

Comparison Between The Efficacy of Different Medical Herbs on *Cryptosporidium* spp.

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Summary:

Objectives:

The study was planned to show the effect of different medicinal plants extracts on *Cryptosporidium* spp.

Materials and Methods:

The experimental study was performed in laboratory mice to show the efficacy of different medical plants extracts (*Achillea fragrantissima*, *Artemisia herba-alba*, *Cardaria draba*, *Mentha longifolia*, *Olea europea*, *Prosopis farcta*, *Punica granatum*, *Teucrium polium* and *Ziziphus spina-charisti* on shedding of *Cryptosporidium* spp. oocysts.

Results:

In a study on the effect of medical plant extracts on the shedding of oocysts in mice, it was found that different medical plants extracts decrease the shedding of *Cryptosporidium* oocysts in mice. Using 250 mg / kg B.W., route it was shown that the efficacy of *Prosopis farcta* (67.6 %) followed by *Artemisia herba alba* (57.7 %), *Cardaria draba* (35.9 %) and the lowest was *Teucrium polium* (12.7 %) but *Mentha longifolia* had no effect. It was also shown that the efficacy of medical plants extracts (500 mg / kg B.W.) was higher than (250 mg / kg B.W.) in shedding of the parasite oocysts.

In a study of the effect of plant extracts on the development of oocysts of *Cryptosporidium* in the macrophage in Rpm medium, it was found that the efficacy of *Punica granatum* (75.9%) was highest, followed by *Prosopis farcta* (67.1%) and *Artemisia herba- alba* (55%).

Conclusions:

The medical plant extracts have an effect on *Cryptosporidium* spp. Infection in albino mice. The possibility of studying the therapeutic research on *Cryptosporidium* spp. in RPM medium.

Key words: Efficacy, medical herbs, *Cryptosporidium*

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Introduction:

Cryptosporidiosis is self-limited illness in immune-competent patients, while it is life threatening in immune-compromised ones. No effective drugs are known for complete recovery. Some used paromomycin others achieved promising results with azithromycin and nitazoxamide (1).

Medical plants are natural sort of treatment and their importance is increasing all over the world. The civilization settled in different parts of the world e.g. Mesopotamia,

Greece, Nile Valley, China and others, registered different plants as antibacterial, antiviral and antiparasitic agents (2).

Scientists during Islamic era introduced numerous plants for treatment of clinical disease of malaria, tapeworms, roundworms and others.

In many countries, due to high cost and side effects of chemical drugs, people returned to herbal treatments instead of chemical ones. Recently, the attention of many scientists is diverted toward the medical plants for treatment of parasitic diseases.

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Several medical plants are described for purpose of treatment of parasitic diseases.

The present study aimed to trial treatment by using different extracts of medicinal herbs.

Materials and Methods:

Nine species of plants used in this study, were collected from Tikrit city and near by areas, they were:

1-*Achillea fragrantissima*. 2-*Artemisea herba-alba* 3-*Cardaria draba*. 4-*Mentha longifolia*. 5-*Olea europea*. 6-*Prosopis farcta*. 7-*Punica granatum*. 8-*Teucrium polium*. 9-*Ziziphus spina-christi*.

The leaves were the only part that was used in this study except *Punica* and *Prosopis* fruit were used.

Collected parts of plants were dried in the air in shade and milled in mortar and pistol. The milled substances were sieved by using fine mesh sieve and the powder material adjusted in dissector containing CaCl_2 in order to complete dryness. All the types of powder were extracted in ethyl alcohol 96% according to Harboren (3). The dried plant powder was extracted in ethyl alcohol (96%) in ratio 1-10 (W/V) mixture was shaken continuously up to 24 hours under 4°C .

The solution filtrate under high pressure in Buchner funnel for expression of the filtration for 15 minutes. The filtrate was collected and evaporated in vaccum rotary evaporator (Buch Rotavapor oil RE 11) under less than 60 C. The rest of the extracted substance (chlorophylic extracts) and water was filtered by using whatman filter paper no.4. The filtrate was returned to evaporator to remove the water. The extracted substances (in dark bottle) were sent to Medical Research Center of Al-Nahrin, College of Medicine for lyophilization. The lyophilized material was stored at 4°C until used.

The effect of lyophilized extracts on the oocysts of *Cryptosporidium* sp. was achieved in two lines in vivo and in vitro.

In vivo study:

A-Nine groups of 5 white Balb c mice, 1 week old were infected with 103 oocysts of *Cryptosporidium* sp. (Collected from naturally infected calf and purified in ether) per os using stomach tube. Feces of infected mice were examined daily for oocysts and counted. After 3 days post infection when the maximum

number of the oocysts was reached, the mice groups were treated with the lyophilized extract in dose equivalent to 250 mg/kg body weight by os using stomach tube. At the 6th day post infection the oocysts were enumerated.

B-The lyophilized extract of higher effect on shedding of oocysts were chosen to another run of treatment with dose of 500 mg/kg body weight as in A.

C-Six groups of 5 mice were infected with 10 4 oocysts, five groups were treated with medical herbs and a sixth group kept without treatment as a control group. The treated and control groups of mice were sacrificed and the intestine were separated for collection and enumeration of the total number of oocysts.

In vitro study:

The cultivation of the oocysts was processed according to Martinez et al. (4).as follows:

A-preparation of the oocysts: oocysts were obtained from naturally infected calf and purified.

B-Preparation of macrophage: Mice of 4-5 weeks old were injected intraperitoneally (I/P) with 5-10 ml of Hank's solution (PBS) pH 7.2. The peritoneal fluid was centrifuged at 2500g for 5 minutes. The sediments, which contained the macrophage were suspended in RPM medium containing 10% of inactivated fetal calf serum (IFCS) incubated at 56°C in water bath.

The incubated macrophages in RPM medium were distributed in wells of microtiter plate of 96 wells as 0.2/ml . The incubation of microtiter plate continued for 24 hours and examined to insure presence of macrophages.

C- Infection of macrophages with oocysts in rate of 1 oocyst / macrophage. The incubation continued in 37°C in the presence of 002 using candle in dessicator (5% 002).

D- Treatment of oocyst with lyophilized extracts: The infected macrophages in micro titer plate were inoculated before incubation with lyophilized extract in concentration 2.5% and 5% in sterilized distilled water with antibiotics 5000 u penicillin (5000 IU) and 5 mg/ ml streptomycin.

Examination of the treated cultures was achieved after 72 hours by spreading drop from the macrophage suspension on a clean slide and stained with Giemsa's stain under

light microscope at 40X, I OOX oil immersions.

Hundred macrophages were enumerated and the development of the parasite *Cryptosporidium* inside the macrophages was examined. The percentage of development of *Cryptosporidium* stages were registered. The rates of three successive readings were done for all the treated and the control wells.

Counting of *Cryptosporidium* oocysts were made by using neubar slide chamber for counting.

Evaluation of efficacy % of the extract:

The efficacy of the extract was done according to Ergon (5) by using the following formula:

$$\text{Efficacy \%} = \frac{\text{Rate No. of the control group} - \text{Rate No. of the treated group}}{\text{Rate No. of the Control group}} * 100$$

Statistical analysis: The student t-test was done to show significant difference between any two groups.

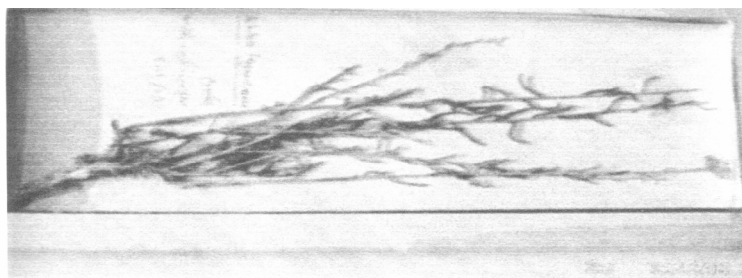


Fig 1. Achillea fragmentisma, upper part

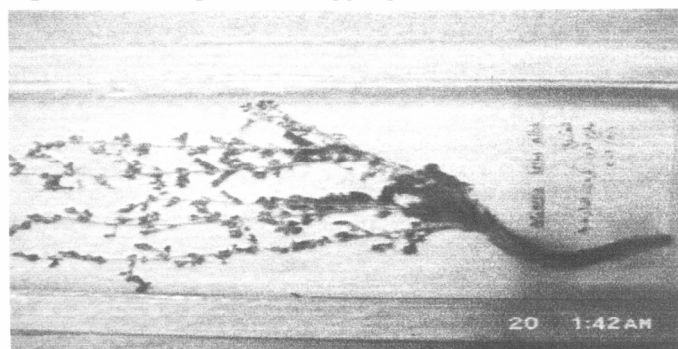


Fig 2. Artemisea herba-alba, whole parts



Fig 3. Cardaria draba, upper parts

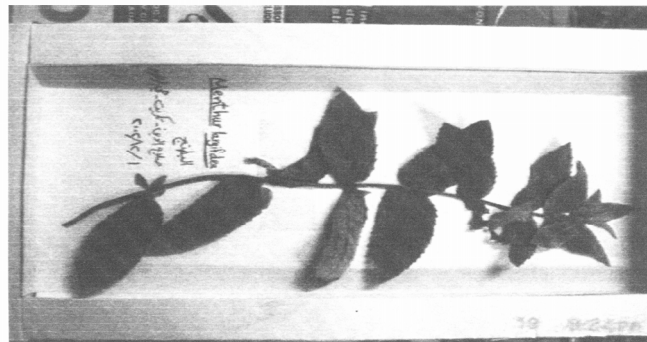


Fig 4. Mentha longifolia, upper part

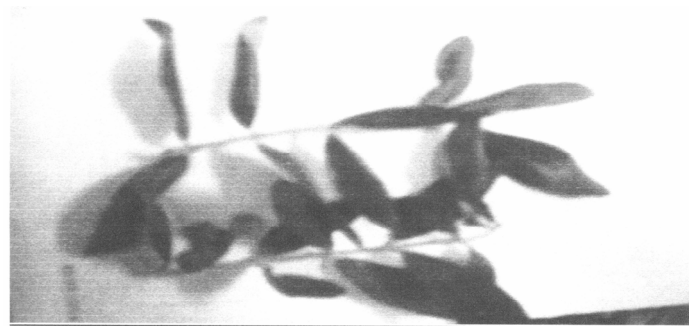


Fig 5. Olea europea, upper part

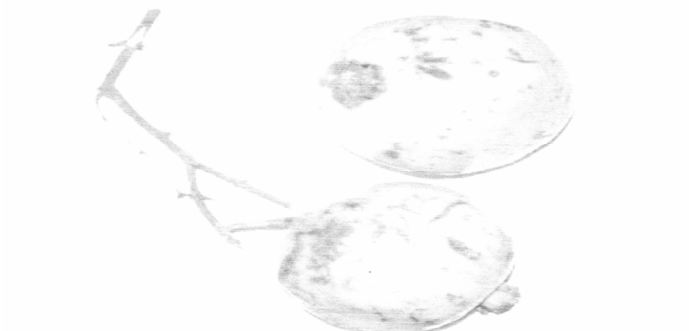


Fig 6. Punica granatum, fruit

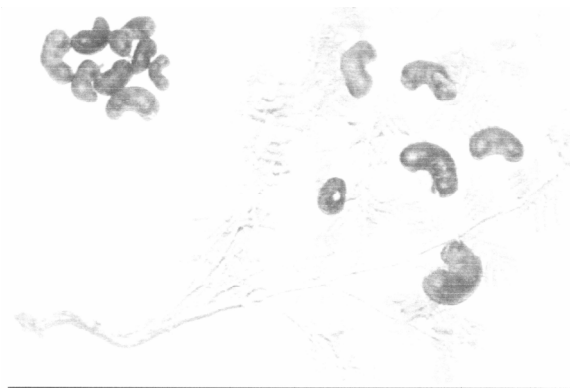


Fig 7. *Prosopis farcta*, whole parts

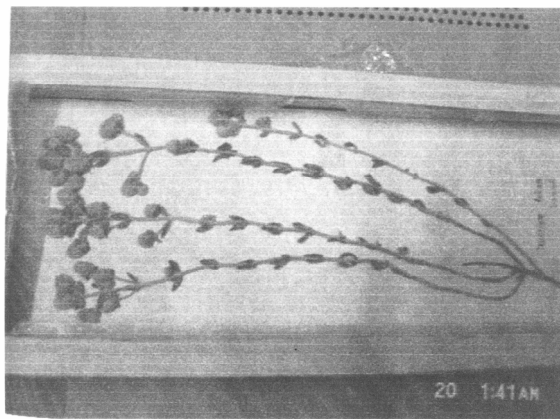


Fig 8. *Teucrium polium*, upper part

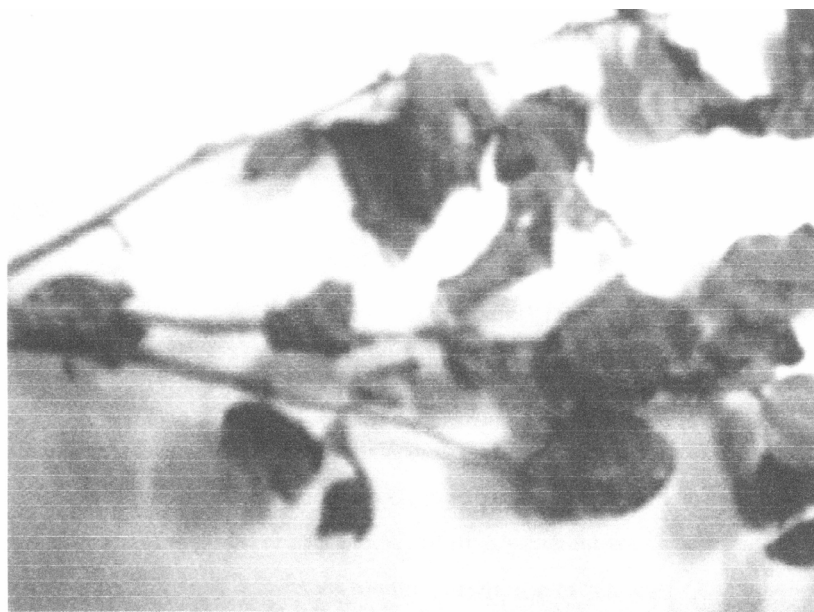


Fig 9. *Ziziphus spina-christi*, upper part

Results:

Plants extracts:

The effect of plants extracts on the shedding of oocysts in mice is indicated in (Table 1), that 250 mg / kg body weight of different plant extracts decrease the shedding of *Cryptosporidium* oocysts in infected mice, with significant difference in the number of oocysts between pre and post treatment (P<0.05).

Table (2) indicates that, the efficacy of 500 mg/ kg B.W. of plants extract on the shedding of oocysts in mice. The efficacy of *Propolis farcta* was highest (70.9 %) followed by *Punica granatum* (66.7%) and the lowest was *Olea europea* (34.5%).

It is clearly observed that the efficacy of 500 mg/ kg B.W. of different plants extract on the total number of *Cryptosporidium* oocysts in the intestine of infected mice was greater than those treated with 250 mg / kg B.W. of the plants extract (Table 3). The difference

between 250 mg / kg B.W. and 500 mg / kg B.W. of plants extract was statistically significant (P <0.05).

In using 250 mg/kg B.W. of plants extract, the efficacy of *Olea europea* was highest (51.7%), followed by *Ziziphus spina-christi* (50%), and the lowest was *Punica granatum* (45%). While using 500 mg / kg B.W. of plants extract, the efficacy of *Propolis farcta* was highest (75.0%), followed by *Olea europea* and *Punica granatum* (71.7%) for each and the lowest was *Ziziphus spina-christi* (41.7%).

Table (4) shows the effect of 2.5% and 5% of *Artemisea herba-alba*, *Propolis farcta* and *Punica granatum* on the development of *Cryptosporidium* oocysts in the macrophage in RPM medium. It was indicated that the efficacy of 2.5% and 5% of *Punica granatum* (52.9% & 75.9%) was highest followed by *Propolis farcta* (43.4% & 67.1%) and *Artemisea herba-alba* (26.3% & 55%) respectively.

Table 1: Effect of plant extracts 250 mg/kg body weight on the shedding of *Cryptosporidium* oocyst in infected mice.

Plant Sp.	No. Oocysts (* 10 ³)		Efficacy	Control Group
	* Prt	6 th day **Post		
<i>Achillea fragrantissima</i>	3.5	2.8	20	3
<i>Artemisea herba-alba</i>	2.6	1.1	57.7	-
<i>Cardaria draba</i>	3.9	2.5	35.9	-
<i>Mentha longifolia</i>	2.6	2.7	0.0	-
<i>Olea europea</i>	3.8	1.7	34.4	-
<i>Propolis farcta</i>	3.7	1.2	67.6	-
<i>Punica granatum</i>	4.8	3.1	35.4	-
<i>Teucrium polium</i>	5.5	4.8	12.7	-
<i>Ziziphus spina-christi</i>	3.1	1.8	41.9	-

* Prt=Pre-treatment

** Post=Post-treatment

P<0.05

Table 2: Effect of some chosen plant extracts in dose 500 mg/kg body weight on the shedding oocysts in infected mice.

Plant Sp.	No. Oocysts (* 10 ³)		Efficacy	Control Group
	* Prt	**Post		
<i>Artemisea herba-alba</i>	3.1	1.5	51.6	3.5
<i>Olea europea</i>	2.9	1.9	34.5	-
<i>Punica granatum</i>	3.0	1.0	66.7	-
<i>Propolis farcta</i>	3.4	1.0	70.9	-
<i>Ziziphus spina-christi</i>	2.8	1.5	46.4	-

* Prt=Pre-treatment

** Post=Post-treatment

P<0.05

Table 3: Effect of chosen plants extract on the total number of *Cryptosporidium* sp. oocysts (*10⁴) collected from intestines of infected mice, using two doses 250 and 500 mg/kg body weight.

Plant Sp.	250 mg	Efficacy %	500 mg	Efficacy %	Control Group
<i>Artemisea herba-alba</i>	3.2	46.7	2.0	66.7	6
<i>Olea europea</i>	2.9	51.7	1.7	71.7	-
<i>Proposis farcta</i>	2.2	46.7	1.5	75.0	-
<i>Punica granatum</i>	2.3	45	1.7	71.7	-
<i>Ziziphus spina-christi</i>	3.0	50	2.5	41.7	-

P<0.05

Table 4: Effect of three plants extracts 2.5% and 5% on the development of *Cryptosporidium* spp. oocysts in the macrophages in RPM medium.

Plant Sp. Extract	Untreated	Treated			Efficacy
		2.5%	Efficacy	5%	
<i>Artemesia herba-alba</i>	80	58	26.3%	36	55.0%
<i>Proposis farcta</i>	76	43	43.4%	25	67.1%
<i>Punica granatum</i>	87	41	52.9%	21	75.9%

Discussion:

Several chemical compounds were tried to cure cryptosporidiosis in man and animals, but their efficacy were controversial. This might be due to mechanisms of colonization of *Cryptosporidium* spp. are not well understood, but it has been suggested that the parasite may utilize a lectin like receptor (6).

Several workers tried medical treatments with chemical drugs. In ruminants, it has been reported that sulfonamide (7), lasalocid (8), halofuginone (9, 10), Paromomycin (II), Deccox R (12), and decoquinate (13).

In man, it is shown that symptomatic improvement of cryptosporidiosis has been achieved by spiramycin in immunodeficient patients (14, 15). Russell et al. (16) reported that azithromycin was beneficial in the treatment of intestinal cryptosporidiosis in immunocompromised patients. In the present study several medical plants extracts were tried (*Achillea fragmentisma*, *Artemisea herba-alba*, *Cardaria draba*, *Mentha longifolia*, *Olea europea*, *Prosopis farcta*, *Punica granatum*, *Teucrium polium*, and *Ziziphus spina-christi*), as alternatives of chemical drugs due to their toxicity and unavailability in the country. The medical plant extracts efficacy was tried in mice as alternative of human and economically important live stocks.

Using 250 mg/kg B.W. of plant extracts, the highest efficient plant extract was *Prosopis farcta* (67.6%), followed by *Artemisea herba-alba* (57.7%); *Ziziphus spina-christi* (41.9%); *Cardaria draba* (35.9%) and *Punica granatum* (35.4%).

Prosopis farcta is a medical plant, its chemical contents are glucosinolate and Prospering and it is used as antidysentric (17). The second effective drug was *Artemisea herba-alba*, its efficacy might be due to its contents of artemisin and santonin. It was used against *Ascaris* parasite (18, 19) and was found to be active against hydatidosis in laboratory mice (20). The anti-parasitic action of *Ziziphus spina-christi*, is also reported and suggested that it might be due to its chemical contents of glucose and fructose. The anticryptosporidial activity of sugar has been found by Harp (21), who found that sucrose and to a lesser extent isomaltose reduced intestinal colonization of *C. parvum* in neonatal mice.

The efficacy of *Cardaria draba* was also high, which also included glucose in its content in addition coumarine, volatile oil, sulphur, isothiocyanate, and glucosinolate and it acted as vermifuge and intestinal carminative (22).

Al-Aubaidy (23) studied the effect of alcoholic extract of *Cardaria draba* plant extract on *Hymenolepis nana*, *Taenia saginata*

and *Enterobius vermicularis*, she observed 10-14 compounds in both the dry and fresh parts of plants, among the compounds determined was *Esculetin* as a coumarine compound. She concluded that the dry and wet extracts of *Cardaria draba* were effective on infertility, egg development, and average eggs output of *H. nana*. In addition to infectivity of hexacanth embryo of *T. saginata* and to eggs, larvae and adults of *E. vermicularis*. She also found the efficacy of alcoholic extract of *Cardaria draba* on *Hymenolepis nana* was (100%), on days fifth and sixth post-infection using 500 and 250 mg/kg B.W. respectively.

Regarding the efficacy of *Olea europea*, it was (34.4%), which contents include Mannite, glucose, oleosterol, resine, olivine, and resine (24). It has an active antiparasitic effect (25).

It is also reported that the efficacy of *Teucrium polium* was (12.7%), which is also reported to be parasite killer. Its chemical contents include beta caryophyllena, Humlene Teucrine (18).

Increasing the dose of plant extracts from 250 to 500 mg/kg B. W. to show the effect of five plants (*Artemisea herba-alba*, *Olea europea*, *Punica granatum*, *Prosopis farcta*, and *Ziziphus spina-christi*) on *Cryptosporidium* oocysts. It was clearly indicated that the efficacy of plant extracts was

increased with increasing the doses of plant extracts.

On a trial to show the effect of three plant extracts (2.5%) and (5%) on the development of *Cryptosporidium* sp. strain in the macrophage in Rpm medium. It seemed that the efficacy of *Punica granatum* was greatest followed by *Prosopis farcta* and *Artemisea herba alba*.

The higher viability and infectivity of freshly isolated *Cryptosporidium* spp. oocysts than those preserved in 10 RPM reflect that the efficacy of fresh oocysts was greater than the preserved ones. In addition to that, this study confirms the possibility of culturing of *Cryptosporidium* oocysts in Montanans for purpose of therapeutic researches.

From the results of this study, it is concluded that the medicinal plant extracts of *Prosopis farcta* have an effect on *Cryptosporidium* spp. infection in albino mice and the possibility of studying the therapeutic research concerning cryptosporidiosis in RPM culture medium.

It is recommended to use various concentrations of medical plant extracts for longer period to establish their efficacy on *Cryptosporidium* spp. oocysts. Further studies should be done about the efficacy *Prosopis farcta* using various doses and study LD50 of its extract and related pharmaceutical studies.

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