ejpmr, 2018,5(9), 81-84

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

CONTRIPUTION STUDY TO THE CHEMICAL COMPOSITION OF THE *RUMEX CRISPUS* L. FAMILY POLYGONACEAE, WIDE SPREAD IN EUPHRAT REGION IN SYRIA, AND DETERMINATION OF THE ANTI-PROTOZOA ACTIVITY

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Article Received on 29/06/2018 **Article Revised on 19/07/2018** **Article Accepted on 08/08/2018**

ABSTRACT

Rumex crispus L. Family Polygonaceae is widely spread plant in the Euphrates region in Syria, where is make a big trouble for the farmer and viliger in this region. We conducted this study to find out the benefits of this plant, by determination the main phenolic components in the aerial parts and the anti oxidant and anti leishmania activity. The samples of *Rumex crispus* were obtained from the Euphrat river by the city of Alraqqa in north east of Syria, dried and the phenolic compounds were extracted with ethanol 80% using soxhlet extraction method. The extracts was concentrated, the main phenolic compounds were detected using chromatographic methods and general reagents for phenols. Total phenolic compounds evaluated using Folin-Ciocalteu method. The Anti oxidant activity was determined using *β*-carotene bleaching method. Anti leishmania activity is estimated on isolates from pathogenic leishmania prepared from public health laboratories and Hospital of Dermatology in Damascus, where determnined the anti leishmania activity. In this study, found that the main phenolic components present the extracts were flavonoids, anthraquinones in the free and combeined state, and high concentration of chlorogenic acid and other organic acids. The percentage of the phenolic compounds was 6.3% and 1.7% (ether extract and ethyl acetate extract). The anti oxidant activity was 94.5% and 22.1% in both extracts. The anti Leishmania activity was determined in both extracts but the the ethylacetat extracts showed higher activity against growth of leishmania than the ether extract.

KEYWORDS: *Rumex crispus* L., phenolic compounds, anti oxidant activity, anti Protozoa activity.

INTRODUCTION

Rumex crispus L. Family Polygonaceae Native to northern Africa (i.e. Algeria, Egypt, Libya, Morocco and Tunisia), the Canary Islands, Europe, western and northern Asia (i.e. Afghanistan, Iran, Iraq, Lebanon, Syria, Turkey, Armenia, Azerbaijan, Georgia, Kazakhstan, Kyrgyzstan, Tajikistan, Uzbekistan, Russia, Mongolia, China, Japan, Korea and Taiwan), the Indian Sub-continent (i.e. Pakistan) and parts of south-eastern Asia (i.e. Myanmar and Thailand). (AnjenLi, Alisa E.et al).

In Syria R. c. plant is widely distributed in Euphrat region especially in the river, so it make a big problem to the farmer and viliger.

Rumex c. is long-lived (i.e. perennial) herbaceous plant that forms a basal rosette of leaves at first, and later produces upright (i.e. erect) flowering stems with much-branched flower clusters up to 1.5 m tall. The plant has basal rosette of large curly leaves at first; it eventually produces a number of upright leafy stems, which branch into densely clustered inflorescences. The stem leaves are smaller, narrower and stalkless. Its inflorescences are green at first, but become reddish-brown as the fruit mature. The Achenes brownish, ovoid, trigonous, 2.3 mm, apex acuminate. (fig. 1).

Fig. 1: Rumex crispus plant in Euphrate river origin.

Physiological activity and uses of Rumex crispus
Root and Seed of rumex c. used in Dentrifrice, Chronic dysentery and nausea and in Hepatic disorders. (Suleyman H, et ale 1999).

Chemical Constitutents of Different Species of Rumex
Litereture study showed that the main chemical compounds of some of Rumex spp. were investigatigated and determined as 1,5-Dihydroxyanthraquinones and an Anthrone from Roots of Rumex Crispus. The separation of 1,5-dihydroxy-3-methyl anthraquinone;1,3,5-trihydroxy-6 hydroxymethyl anthraquinone;1,5-dihydroxy-3-methoxy-7-methyl anthraquinone by micellar electrochromatographic method from the root of Rumex crispus. (Günaydin K, et ale).

The aim
1-Invistigation and determination the Phenolic compounds in the aerial parts of Rumex crispus L.
2- Determination of the antioxidant and anti -Protozoa activities

MATERIALS AND METHODS

1- Plant material
The plant material of *Rumex crispus* L. Family Polygonaceae was gatherd in autumn 2015 from Euphrates region in Syria L.

2- Chemicals
All used chemicals have analytical purety from Fluca.

3- Apparatus: Soxhlet (100 g), rota vapore and spectrophotometer 5100 from Secomam.

4- Extraction of the Plantmaterial
Plant material was dried and the phenolic compounds extracted from the aerial parts using soxhlet extraction method. 100 g of powederd dry plant material extracted using 500 ml of Methanol/ Water mixture (80%) for about 6 circles, and the extract was concentrated and dried under vaccum. (Mhd isam hasan agha, et ale 2016).

5- Determination of total phenolics content
Total phenolic compounds of *R. crispus* plants determined by Folin-Ciocalteu, using colorimetric method (Gulcin et al., 2002).

Dried extracts deluted with about 10ml. methanol and 1 mL of the solution (10 mg dried extract of each) added into a flask. Then, 46 mL distilled water and 1 mL Folin-Ciocalteu reagent was added and mixed thoroughly. The mixture left to stand for 3 min, and 3.0 mL of 2% sodium carbonate added. After 120 min. incubation at ambient temperature with shaking, the resulting.

Absorbance measured at 760 nm. Measurements carried out in duplicate, the calibration curve performed with gallic acid, and the results expressed as μg Gallic Acid Equivalent (GAE)/mg.

6- Determination of antioxidant activity
The anti oxidant activity was determind in both extracts (ether, Ethylacetate) using β-carotene bleaching method described by Kaur and Kapoor (2002). BHA and BHT used as standards. All samples assayed in duplicate. Degradation rate (DR) calculated according to first-order kinetics, using the equation:
$\ln(a/b) \times 1/t$ = DRsample or DRstandard where ln is natural log, a is the initial absorbance (470 nm) at time 0, b is the absorbance (470 nm) at 100 min, and t is time. Antioxidant activity (AA) was expressed as percent of inhibition relative to the control, using the formula:
AA = (DRcontrol − DRsample or DRstandard / DRcontrol) × 100

7- Anti-leishmania activity
Pathogenic leishmania and standard of Leishmania donovani strain AG 83 was collected frome patients in Alraqqa and supplemented with 10% fetal bovine serum

of pH 7.2. The logarithm phases of promastigotes (2×106 cells/ml) incubated with or without the volatile oile along with Medium-199 at 22 °C.

The tested dried extracts dissolved in 0.2% dimethyl sulphoxide (DMSO), it added to the culture in graded dose. After 2 h of treatment, the tubes centrifuged at 8000 g for about 10 min. The supernatant was decanted and the pellets washed with 20mM phosphate buffer saline (PBS). Each pellet was dissolved in 100µl (2mg/ml) MTT solution, the tubes incubated at 22°C for 4 h and then centrifuged at 8000 g for 10 min. The resulting pellets were dissolved in 500µl 0.2% DMSO and the absorbance measured spectrophotometrically at 570nm. (Mhd. Isam Hasan Agha et ale 2018).

% lysis = 100 – {(test – positive control)/(control – positive control)} × 100 ...(1)

4- RESULT AND DISCUSSION

- Total phenolic content and antioxidant activity

Total phenolicin ether extract of the aerial part of *R. crispus* found to be 63 *µ*g/mg dry weight (DW). Phenolic compounds considered the most important antioxidative plant components, and the antioxidant activity of plant materials, well correlated with the content of their phenolic compounds (Elzaawely et al., 2005). Phenolics have also played an important role against chronic diseases (Simopoulos, 2004). The Folin-Ciocalteu procedure is nonspecific because it detects all phenolics (phenolic acids, flavonoids, and tannins) (Niklova et al., 2001), so it does not give details of the quantity and quality of the phenolic constituents of the extracts. Nevertheless, this widely used method provides a rapid and useful overall evaluation of the phenolic content of extracts (Luximon-Ramma et al., 2003). The results for total phenolics of aerial parts of *R. crispus* clearly outline it as a rich phenolics source. Elzaawely et al. (2005) previously reported that total phenolic contents of *Rumex japonicus* Houtt grown in Japan was 200 mg GAE/g extract. The variability could be due to plant species used, environmental factors, and collection period. The antioxidant assayed using the discoloration of *β*-carotene is widely used to measure the antioxidant activity of plant extract, because *β*-carotene is extremely susceptible to free radical–mediated oxidation of linoleic acid (Kumazawa et al., 2002, MHD Isam Hasan Agha et al 2016).

Antioxidant activity of the aerial part of *R. crispus* is coparable with the standard and is no big differences among the anti oxidant activity of rumex c. extract, BHA, and BHT and the result showed statistically importance. Antioxidant activity found to be 94.5% in *R. crispus* extract and 97.5% in BHA and 96.1% in BHT.

4-3- Anti-Protozoa activity of the extract

In vitro anti- Protozoa activity against standard of Leishmania (strain AG83) (tab-1).

Table 1: Shows that the extract inhibite growth of Leishmania in a dose-depended manner.

Concentration	Vitality %
0%	100
16%	81
32%	70.61
48%	51
64%	38
164%	0

Frome the line formula y=-0.542 +82.72 we can conclude the IC50 of the extract of rumex c. as 60 µg/µL (Hmaidan A. et ale 2007).

5-CONCLUSION

Rumex c., which make problem in some origin of Syria it could used as a source for antioxidants and anti protozoa.

ACKNOLEDGMENTS

Dermatological hospital in Damascus University.

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