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Evaluation of *in vitro* antitoxoplasmal activity of some medicinal plants collected from Al Qassim, Saudi Arabia

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Toxoplasmosis is a protozoal parasitic infection with serious consequences for immunocompromised people. The commercial pharmaceuticals used for treatment cause adverse effects and cannot provide a 100% cure. We investigated on antitoxoplasmal activities of eleven medicinal plants from seven different families used in the Al Qassim region of Saudi Arabia against infectious diseases. The plants were extracted in methanol. The methanolic extracts were evaluated against *Toxoplasma gondii* and the Vero cell line using the MTT assay. The results obtained revealed the maximum inhibitory effects of extracts of *Citrullus colocynthis*, *Blepharis ciliaris* and *Aerva javanica* with IC_{50} values of 27.7, 65.2 and 78 µg/ml, respectively against the parasite. However, at the effective levels of antitoxoplasmal activity extracts, of all the above plant species showed different degrees of cytotoxicity against the Vero cell line with SI values of 1.3, 0.8 and 1.1. Hence, we suggest further studies to isolate the active ingredients are highly recommended.

Keywords: Al Qassim, Anti-*Toxoplasma*, *in vitro,* Medicinal plants, Saudi Arabia
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Natural product research shows the potential to serve as a niche for novel active ingredients, and plants in particular are regarded as highly valuable sources for the screening of bioactive compounds against various pathological conditions, including parasitic diseases. For instance, *Citrullus colocynthis* has also been subjected to phytochemical analysis and applied traditionally in many countries for the eradication of various diseases such as diabetes, cancer, enteric conditions, arthritis, respiratory inflammation and mastitis[1-5]. Similarly, plants of the genus *Aerva* and *Blepharis* have been used as medicinal herbs in several traditional systems of medicine all over the world as diuretics, demulcents, purgatives, emetics and tinder [6-8].

Toxoplasmosis is a cosmopolitan protozoal parasitic infection with serious symptoms, particularly for immunocompromised people[9-11]. In cases of efficient immunity there are no symptoms associated with the disease, but in people of insufficient immune system the disease becomes more fatal with sever and dangerous symptoms. In Saudi Arabia, there is no national systemic serological toxoplasmosis screening program; however, some researchers have conducted studies on the sero prevalence in many areas inside the country. These studies indicated that the sero prevalence ranges between 25 and 51.4% in different regions of Saudi Arabia[12-19]. The best medication for anti-*Toxoplasma* must be efficient against all the stages of the parasites with ability to penetrate inside the cysts as well as its ability to pass via placental barriers and without maternal and fetal toxicity and free from teratogenicity. Never the less, all the applied therapeutics at these days cannot fulfill these criteria[20].

With this mentioned information, the current work is planned for the evaluation of the *in vitro* anti-*Toxoplasma* activity of a select group of 11 medicinal plants from 4 orders and 7 families in the Al Qassim region to explore new, affordable and sustainable therapeutic treatments against *Toxoplasma gondii*. The plants were also tested for their potential cytotoxicity using a Vero cell line.

Methodology

Plant samples and preparation of extracts

The selected plants were collected from the fields in and around the Al Qassim region during their growing seasons (Table 1). Subsequently, the collected plants were identified and authenticated by a

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Table 1 — Plants subjected for the present work

#	Plant Name and Family	Common name	Speciemn Number	Distribution	Phytochemistry	Traditional usage
1	*Heliotropium bacciferum* (Boraginaceae)	(Ar.) Ramram, (Engl.) Heliotrope, Turnsole	HBQU-041511	Najd, South Hejaz, Eastern and Southern Regions.	Alkaloids, saponins, tannins, steroids, terpenoids, flavonoids, glycosides, phenols, sterol, isoprenoids, terpene lactones, monoterpene, monoterpene glucoside, phenolic compounds.	Ulcers, snake bites[26].
2	*Salsola imbricate* (Chenopodiaceae)	(Ar.) Khareit	SIQU-041512	Najd, South Hejaz, Eastern and Southern Hejaz	Triterpene glycoside derivates, scopoletin, bergapol, daphnoretin, bergaptol , isorhamnetin derivatives.	Contraceptive, rheumatic pain, anthelmintic [27].
3	*Bassia eriophora* (Chenopodiaceae)	(Ar.) Qutaynah	BEQU-041513	Widespread	Alkaloids, carbohydrates, glycosides, phytosteroids, phenolic compounds, saponins, terpens, tannins and flavoides .	Wound healing[28].
4	*Aerva javanica* (Amaranthaceae)	(Ar.) Ara, (Engl.) Dessert Cotton	AJQU-041514	Widespread	Hentriacontane, nonacosane, heptacosane, pentacosane, octacosane, triacontane and hexacosane, steroids, terpenoides, fats, flavonoids, tannins, saponins, alkaloids, sulphates, carbohydrates and glycosides.	Antidiarrheal, Tooth pain[29].
5	*Rumux vesicarius* L. (Polygonaceae)		RVQU-041515		Proteins, organic acids (malic, citric, oxalic acids), ascorbic acid and tocopherols, lipoid constituents.	hepatitis, digestive diseases, nausea, tooth pain, against inflammation, anticancer as well as antiparasitical, and antibacterial activities[30].
6	*Zilla spinosa* (Brassicaceae)	(Ar.) Shubrum, Silla, (Engl.) Spiny Zilla	ZSQU-041516	Najd, South Hejaz, Eastern and Northern Regions	Campestero, spinasterol, Beta-sitosterol, Alpha-amyrine, B-amyrine, squalene, quercetin, kaempferol.	Kidney stones[31].
7	*Eremobium aegyptiacum* (Brassicaceae)	(Ar.) Ghurayra, Sleila.	EAQU-041517	Najd, Nufud Region	Flavonoids[32]	Not found
8	*Morettia parviflora* (Brassicaceae)	(Ar.) Rabol	MPQU-041518	Najd, North Hejaz, Southern Region.	Not Found	Not Found
9	*Cleome amblyocarpa* (Capparidaceae)	(Ar.) Kenaza, Efana, (Engl.) Spider flower	CAQU-041519	Najd, Eastern Region	Dammaraneterpenoid.	Anti-inflammatory, antidiuretic[33].
10	*Citrullus colocynthis* (L.)Schrad in L. (Cucurbitaceae)		CCQU-041520		Flavonoid quercetin, Flavone c-glucosides and other alcohols, ketones, acids, epoxy compunds and hydrocarbons.	Antidiabetic, anti-inflamatory, mosquito larvicidal and anticancer[34].
11	*Blepharis ciliaris* (L.) B.L. Burtt in Tackh. (Acanthaceae)		BCQU-041521		Phenols, apigenin glucoside, apigenincoumaroyl glucoside, flavones, flavonones and isoflavone glycoside caffeic acid ester.	Antimicrobial, anti-inflammatory, treatment of cough and kidney disorders[35].

taxonomist and specimen vouchers were prepared and preserved at the Botany Department Herbarium, Qassim University, Saudi Arabia. The aerial parts (whole plants except the root) of collected plant material were shade dried, reduced to fine powder using a laboratory blender, passed through a 60-mesh sieve (BS), packed in screw caped containers then stored at 4°C in order to be used later[21].

The material of each plant (500 g) was soaked in 5 L of analytical grade methanol at 25°C temperature for overnight. Whatman No.1 filter paper was used for filtration of the extract. The residue was transferred to a container, extracted again with 2.5 L of fresh methanol for 16 h and then followed by filtration. Then all filtrates were pooled and evaporated under vacuum using a rotary vacuum evaporator at 45°C until dryness occurred. Then the weight of dried extract was calculated to note the yield % and stored in airtight containers at 4°C for later use[21].

Evaluation of the anti-Toxoplasma activity of crude extracts

An active form of *T. gondii* RH strain was obtained from Dr. S. El-Ashram (State Key Laboratory for Agrobiotechnology, China Agricultural University, Beijing, 100193, China) and proliferated in a Vero cell line (ATCC® CCL-81™, USA). 100-cm^2 culture flasks were used for culturing parasites in complete RPMI medium supplied with 10% fetal bovine serum (FBS) and then incubated at 37°C and 5% CO_2. *T. gondii* tachyzoites (RH strain) were maintained, by serial passage, in Vero cells grown in RPMI medium with 10% FBS. Tachyzoites were collected and preserved in liquid N_2 at a concentration of 6×10^6 parasites/ml.

Plates of 96 wells were used for culturing Vero cells (5×10^3 cells/well in 200 µL RPMI 1640 medium with 10% FBS) at 37°C and 5% CO_2. Then one day later, after removing the medium, PBS was used for washing the cells and getting rid of non-adherent ones. After that, RPMI 1640 medium supplied by 2% FBS containing *T. gondii* tachyzoites at a parasite to cell ratio of 5:1 was added. Followed by incubation at 37°C and 5% CO_2 to 4 h, then PBS was used for washing the cells to get rid from free parasites. The cells were then treated as described below in RPMI 1640 medium with 2% FBS and incubated at 37°C and 5% CO_2.

1	Control	Medium
2	Positive Control	Medium + Atovaquone (Reference: 50, 25, 12.5, 6.25µg/ml)
3	Experimental	Medium + Plant Extract (50, 25, 12.5, 6.25µg/ml)

After 72 h, toluidine blue with concentration of 1% was used for staining the cells. Inverted photomicroscope was used for the determination the *T. gondii* infection index (the number of cells infected from each 200). The inhibition percentages of the infection index were calculated according to the following equation.

Inhibition % = ((1control-1experimental)/1control) X 100

Wherein, I *Control* means the infection index in untreated cells, while I *Experimental* means the infection index in drug/extract-treated cells. IC_{50}: 50% reduction of infected cells compared to the control cells (those exposed to culture medium alone, without extracts or reference drug)[21].

Toxicological evaluation of crude extracts in vitro using the MTT assay

Toxicological evaluations were performed to confirm the safety or toxicity of the plant extracts against the cells of the host according to the plant extract concentration that can be safely used without negatively affecting cell viability. Plates of 96-well were used for culturing Vero cells at concentration of 5×10^3 cells/well/200 µl for one day in complete RPMI 1640 medium supplied by 10% FBS and 5% CO_2 at 37°C. PBS was used for washing the cells. The cells were treated with atovaquone for 72 h (positive control) or plant extracts at varying concentrations (50, 25, 12.5, 6.25 µg/ml) in 10 % completed medium with serum. This medium was used with cells as negative control. Then, supernatant was removed, and 50 µl of plain RPMI 1640 medium with 14 µl of MTT (5 mg/ml) was applied, and allowed to stay at room temperature for incubation period of 4 h. Next, the supernatant again removed gently. For dissolving the water-insoluble formazan salt, 150 µl of DMSO was added. FLUO star OPTIMA spectrophotometer was used at 540 nm in order to read the colorimetric reaction values produced from MTT. Cytotoxic results were presented as the concentration that may give 50% reduction in the viability of the cells (CC_{50}) compared to the control cells (those treated only with medium, without an extract or reference drug)[21].

Statistical analysis

The data were presented as the mean ± SD of triplicate determinations. Wherever applicable, the data were analyzed by ANOVA and significant differences between the groups were analyzed by Tukey's post hoc test. Values were considered

significant at $p \leq 0.05$ and $p \leq 0.001$. IC_{50} values, wherever applicable evaluated by linear regression.

Results and Discussion

The anti-*Toxoplasma* assay demonstrated that all the plant extracts possess toxoplasma inhibitory properties of varying degrees.

The maximum inhibitory percentage was recorded for *C. colocynthis*, which inhibited *T. gondii* up to 84% with a 50 µg/ml concentration, while the minimum was recorded as 5% for *B. eriophora* at same extract concentration of 50 µg/ml. As expected, the inhibitory percentage decreased with decreases in extract concentration, with *A. javanica* having the highest antitoxoplasmal activity of 24% at 6.25 µg/ml. Only 2 other plant had inhibitory percentages of ≤ 10%, while the remaining 8 plants were completely ineffective at the low extract concentration of 6.25 µg/ml as shown in Figure 1.

The cytotoxicity assay revealed that all 11 plants extracts show varying degrees of cytotoxicity, ranging from 35% to 83% for the highest extract concentration of 50 µg/ml. The minimum extract concentration at which all the samples were cytotoxic was 12.5 µg/ml. However, at 6.25 µg/ml, 6 plant samples demonstrated cytotoxicity ranging from 2% (*B. eriophora)* to 16% (*C. amblyocarpa* and *R. vesicarius*), while the rest of the 5 samples were non cytotoxic. The cytotoxic trends are further elucidated in Figure 2.

Calculations of the IC_{50}, CC_{50} and Selectivity Index (SI) of the plant extracts highlighted the therapeutic efficacy of *A. javanica* and *C. colocynthis* with SI values of 1.111 and 1.317, respectively.

The other 9 plant extracts demonstrated relatively low therapeutic efficacy with SI values falling below 1. Although *M. parviflora* had a calculated SI value of less than 1.277, its higher CC_{50} value indicates that the extract is the least toxic among all 11 samples. Table 2 provides a detailed overview of the IC_{50}, CC_{50} and SI values for each plant extract.

From time immemorial, medicinal plants have proved to be a better source for drug discovery and the elimination of various types of diseases[22]. Many of the highly active and efficient drugs against protozoan diseases were developed from the products of medicinal plants such as quinine and artemisinin[23]. In the present work a group of eleven medicinal plants commonly used locally in Al Qassim, KSA for the eradication of various types of infectious disease were examined for antiparasitic activity against the *T. gondii* RH strain *in vitro*. Among them, only *C. colocynthis* has an IC_{50} less than 30 µg/ml (27.7 µg/ml), which indicates good activity and promising results for future isolation of the active ingredient/s. In previous studies, *C. colocynthis* proved to be very potent, with many types of biological activities, e.g., the crude ethanolic extracts of the aerial parts of the plant was found to possess potent antimicrobial

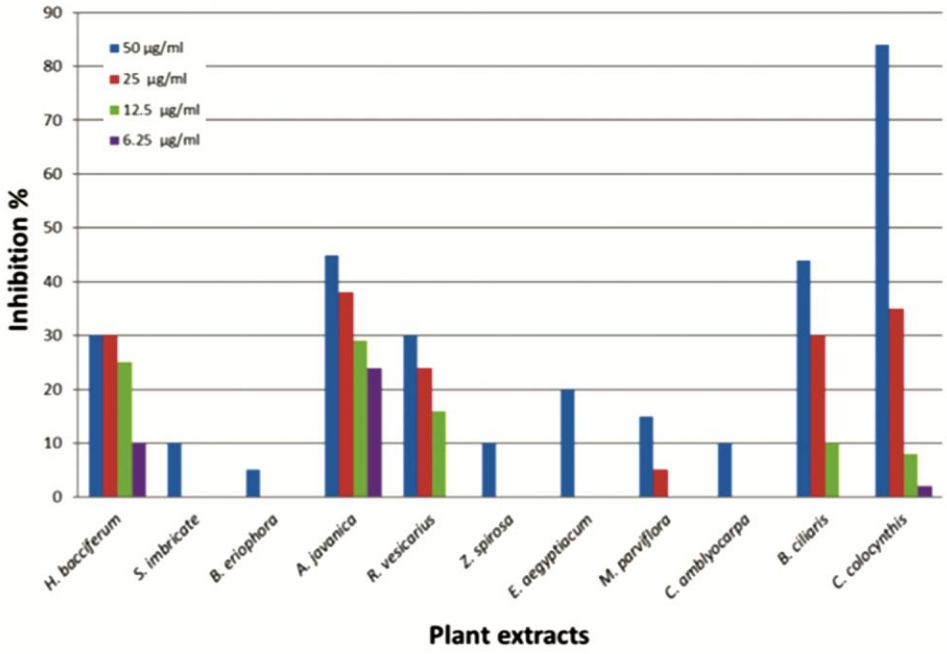

Fig. 1 — Antitoxoplasmal activity of selected plant methanol extracts

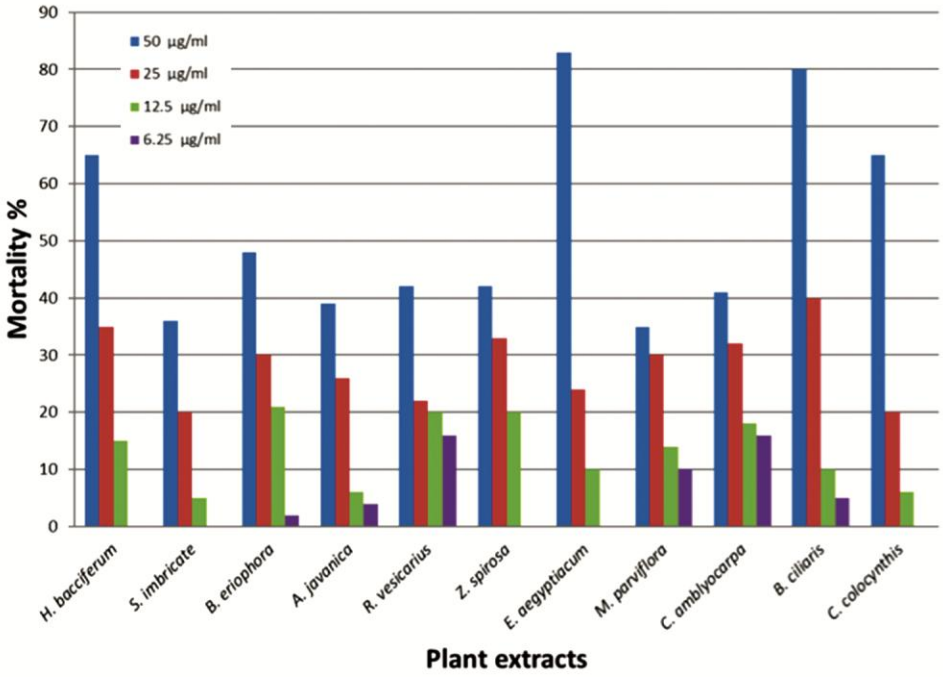

Fig. 2 — Cytotoxic activity of selected plant methanol extract against the Vero cell line

Table 2 — IC_{50} (antitoxoplasmal activity), CC_{50} (cytotoxicity) and SI of plant extracts

S. NO	Plant name	IC_{50} (µg/ml)	CC_{50} (µg/ml)	SI=CC_{50}/ IC_{50}
1	*Heliotropium bacciferum*	> 100	38.28	> 0.38
2	*Salsola imbricate*	>100	> 100	-
3	*Bassia eriophora*	>100	54.1	<0.54
4	*Aerva javanica*	78	86.7	1.112
5	*Rumux vesicarius*	> 100	98.7	< 0.98
6	*Zilla spirosa*	>100	63.4	<0.63
7	*Eremobium aegyptiacum*	>100	29.04	<0.29
8	*Morettia parviflora*	> 100	>100	-
9	*Cleome amblyocarpa*	> 100	96.4	< 0.964
10	*Blepharis ciliaris*	65.16	54.25	0.833
11	*Citrullus colocynthis*	27.69	36.48	1.317

property against different types of standard and clinically isolated bacteria and fungi[24]. The plant is rich in groups of secondary metabolites such as polyphenols, glycosides and fatty acids, which were identified as the main agents acting against *T. gondii*[25].

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Conclusion

Therefore, we can consider the presence of these secondary metabolites in *C. colocynthis* as the major source for its antitoxoplasmal activity. We can conclude that *C. colocynthis* methanol extracts have good antitoxoplasmal activity, but further study for isolation of the active ingredient is highly recommended, particularly to avoid the cell toxicity observed in this study.

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Conflict of Interest

None

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