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Original paper

Thymus vulgaris, *Mentha piperita* and *Elettaria cardamomum* against *Trypanosoma evansi in vitro* and in an animal model with new insights for the treatment of trypanosomosis

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ABSTRACT. Trypanosomosis is a worldwide disease that affects human and livestock populations with limited availability and high cost of trypanocides. The study aims to evaluate the possible *in vitro* and *in vivo* anti-trypanosomal activity of *Thymus vulgaris* (thyme), *Mentha piperita* (mint) and *Elettaria cardamomum* (cardamom) aqueous extracts against *Trypanosoma evansi* in experimentally infected rats using Intropar as a reference drug. The crude extracts of the selected plants were used in three concentrations (2500, 2000, and 1000 µg/ml). The *in vitro* trypanocidal activities were assessed regarding parasite motility, count, and infectivity. The *in vivo* susceptibility of *T. evansi* was evaluated by assessing the level of parasitemia in the experimental rats. The packed cell volume (PCV) was also monitored. Both the *in vitro* and *in vivo* experiments showed trypanocidal activity, of all the tested extracts, higher than that of Intropar. The *in vitro* trypanocidal effects were dose-dependent and represented by a significant reduction of the parasite count together with immobilizing effect within 3 hours incubation period, compared to the negative and positive controls ($p < 0.05$). The *in vivo* trypanocidal effects of the different concentrations of all the tested extracts were represented by the significantly lowered levels of parasitemia, compared to the negative control (NC) group with varying degrees; in a dose-dependent manner concerning the time. They exhibited also a significantly higher level in PCV recovery compared to the NC group ($p < 0.05$). This study initially confirmed the potent *in vitro* and *in vivo* trypanocidal effect of the three extracts, with a potentially promising future for the treatment of trypanosomosis.

Keywords: anti-trypanosomal, *Thymus*, *Mentha*, *Elettaria*, rat

Introduction

Trypanosome is a single-celled flagellar protozoon belonging to the Family Trypanosomatidae genus *Trypanosoma* that comprises several pathogenic species: *Trypanosoma brucei rhodesiense* and *T. brucei gambiense* are causing human African trypanosomosis (HAT) or human sleeping sickness, whereas *Trypanosoma brucei brucei* causes nagana in cattle. In the last decades, there is an alarming increased number of cases of trypanosomosis in both humans and animals with a high economic burden [1,2].

Trypanosoma evansi is the etiological agent of surra; a form of animal trypanosomosis [3] that has been classified by WHO as an OIE notifiable multispecies animal disease [4].

It has the widest world's geographical distribution among all the pathogenic trypanosomes and can naturally parasitize almost all domestic and many wild mammals [5]. umans have long been considered to be refractory to *T. evansi* infection [6]. This is due to the presence of a trypanolytic factor in human serum that makes human infection by animal species of *Trypanosoma* impossible. However, the zoonotic potential and the capability of *T. evansi* to resist

human plasma in certain circumstances have been demonstrated [7].

According to Gupta et al. [8], the increasing number of human cases over time [9–15] may herald an era when we will observe that the parasite is spreading to humans and cause disease with a broader distribution even more than *T. brucei* [16].

Despite the economic and animal health burdens of *T. evansi*, it has been still severely neglected in terms of awareness, control and research into improved control means [17]. Since it has been difficult to develop an effective anti-trypanosomal vaccine in the immediate future due to the problem of antigenic variation produced by the strains of the parasites, the control of human and animal trypanosomosis has depended mainly on a few old chemicals [18,19]. Unfortunately, currently available drugs are toxic, expensive, lack efficacy and suffer from the rapid resistance and high recurrence rates developed by the parasites [20,21].

For example, trypanocides as diminazene aceturate, suramin, and imidocarb dipropionate have a destructive toxic effect on both the trypanosomes and the host organs, as they deposit metabolic wastes in the liver and kidneys, causing damage of these organs [22]. On the other hand, these drugs are not easily accessible to rural African patients, who have great suffering from the disease [23]. In this context, more effective and accessible natural products for the treatment of trypanosomosis are needed [24].

The WHO estimated that 80% of the population of developing countries including those where sleeping sickness occurs, rely on traditional medicine mostly plant drugs for the treatment of this disease and other parasitic diseases [25,26]. However, only a few of these herbs have been evaluated for their in vivo trypanocidal effect [19] and there are not enough data available on their safety and efficacy [27].

Thyme (*Thymus vulgaris*) is one of the most thoroughly investigated aromatic plants. It contains thymol, an active component that is known with its broad anti-microbial activates [28], antioxidant effect [29,30], fungicidal [31], anti-mutagenic properties [32] and antitumor activities [33]. Furthermore, *Thymus vulgaris* possesses potential anti-protozoal activities against *Giardia lamblia* and *Trichomonas vaginalis* [34]. In addition, the recent immunomodulatory effects of *Thymus vulgaris* in the experimental autoimmune encephalomyelitis model have been demonstrated [35].

Elettaria cardamomum (cardamom) a cooking spice of Zingiberaceae family has its seeds used commonly. It possesses a wide range of potential medicinal applications as an antioxidant, anti-inflammatory, anti-diarrheal, anticancer, a cytotoxic, and a cardio-protective [36].

Mentha is one of the world's oldest and most popular herbs. It is a member of the Lamiaceae with almost cosmopolitan distribution [37]. Extracts and essential oil of *Mentha* species have been documented to possess different pharmacological activities encompassing antimicrobial activity, anti-inflammatory, anti-carcinogenic, antioxidant, analgesic, and insecticidal properties [38–40].

The current study demonstrates, for the first time, the trypanocidal activity of thyme (Thymol), mint (*Mentha piperita*) and cardamom *(Elettaria cardamomum*) aqueous extracts against *T. evansi in vitro* and in rat models of infection.

Materials and Methods

Plant material collection and identification

Thymus vulgaris, *Mentha piperita,* and *Elettaria cardamomum* were collected in November 2019 from a local market in Assiut Governorate, Assiut, Egypt. Plants verification was done by Dr. Zedan Z. Ibraheim with voucher numbers of 3-2015, 6-2016 and 10-2016, at the Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Egypt.

Preparation of extracts

Aqueous extracts were prepared according to the method described by Sokmen et al. [41]. Briefly, plant materials were washed 2–3 times with running water and once with distilled water. Shade-dried powdered aerial parts of all plants (200 grams) were extracted separately with 70% ethanol by maceration and percolation at laboratory temperature for 24 hr. The process of extractions was repeated twice. The alcohol extract of each plant was pooled together and evaporated under reduced pressure at 45°C till free from solvent. The alcohol-free residue of each extract was weighted. 2.5 mg of each extract was dissolved in 1 ml of distilled water and 3-5 drops of tween®80 were added to dissolve the extracts. Serial dilutions of these stock solutions were done using distilled water to obtain concentrations of 2500, 2000, and 1000 µg/ml.

Standard trypanocidal drug

Commercial diminazene aceturate (Intropar®,

Pharma-Swede-Egypt), containing 1.05 g diminazene aceturate and 1.31 g phenazone, was used to validate the bioassays and to provide reference values [42].

Experimental animals

Healthy Wistar male rats (150–200 g), aged 3–4 weeks, purchased from the animal house, Faculty of Medicine, Assiut University, Assiut, Egypt, were used for conducting experiments. Stool and blood samples from each rat were examined microscopically three days before the experiment to ensure that all rats were free from any infections. The animals were maintained in well-ventilated laboratory cages with 12 hours day/night cycles and in standard environmentally controlled conditions (22±1°C, relative humidity 65±5%). The rats were fed on a standard pellet diet containing 24% protein with water *ad libitum*.

Test organism

The strain of *T. evansi* (JX888091) originated from a naturally infected dromedary camel (*Camelus dromedarius*) [43] was used throughout the experiments. *Trypanosomes* were kept in experimental rats by a continuous passage at the massive parasitemia (every 3 days) using intraperitoneal (i.p.) injection of 0.1 ml heparinized infected blood from a donor rat's eye. To detect parasitemia, the tail snip method was used. Blood was examined using wet blood film [44].

Preparation of trypanosomes for the in vitro assessment of anti-trypanosomal activity

At massive parasitemia of rat, when trypomastigotes reached 63 tryp/microscopic field (x 1000) magnification, 2 ml blood with about 1×10^6 *T. evansi*/ml was collected from a donor rat's eye in EDTA tube.

Assessment of the in vitro anti-trypanosomal activity was performed in triplicates in 96 wells flat bottom microtitre plates (Flow laboratories Inc., Mclean, Virginia, USA). In the wells of the plate, 40 µl of the infected blood was incubated at 37°C with 20 µl of each extract concentration (2500, 2000, and 1000 µg/ml). 20 µl of Tween® 80 and EDTA separately were used as negative controls (NCs) while intropar was used as a positive control (PC).

The susceptibility of *T. evansi* trypomastigotes to treatments was assessed through motility assessment, parasite count, and infectivity. Motility assessment was performed by the rapid matching method according to Herbert and Lumsden [45]. Trypanosomes were counted using a Neubauer chamber according to the methodology described by Da Silva et al. [46] and observed under a powered light microscope (Olympus CX 22) (x 400) magnification. Both assessments were performed each 15 min for 3 h.

The infectivity assessment

For infectivity assessment, the drug incubation infectivity test (DIIT) [47] was used where 0.1 ml/per 100 g rat's bodyweight (b.w.) of the content of each well, after 3 h, was inoculated i.p. into three non-infected experimental rats. Tail snip was used daily from each rat and checked for the presence of trypanosomes using a wet blood test. Rats that survived for 10 days without detected trypanosomes; were not further checked; assuming that they were well beyond the incubation period of the trypanosome. Those acquiring the infection were further checked until the death of the infected rats [18]. The experiments were carried out in triplicates and repeated three times.

The in vivo assessment of anti-trypanosomal activity

Sixty rats were divided into twelve groups (5 rats each). Eleven groups were i.p. infected with 0.1 ml *T. evansi* per 100 g b.w. Exactly 1 ml of each extract concentration (5, 10, and 15 µg/kg/day) was administered i.p. once daily for 10 days starting from the day that parasites were first detected in the bloodstream (day 2 post-infection) for nine groups. 0.5 ml/100 g b.w. of Intropar was given i.p. to an infected group to be a positive control (PC) group. Another infected group was left untreated as a control negative one (NC). The last group was kept uninfected nontreated for comparison.

Motility assessment and parasitic count (degree of parasitemia) were monitored every 2 days by tail blood examination method for 30 days, the period of the experiment. The presence and degree of parasitemia were confirmed by Giemsa staining of thin blood smear in each examination and counting the parasite under a light microscope in 10 fields (×1000) magnification. An average mean trypanosomes count was taken as the number of trypanosomes per field after counting parasites. All samples were run in triplicate [44].

Acute toxicity study

This was carried out by intraperitoneal injection of 5000 mg/kg of each extract into three healthy

Table 1. The *in vitro* effect of thyme (*Thymus vulgaris*), mint (*Mentha piperita*) and cardamom (*Elettaria cardamomum*) aqueous extracts on the motility of trypanosomes

	Conc. (µg/ml)	Trypanosomes motility after being incubated for (h)					
		0.5	1.0	1.5	2.0	2.5	3.0
Thymus vulgaris	2500	+++	++	++	++	-	-
	2000	+++	++	++	++	-	-
	1000	+++	+++	++	++	++	-
Mentha piperita	2500	++	+	-	-	-	-
	2000	++	+	-	-	-	-
	1000	+++	++	++	+	+	-
Elettaria cardamomum	2500	++	+	-	-	-	-
	2000	++	+	-	-	-	-
	1000	+++	++	++	+	+	-
Intropar		+++	++	+	-	-	-
Tween 80		+++	+++	+++	+++	+++	+++
Sodium heparin		+++	+++	+++	+++	+++	+++

Explanations: +++: many actively motile trypanosomes; ++: reduced motility of trypanosomes; +: very few motile trypanosomes (very sluggish motility); -: full inhibition of motility

rats. The extract was considered non-toxic if none of the rats died or showed signs of acute toxicity during the 30 days observation period [23].

The packed cell volume (PCV)

Blood was taken from each rat by tail snip and the PCV was estimated for each rat in all the experimental groups. The PCV of all rats was determined by the microhematocrit method three times during the experiment; pre-infection, during high parasitemia, and terminal after treatment. The PCV was determined by centrifuging heparinized blood in a capillary tube (also called a micro-hematocrit tube) at 10,000 RPM for 5 minutes. This separates the blood into layers. The volume of packed red blood cells divided by the total volume of the blood sample gave the PCV. Because a tube is used, this was calculated by measuring the lengths of the layers [48].

Ethical considerations

Protocols for this experiment were approved by the research and ethics committee of the Faculty of Medicine, Assiut University, with ethical approval No. 17300375. A standard protocol was drawn up following the Good Laboratory Practice (GLP) regulations of the World Health Organization (WHO). The principles of laboratory animal care were also duly followed in this study.

Statistical analysis

The results were presented as the mean (±SEM). Differences among the means were compared by the one-way analysis of variance (ANOVA), using the SPSS version 11.5 software program. Differences were considered significant when $P < 0.05$.

Results

In the present study, the crude aqueous extracts of thyme (*Thymus vulgaris*), mint (*Mentha piperita*), and cardamom *(Elettaria cardamomum*) showed trypanocidal potentials against *T. evansi*.

The in vitro test

Motility assessment

The *in vitro* effect of the aqueous extracts of thyme (*Thymus vulgaris*) on *T. evansi* revealed that thyme, at concentrations of 2500 and 2000 µg/ml, was capable of inducing immobilization of the parasites after 2 h. In a subsequent concentration of 1000 µg/ml, there was no parasite motility after 2.5h. Both *Mentha piperita* and *Elettaria cardamomum* induced immobilization of the parasite at the concentrations of 2500 and 2000 µg/ml after one h, while after 2.5 h. at a concentration of 1000 µg/ml. In the PC, the reference drug had immobilized the parasites in 2 h., while the NCs showed parasites motility for more than 3 h (Table 1).

Effect of the crude extracts on parasite count

All the tested extracts showed a trypanocidal dose-dependent effect on *T. evansi* trypomastigotes. The concentrations of 2500 µg/ml and 2000 of thyme extracts significantly reduced the number of parasites after 150 min observational period. Parasite count was also significantly reduced at the concentration of 1000 µg/ml but to a lesser degree (Fig. 1). Both *Mentha piperita* and *Elettaria cardamomum* induced a significant reduction of the parasite count at 2500 µg/ml and 2000 µg/ml concentrations after 120 min and to a lower extent at the concentration of 1000 µg/ml (Fig. 2,3). In the PC

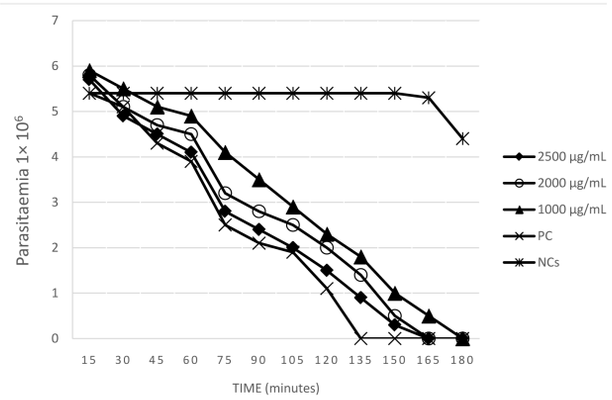

Figure 1. The *in vitro* dose-dependent trypanocidal effect of thyme (*Thymus vulgaris*) on parasitemia, compared to the reference drug. PC: positive control; NCs: negative controls

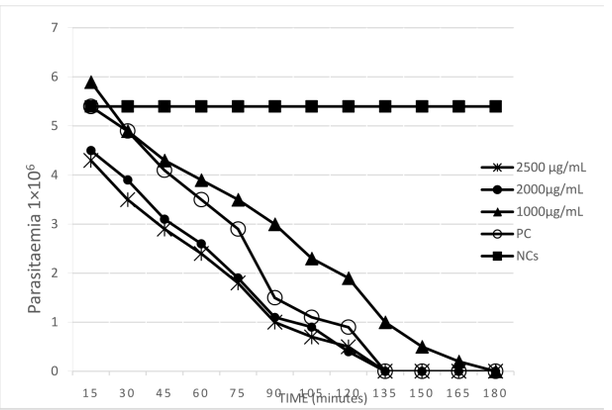

Figure 2. The *in vitro* dose-dependent trypanocidal effect of mint (*Mentha piperita*) on parasitemia, compared to the reference drug. PC: positive control; NCs: negative controls

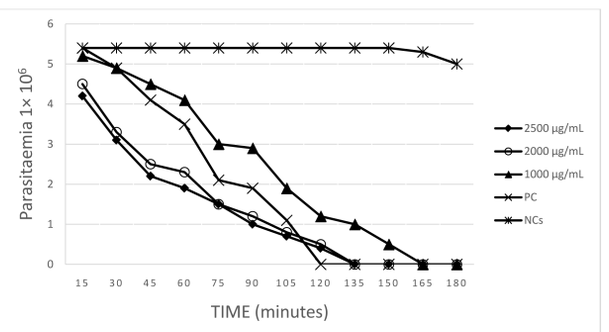

Figure 3. The *in vitro* dose-dependent trypanocidal effect of cardamom (*Elettaria cardamomum*) on parasitemia, compared to the reference drug. PC: positive control; NCs: negative controls

Table 2. Effect of thyme (*Thymus vulgaris*), mint (*Mentha piperita*) and cardamom (*Elettaria cardamomum*) aqueous extracts on the infectivity of trypanosomes to experimental animals, after incubation period

	Conc. (µg/ml)	Trypanosomes infectivity after incubation period		
		No. of mice inoculated	Infection/ parasitemia	Survival of mice
Thymus vulgaris	2500	3	N	S*
	2000	3	N	S*
	1000	3	N	S*
Mentha piperita	2500	3	N	S*
	2000	3	N	S*
	1000	3	N	S*
Elettaria cardamomum	2500	3	N	S*
	2000	3	N	S*
	1000	3	N	S*
Intropar		3	N	S*
Tween 80		3	P•	NS•
Sodium heparin		3	P•	NS•

Explanations: N: parasitemia and infection negative; P: parasitemia and infection positive; S: all rats survived the 10 days infectivity observation period; NS: none of the rats survived the 10 days infectivity observation period; •: rats died 4 days after inoculation; *: statistically significant

Table 3. The *in vivo* effect of thyme (*Thymus vulgaris*), mint (*Mentha piperita*) and cardamom (*Elettaria cardamomum*) aqueous extracts on parasitemia in rats experimentally infected with *T. evansi* through 30 ds observational period

	Conc. µg/ml	Mean number of parasites per microscopic field /d															
		d/N	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30
Thymus vulgaris	2500	5	39	44*	33*	26*	19*	23*	16*	19*	7*	3*	0	0	0	0	0
	2000	5	45	49	53*	44*	29*	31*	27*	16*	9*	5*	2*	0	0	0	0
	1000	5	49	55	63	47*	42*	39*	31*	43*	29*	13*	12*	8*	0	0	0
Mentha piperita	2500	5	33	37*	34*	15*	26*	19*	23*	9*	4*	0	0	0	0	0	0
	2000	5	39	45	35*	29*	17*	22*	25*	18*	7*	3*	0	0	0	0	0
	1000	5	44	39*	32*	28*	19*	15*	23	13*	9*	5*	4*	0	0	0	0
Elettaria cardamomum	2500	5	35	56	49*	33*	28*	19*	13*	9*	7*	0	0	0	0	0	0
	2000	5	40	35*	33*	39*	31*	25*	18*	12*	8*	3*	0	0	0	0	0
	1000	5	42	37*	37*	32*	29*	27*	22*	19*	13*	8*	7*	0	0	0	0
PC		5	38	27*	18*	11*	4*	0	0	0	0	0	0	0	0	0	0
NCs		5	43	55	65	75	82	96	110	122	135	143	154	163	175	220	245

Explanations: d: day of infection; N: number of rats per group; *: significantly lowered the level of parasitemia (P<0.05)

group, there was a significant reduction of trypomastigotes count and total lysis of them after 120 min. The number of parasites remained constant in the NC group in the 3 h observational period and started to decrease gradually onward.

The infectivity assessment

As the extracts could immobilize the trypanosomes, they rendered them not infective to rats (Table 2). All rats of the treated groups survived the 10 days infectivity observational period. While none of the rats of the NCs survived and all died within the observational period with a statistically significant difference (P<0.05).

The in vivo test

The parasites were detected in the peripheral blood of all the infected groups on the 2nd d. post-infection. In the NC group, there was a progressive increase in parasitemia observed until the end of the experiment (30 d.). One out of the five rats of this group died in the 24th d. and two of the remaining four rats died in the 26th d of the experiment. Whereas in the PC group, the parasites started to decrease per high power fields from the 2nd d. post-treatment till disappeared from the bloodstream on day 12 p.i. and the rats survived till the end of the observational period. Treatments with all the tested extracts, with the different concentrations, significantly lowered the level of parasitemia (P<0.05), compared to the NC rats, with varying degrees, in a dose-dependent manner concerning the time (Table 3). Only 2 rats, treated with thyme (*Thymus vulgaris*) and mentha (*Mentha piperita*) at the concentrations of 2000 µg/mLand 1000 µg/mL respectively, died in the middle of the experiment.

Acute toxicity study

None of the tested extracts showed signs of acute toxicity or fatality in the rats that were inoculated by 5000 mg/kg of each extract.

The packed cell volume

The NC rats showed a significant fall in the PCV, during the high parasitemia; indicative of anemia (compared to the uninfected non treated group). However, the administration of the drug control to the PC animals had significantly reduced the magnitude of the decline in the PCV after treatment. The administration of the extracts at the various concentrations, in this study, showed significantly (P<0.05) higher level in PCV recovery compared to the NC group (Table 4).

Table 4. The effect of thyme (*Thymus vulgaris*), mint *(Mentha piperita)* and cardamom (*Elettaria cardamomum*) aqueous extracts on the PCV in rats experimentally infected with *T. evansi* at the period of recovery

Extracts and controls	Conc. (µg/ml)	N	PCV%
Thymus vulgaris	2500	5	*43.20±5.12
	2000	5	*41.13±2.5
	1000	5	*39.34±4.72
Mentha piperita	2500	5	*45.00±3.9
	2000	5	*43.80±1.4
	1000	5	*35.52±1.31
Elettaria cardamomum	2500	5	*49.80±3.9
	2000	5	*45.00±2.8
	1000	5	*42.13±1.64
PC		5	*51.24±3.2
NC		5	•28.00±2.45
Uninfected nontreated		5	53.20±4.72

Explanations: PCV: packed cell volume; N: number of rats per group; PC: positive control; NC: negative control; * significant increased PCV (P< 0.05) compared to NC group; • significant reduction in PCV (P< 0.05) compared to the uninfected nontreated group

Discussion

Trypanosomes are parasitic haemoprotozoa that infect humans and animals, cause morbidity, mortality, and economic losses worldwide [42]. The currently used treatments of trypanosomiasis are challenged with many problems as drug resistance, expensiveness, and toxicity [20].

There is a great resemblance in the morphology and the molecular characterizations between *T. evansi* and other trypanozoon species, including *T. brucei brucei*, *T. b. gambiense* and *T. b. rhodesiense* [49]. Some studies suggested that *T. evansi* could be originated from *T. brucei* [50], others suggested that they are closely related at the genetic level [51]. So, *T. evansi* was used as a model for controlling the human sleeping sickness because of its morphological and molecular similarities to *T. b. gambiense* and *T. b. rhodesiense*. It was previously said that humans cannot be infected with animal species of *Trypanosoma* because of the presence of a factor in the human serum which has a trypanolytic effect. However, *T. evansi* might have the power to overcome the species barrier and resist human plasma [51,15].

In the present study, the aqueous extracts of thyme (*Thymus vulgaris*), mint *(Mentha piperita*) and cardamom *(Elettaria cardamomum*) were initially tested for their anti-trypanosomal potentials against *T. evansi* and showed a powerful in vitro and in vivo trypanocidal effect which exceeds in some concentrations the effect of the reference drug.

Parasite motility represents a critical indicator of the viability and virulence of most flagellate parasites. Therefore, the degree of immobilization of trypanosomes may be considered as a measure of the trypanocidal potential of the tested extracts [52]. In the present study, our aqueous extracts showed the ability to immobilize the trypanosomes and rendered them not infective to rats, evidence on their *in vitro* activity against trypanosomes. Although the mechanism of immobilization is not well developed, at this stage of the work, it was reported that many extracts could block glycolysis and cell division [44]. Such effects might make the trypanosomes completely immobilized after the 3 h incubation period and not able to initiate infection in the experimental rats in our study.

Some studies suggested that complete in vitro immobility of the parasites may not necessarily confirm the death of the parasites, but rather they might have lost their infectivity. As some extracts, in some concentrations, could only immobilize but not kill the parasite through initialization of unfavorable conditions. Then the parasite may have recovered and becomes infective for a new host when it comes in contact with suitable physiological conditions [53]. However, the *in vitro* anti-trypanosomal activities of our tested extracts were confirmed by the blood infectivity test where they rendered the trypanosomes not infective to rats, suggesting that these extracts may belong to the group that acts by killing the trypanosomes rather than affecting the static action that interferes with the growth and multiplication of the parasite.

The steady clearance of parasitemia achieved by our extracts, in the in vivo experiment, was not surprising since many previous reports [48] have demonstrated that some plants of different families could exhibit an *in vivo* anti-trypanosomal activity. However, it is not logical to compare our results with those of the previous reports because the plant evaluated here was not previously investigated for their trypanocidal effect. Although some

researchers have reported treatment failure with certain herbal extracts when given with massive parasitemia. Treatments with lower concentrations of some crude extracts may have favored this failure and the parasitemia could be eliminated if the treatment was initiated early and at high doses [54]. Such differences in the effectiveness of the plants may also due to the variation in their chemical composition according to the geographical areas and the seasons of collection of these plants [55].

The exact mechanism of the *in vivo* action of the tested extracts cannot be determined here since the active ingredients of the plants were not isolated. Many previous reports attributed the anti-trypanosomal activity of some plants to the presence of some active ingredients like flavonoids, alkaloids, saponins, barbarine, harmaine, and tannins [48]. Different accumulated evidence speculated that several plants exhibited their anti-trypanosomal activity by disturbing the redox balance of the parasites and interfering either with the respiratory chain or with the cellular defense mechanisms against the oxidative stress [52]. It was also reported that some products act by binding the kinetoplast DNA of the parasite [56]. It is thus possible that our extracts could act through one or more of those mechanisms together with a negative effect on motility that is essential for evasion of the host immune response during bloodstream infection.

Anemia is an important clinical and laboratory feature of African trypanosomosis and is considered the primary cause of death in such disease. It is usually characterized by a corresponding reduction of the PCV during the high parasitemia [54]. The significantly higher level in PCV recovery of the treated groups, in our study, compared to the negative control group, was in agreement with the results of other studies used other plants like that reported by Mann et al. [55] who explained the effect of their plants, on PCV reduction, by decreasing the parasite load and neutralizing the toxic metabolites produced by the trypanosomes. Another explanation returned to the enhanced resistance of the host erythrocytes, after treatment, to hemolysis [24]. However other studies, where their tested extracts did not affect the severity of anemia or the PCV recovery in the infected animals, attributed this effect to the persistent parasitemia and the establishment of the etiological factors involved in the hemolysis before starting the treatment [44]. While, in our study, the treatments started daily from the parasites were first detected in the bloodstream.

In conclusions, in light of the results, the present study initially confirmed the in vitro and in vivo anti-trypanocidal effect of the aqueous extracts of thyme (*Thymus vulgaris*), mint *(Mentha piperita*), and cardamom *(Elettaria cardamomum*). Further work is required to purify and isolate the bioactive compound/s using chromatographic and spectroscopic techniques to identify the main constituents of each extract and to better understand the mechanism of such actions precisely that could lead to the development of a safer and cost-effective alternative drug for human trypanosomosis.

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