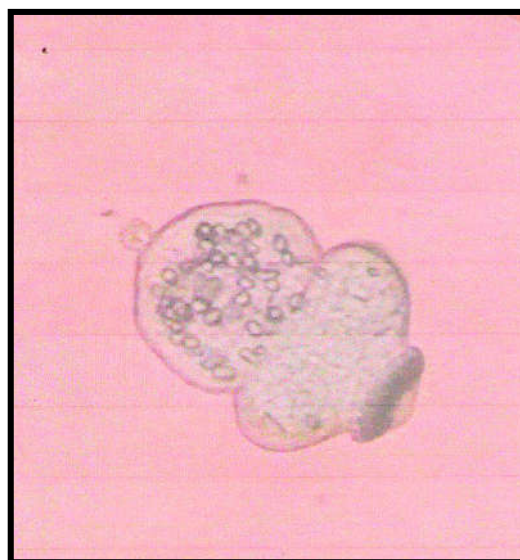


A

Image of the plant (semmar) *Juncus rigidus* (A & B)



B



A

Fig -1- Protoscolices of *Echinococcus granulosus*

A - Life protoscolex

B – Dead protoscolex



Image illustrate the axial part of the plant *Juncus rigidus*



Image illustrate the Rhizomes of the plant *Juncus rigidus*

DISCUSSION

Results of the present study showed that various concentrations of the aqueous extract of *Juncus rigidus* have protoscolicidal activity against protoscolices of *E. granulosus*. This finding agree with that reported in folk Arabic medicine, that this *Juncus rigidus* could be used in treatment of many infections and physiological disease .[10]

The antidermal and antimicrobial activity of *Juncus rigidus* may due to the presence of phenolic glycosides which responsible for this activity . [3]

There is another study on the isolation and identification from *Juncus rigidus* of the phenanthrene , dehydroeffusol [1] and the dihydrophenanthrene , Juncusol 2, compounds that enhance antimicrobial activities in light. [2]

The death of protoscolices after treatment with *Juncus rigidus* seeds extract may be resulted from interaction with the biotic components of protoscolices , such as polymerization of tubulin and blind the same site on the tubulin dimmer as that for colchicines .

الفعالية المضادة للرؤيسات الأولية لطيفلي المشوكة الحبيبية *Echinococcus granulosus*

للمستخلصين المائي والكحولي لنبات الأسل (السمار) (*Juncaceae*) *Juncus rigidus* مختبرياً

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الخلاصة

تم في الدراسة الحالية تقييم الفعالية المضادة للرؤيسات الأولية لطيفلي المشوكة الحبيبية للمستخلص المائي والكحولي لبذور نبات الأسل (السمار) *Juncus rigidus* بتركيزات مختلفة (٥ ، ١٠ ، ١٥ ، ٢٠) % ، أظهرت جميع التراكيز القابلية على قتل الرؤيسات الأولية في (٤ - ٨) أيام من بدء المعاملة بالمستخلص . أظهر المستخلص المائي بتركيز (٢٠) % القدرة على قتل الرؤيسات في اليوم الرابع من بدء المعاملة أكثر من التراكيز الأخرى وحسب التحليل الإحصائي R.L.S.D. . كما إن المستخلص المائي بالتراكيز جميعها أمتلك تأثيراً أكبر من المستخلص الكحولي في قتل الرؤيسات الأولية ولنفس التراكيز . وحفظت الرؤيسات الأولية في محلول هانك كمجموعة سيطرة لأكثر من (٢٠) يوماً .

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