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Research paper

Biological Activity and Cytotoxic Effect of Iraqi Wild *Mentha arvnesis* Total Flavonoid

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Abstract

The major source of flavors and fragrances raw material is *Mentha arvensis*. The flavonoids were extracted from the leaves of the mature *Mentha arvensis* by reflex, and then the flavonoids of the extract were detected qualitatively with TLC technique. The plant was found to be rich in flavonoids, especially Rutin, Quercetin, Kaempferol, luteolin and others. This study conducted to assess the biological and cytotoxic effect of this purified total flavonoid on cell lines (L20B and MCF-7) and *Leishmania* (*Leishmania tropica* and *Leishmania donvani*). The extract inhibited the growth of cell line to reach a maximum growth inhibition rate 64.5 % at the concentration (5 mg/ml) for L20B and 52.4% for MCF-7 at the concentration (10 mg/ml) in comparison with negative control. Also the concentration 5mg/ml of plant extract caused an inhibition in the growth of both type of *Leishmania* to reach to 30.7% for *Leishmania tropica* and 50.2 % for *Leishmania donovani.*

Keywords*: (Leishmania donvani, Leishmania tropica, Mentha arvensis, Total flavonoid).*

1. Introduction

Plants possess many phytochemicals, studied recently as potent antioxidant, free radical scavenging, and may act as immune-modulating agents as long as have being evaluated for their radio-protective effects. This make herbal drugs offer an alternative to synthetic compounds because they are considered to be either non-toxic or less toxic than their synthetic counterparts (Suresh *et al*, 2014). *Mentha arvensis* which called also Wild mint, is classified as a herbaceous perennial plant. The plant is growing to 10–60 cm (3.9–23.6 in) tall, but rarely may grow up to 100 cm. (Blamey and Grey, 1989). The fruit shape is appearing as a two-chambered carpel. In Europe, the plant was traditionally used to treat different problems as cough, gallbladder flatulence and digestive problems. Preclinical studies in the past two decades have shown that some common medicinal plants as antitumor anticancer were used (Huxley, 1992). Flavonoids are a class of compounds presented broadly in nature. Concerns about their extensive profitable bioactive benefits, including anti-viral/bacterial, anti-diabetic, anti-inflammatory, cardio-protective, anti-cancer, anti-aging, have long been received great attention and well supported by numerous studies, and their daily intake varies between 20 mg and 500 mg, mainly from dietary supplements including tea, apples, onions and tomatoes.(Rakers *et al*,2014;, Giuliani *et al*, 2014). This study aimed to qualify and quantify total flavonoids extracted from Iraqi grown *M. arvensis* (Wild mint) and estimate their anti-cancer and antiparasitic effect especially on the *Leishmania* (*Leishmania tropica* and *Leishmania donvani)*

2. Materials and Methods

2.1. Plant collection and classification:

The samples of the plant leaves were collected from the Abu Ghraib/ Baghdad-Iraq area and were classified in the Biology department, Baghdad University, to give the following data for plant taxonomy

Kingdom : plant
Phylum : Angiospermophyta
Class : Magnoliopsida
Order : Lamiales
Family : Lamiaceae
Genus : Mentha
Species : Mentha arvensis

Samples were taken to the laboratory after cleaning well from the suspended soil. They were dried at room temperature (25 ^{o}C) and manually grinded, then packaged in sterile containers and kept away from direct light until use.

2.2. Extract the total flavonoids from the plant:

About 86 g of powdered plant leaves were placed in a 1 liter glass flask and then added 600 ml distilled water with(10% v/ v) HCl. Reflex extraction was performed for 8 hours continuously to ensure that the cleavage and broken of glycoside linkage of the flavonoids and the aglycone part was obtained. The plant extract was Filtered and cooled. The biologic active part of flavonoids (aglycone part), is taken with 50 ml ethyl acetate and repeated three times using a separating funnel. The acetate layer is collected in clean separating funnel again and an equal amount of distilled water is added to remove access HCl residues used in extraction. The acetate layer is then dried using rotary evaporator at

45 °C. The output is saved to complete the rest of the analysis (Harborn, 1984).

2.2.1. Determination of Total Flavonoids (Qualitative assay):

A stock solution from the extracted total flavonoids was prepared by dissolving (0.150) gm. Residue in 5 ml of 50% ethanol. A standard Rutin, Quercetin, and luteolin solutions were prepared in 50% ethanol also. Thin layer chromatography (TLC) was carried out using a silica gel 60- F_{254} plate with a thickness of 0.1 mm with using the mobile phase Toluene: Ethyl acetate :Formic acid (36 : 12 : 5). The type of flavonoids separated can be detected in corresponding to standard flavonoids spots RF value. This value is derived from dividing the distance travelled by each flavonoid in each model phase to the distance traveled by the solvent:

Rf value=
Distance traveled by each flavonoid
_____
Distance traveled by the mobile phase

Each Flavonoid can be detected separately by the exposure of the silica plate to the UV light as a coloured spot at a wavelength of 254nm. The result is shown as bright spots under the UV light (Kato *et al*, 1999).

2.3. Biological Activity of the Extracted Total Flavonoid

2.3.1. Anticancer activity *(in Vitro)*:

In This Study a general protocol by Chli *et al*, 2004 and Freshney, 2012 were preceded against two types of cancerous cell; the L20B cells and MCF-7breast cancerous cells. Briefly different concentrations of (10, 5, 2.5, 1.125, 0.625, 0.3125, 0.15625, 0.078128) mg/ml test solution were prepared by dissolving (10mg/ml) in medium. Aliquot of 100 µl of each prepared plant extracted flavonoid of each concentration had been added to number of cultured wells to be incubated after all additions at 37^0C overnight to about 24 hours. An extracted solvent such as DMSO (50 µl) was added to all cultured wells and the plate was read at 620 nm with aid of ELISA reader. The percentage rate of inhibition was calculated according to the following formula for each cancer cell line.

Growth Rate inhibition (IR %) =

$$\frac{Control - Treated\ cell}{Control} \times 100$$ (Chli *et al*, 2004)

2.3.2. *In Vitro* Anti Leishmanial activity:

The anti-leishmanial activity efficacy of *Mentha* extracted flavonoids against two species of Leishmanial parasite in promastigote forms; *L. Tropica* and *L.donovani* had been studied in this research by a colorimetric method described by Mahmoud *et al.*2015. Initially about 100 µl from both species of *Leishmania* Promastigotes had been suspended in all 96 well of tissue culture plate in a concentration of (10^6 parasites/ ml). Then 100 µl/ of various concentrations of (10, 5, 2.5, 1.125, 0.625, 0.3125, 0.15625, 0.078128) mg/ml test solution were prepared by dissolving (10mg/ml) in distilled water. An aliquot of 100 µl of each prepared solution of the extracted flavonoid at different concentrations was added to each well and incubated at 26 ^{0}C for twenty four hours. The MTT coloring reagent (10 µl) as freshly prepared solution (5 mg/ml) was added to all wells. The plate was incubated at 26 ^{0} C for at least three hours. Finally, about 50 µl of DMSO had been added to all wells to be incubated another10 min. the control was indicated as Promastigotes cultured in complete medium without plant extract. The absorbances of treated and untreated wells were measured at 620 nm with ELISA reader. The growth inhibition ratio was calculated as follows:

Growth inhibition Rate %
$= \frac{Control - Treated\ cell}{Control} \times 100$ (Mahmoud *et al*, 2015)

3. Results and Discussion

3.1. Total flavonoids from the plant qualitative analysis:

Total purified flavonoids have been extracted from *M. arvnesis* and qualitatively detect by TLC, it was show that the plant was rich in flavonoids, especially Rutin (R), Quercetin (Q), Kaempferol (K), luteolin (L) in comparison with standard flavonoids (Figure 1). The Rf value for extracted flavonoids and standard flavonoid was shown in table 1. *M. arvnesis* is a good source of phenolic compounds such as glycosides and flavone, which have a protective effect against inflammation. The two aromatic rings and the bride of three-carbon atom present of the flavonoid structure gives the plant ubiquitous biological activity (Adele *et al*, 2008)

Fig.1: TLC chromotogram used in the identification of the total flavonoid (Tf) corresponding to standard solutions, (R) rutin, (L) luteolin, (K) kaempferol and (Q) Quercetin.

Table 1: Rf value for extracted flavonoids and standard flavonoids

Flavonoid type	Rf value
Rutin	Base time
Querecetin	0.43
Kaempferol	0.6
Luteolin	0.35
Extracted Total flavonoids	All above spots and others

3.2. *In Vitro* anticancer activity:

The cytotoxic effect of *M. arvnesis* total flavonoids on two cancer cell lines was shown in (Figure 2 and Figure 3). The purified total flavonoids caused a significant inhibition in the growth of cell line (L20B) at all concentration to reach to a maximum inhibition (64.5 %) at the concentration 5mg/ml, on the other side the maximum growth inhibition of MCF-7 cell line was show in the concentration 10 mg/ml to reach (52.4%), while the low growth inhibition percentage was shown in the concentration 5 mg/ml to reach to (2.5 %). A phenomenon appeared in the current study and contributed to the behaviour of the cell is called the (Hormetic effect) (Al-shaybanny, 2006). In this type of effect, stimulation in growth was at low concentrations while the growth was inhibited at higher concentration. It was a biological effect in toxicology representing a contrast effect between stimulation of growth (30-60) % in regard to the control cell growth while the higher concentrations showed a growth inhibition, partially or completely (Baldwin and Calabrese, Baldwin, L.A. and Calabrese, E.J., 2003). Plants Polyphenolic compounds among them the flavonoids act to prevent cancer at all stages (initiation, promotion and progression), through dietary intervention that recently has received an increasing interest (Sonia, 2008). Flavonoids in fruits and dietary foods

possess unique chemical structure that makes them of diverse bioactivities relevant to many pathogens beside cancer as some epidemiological studies have validated to show an inverse relation between cancer risks and the consumption of flavonoids. This means that flavonoids possess cancer blocking and suppressing effects which encourage researchers to emphasis on study them deeply. In one study by *Anup et al*, 2008, found that flavonoids modulate various CYPs, which involved in activation of carcinogen and act as scavenging for reactive species (ROS) formed from carcinogens by CYP-mediated reactions (*Anup et al*, 2008). While another study by *Chandan and co*, 2014 declare that the ethanolic extract of *M. arvnesis* possesses potent cytotoxicity against Human liver cancer Hep G2 cell line; (the human cancer liver cell line) *in-vitro* . All above demonstrated that this plant significantly suppresses growth of Hep G2 cell lines by induces these cells toward apoptosis (*Chandan et al*, 2014).

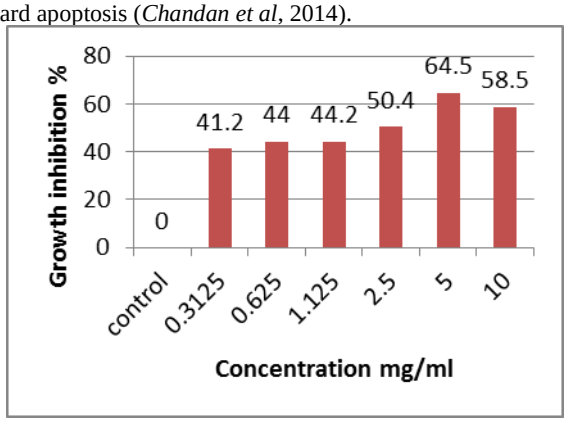

Fig. 2: Cytotoxic effect *Mentha arvensis* total flavonoids extract on the growth of cancer cell line (L20B).

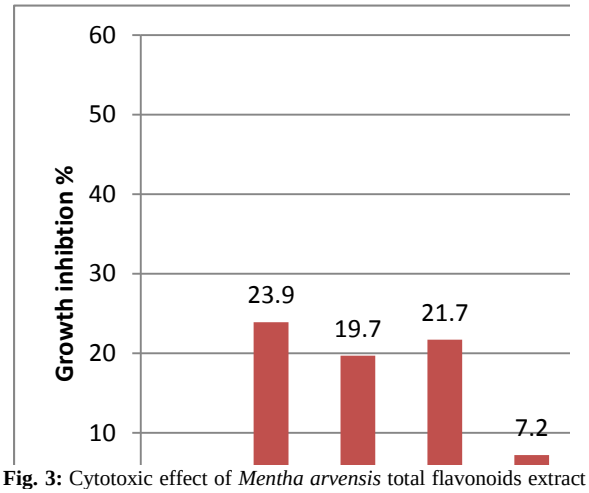

Fig. 3: Cytotoxic effect of *Mentha arvensis* total flavonoids extract on the growth of cancer cell line (MCF-7).

3.3. *In Vitro* Anti Leishmanial activity:

The biological effect of *M. arvnesis* total flavonoids is too obvious toward anti parasitic activity, also the extract showed a decrease in the growth of *L.tropica* and *L.donvani* especially at the concentration (10, 5) mg/ml to reach maximum growth inhibition (29.3%, 30.7%) respectively for *L.tropica* and (35.6%, 50.2 %) for *L. donvani*. (Figure 4, 5). It was shown that different flavonoids of about more than 6000 kinds have been identified. Most of them have an essential function in plants regulation growth and act to provide a protection against various pathogens and may indicated as anti-parasitic agent (Adele, 2008). As the *M. arvnesis* plant was rich with flavonoids, many researches have demonstrated that this species possesses growth inhibitory effects particularly due to the flavonol "Quercetin" and the flavone "luteolin" the plant rich with. For this reason, the wild Iraqi *Mentha* plant show an inhibitory effect against the two type of parasite *Leishmania tropica* (Weiss *et al, 1998*; , Mamani, *et al*, 2004) and *Leishmania donvani* (Sen *et al*,2005) as in figure4 and figure 5.

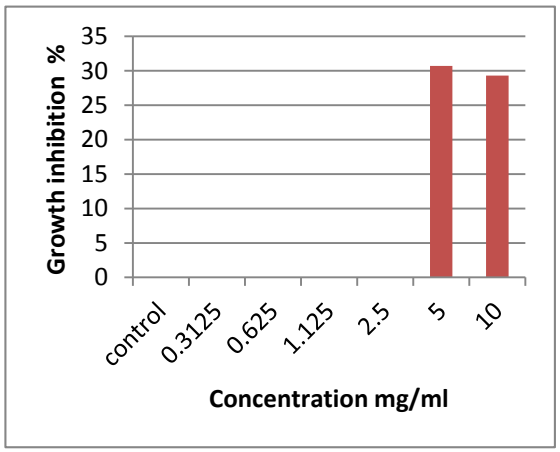

Fig. 4: Effect of extracted total flavonoids from *Mentha arvensis* on the growth inhibition rate% of of *Leishmania tropica*.

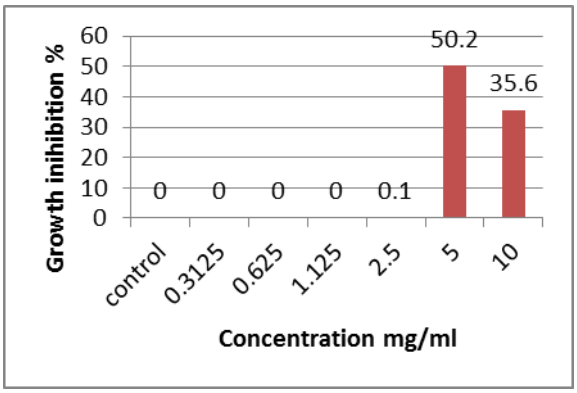

Fig. 5: Effect of extracted total flavonoids from *Mentha arvensis* on the growth inhibition rate% of *Leishmania donvani*.

4. Conclusion

This study conducted to assess the biological and cytotoxic effect of wild Iraqi *M. arvnesis* purified total flavonoid on cell lines (L20B and MCF-7) and *Leishmania* (*Leishmania tropica* and *Leishmania donvani*). The is that the extract inhibited the growth of both cell line in a dose dependent manner in comparison with negative control. Also the concentration 5mg/ml of plant extract caused an inhibition in the growth of both type of *Leishmania*, *Leishmania tropica* and Leishmania *donvani.*

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