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ANTITRYPANOSOMAL ACTIVITY OF ESSENTIAL OILS EXTRACTED FROM ROSMARINUS OFFICINALIS AND SALVIA FRUTICOSA

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ABSTRACT

Objective: The present study aimed to evaluate the antitrypanosomal activity of *Rosmarinus officinalis* L. and *Salvia fruticosa* essential oils (EOs) against *Trypanosoma evansi*. **Methods:** The chemical compositions of these oils were elucidated by GC and GC/MS analysis and assayed for their *in vitro* and *in vivo* antitrypanosomal activity. Different concentrations of EOs were prepared at 10 µg/ mL, 20 µg/ mL, 40 µg/ mL, 80 µg/ mL, 100 µg/ mL, 200 µg/ mL, 400 µg/mL in the diluent and tested for their *in vitro* at different time intervals and, parasitemia monitored along with 30 minutes of incubation. The two oils at the most suitable concentration were inoculated in infected Wester rats with the parasite to evaluate their *in vivo* activities. **Results:** Three concentrates caused significantly earlier and higher mortality than Diminazene aceturate (DA). Out of them, the concentrate of 100 µg/ mL was used for the *in vivo* study. The clinical findings include haemogram, hepatic-renal function tests, as well as, histopathological changes were better in oily treated rats than in the drug and infected controls. It was following the type of treatment and the sex of the experimental rat group. Statistical analysis showed significant differences within all parameters (except ALT). *S. fruticosa* followed by *R. officinalis* L. improved the clinical and immune response against *T. evansi*. **Conclusions:** The two tested oils could be used against *T. evansi* as a potential alternative to substitute commercially available drugs after fractionating each component separately and validating the materials.

KEYWORDS: Antitrypanosomal activity, *Rosmarinus officinalis* L., *Salvia fruticosa*, *Trypanosoma evansi*, Egypt.

1. INTRODUCTION

Trypanosoma evansi, the agent of trypanosomiasis disease, despite its economic and animal health impacts, has been severely neglected in terms of awareness, control interventions, and research into improved control tools.[1] The low prospect for a vaccine is due to antigenic variation of the parasite.[2] For successful control of *T. evansi*, both locally and globally, the need for additional targets and new, less-toxic therapeutics is widely acknowledged.[3] For the moment, four commercial trypanocides are mainstays in our control of the disease.[4] Suramin, diminazene aceturate, isometamidium, and homidium have been in use for well over 40 years,[5] but diminazene aceturate is the most common drug used for controlling trypanosomosis in domestic animals.[6]The most difficulties faced in trypanosomosis therapy are the high toxicity, and the emergence of resistant strains mainly due to that parasite has developed some incredible self-defense mechanisms that make them difficult to eradicate.[7]

In recent years, there is a growing demand for natural products as a source of synthetic and traditional herbal medicine due to their associated adverse public health and environmental effects.[8] Much attention has been directed toward plant extracts, oils, and biologically active compounds isolated from popular plant species.[9] The essential oils and their components, as antimicrobials, have not fully elucidated due to a large number of chemical compounds contained which has no specific but several targets in the cell. [10] Recently, extensive documentation based on their antimicrobial properties on different microorganisms in their hosts, and their constituents were carried out by several workers.[11]

Lamiaceae (Labiatae) is one of the most diverse and widespread plant families in terms of ethnomedicine, and the concentration of volatile oils.[12] Both salvia and rosemary are used for folk medicine and culinary purposes.[13,14] In this paper, we report the chemical composition and the associated *in vitro* and *in vivo* antitrypanosomal activity of *S. fruticosa* and *R. officinalis* L essential oils extracted against *T. evansi*.

2. MATERIALS AND METHODS

2.1. Plant collection: Fresh *Salvia fruticosa* and *Rosmarinus offiicinalis* L. leaves were collected in December 2017 from Romani and Palisium districts in North Sinai Governorate, Egypt. The two plants were identified and confirmed by a taxonomist at the Medicinal and Organic Plants Department, Desert Research Center in Cairo, Egypt.

2.2. Essential oil extraction: The essential oils (EOs) of the two plants were extracted separately according to [15] by the conventional hydrodistillation method. About 250 g fresh plant materials of each *S. fruticosa* and *R. offiicinalis* L. put in two round bottom flasks and 1000 mL distilled water added to each before subjecting to hydrodistillation for 6 hours using a Clevenger-type apparatus. The mixture boiled at 100°C then the temperature was reduced to 60°C and kept for three hours. The EOs were recovered and allowed to settle, and finally withdrawn. After that, the produced oils dissolved in Tween 80 (0.3% v/v) for easy diffusion. Different concentrations of EOs were prepared at 10 µg/ mL, 20 µg/ mL, 40 µg/ mL, 80 µg/ mL, 100 µg/ mL, 200 µg/ mL, 400 µg/ mL in the diluent and kept in a freezer for advance handling.

2.3. Gas chromatography and GC-mass spectrometry conditions: The volatile oil analysis was carried out using gas chromatography-mass spectrometry instrument stands at the Central Laboratories, National Research Center, Egypt by a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., Waltham, MA, USA), coupled with a THERMO mass spectrometer detector (IQS Single Quadrupole Mass Spectrometer), according to. [16] The GC/MS system is equipped with a TG-WAX MS column (30 m x 0.25 mm i.d., 0.25 µm film thicknesses). Analyses carried out using helium as carrier gas at a flow rate of 1.0 ml/min and a split ratio of 1:10 using the following temperature program: 4.0^{o}C for 1 min rising to 160^{o}C and held for 6 min rising at 6 C/min to 210^{o}C and held for 1min. The injector and detector were held at 210^{o}C, and diluted samples (1:10 hexane, v/v) of 0.2 µl of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. Most of the compounds identified using two different analytical methods: relative retention time and mass spectra (authentic chemicals, Wiley spectral library collection, and NIST library).

2.4. Experimental animals and ethical approval: Mixed sexes Albino mice (25 gm) and rats weighing approximately 200 gm. were used to propagate trypanosomes and *in vivo* activity. They were obtained from the Faculty of Science, Ain Shams University in Cairo, Egypt, and kept in well-ventilated plastic cages ten days of acclimatization before the experiment. The experiment was conducted in compliance with internationally accepted principles of the European (EU) Directive 2010/63/EU for animal experiments and the National Institutes of Health guide (NIH Publications No. 8023 revised 1978).

2.5. Parasites and Parasitemia: *Trypanosoma evansi* strain used was previously identified by. [6] It was maintained by the continuous passages in Swiss mice until required at the Parasitology Laboratory, Desert Research Center in Cairo, Egypt. When the parasitemia 5×10^{5}/ml parasites, mice were anesthetized and the blood was collected for *in vitro* and *in vivo* studies.

2.6. In vitro activity: The *in vitro* antitrypanosomal incubation test was carried in triplicates in 96-well microtiter ™ microplates (THERMO Scientific Corp., Waltham, MA, USA), according to. [17] Exactly, 50 μl of infected blood was incubated with 20 μl of each EO concentration, respectively. For comparison, two sets of controls were used; one contained Diminazene aceturate® (DA) (ADWIA Co., Obour City, Kaliobeya Governorate, Egypt), and the other one was heparinized infected blood suspended in phosphate buffer saline (PBS pH 7.2). Parasitemia monitored along with 30 minutes of incubation at 30^{0}C. About one μl of test mixtures were observed every 5 minutes under a microscope 40X, according to. [18] Complete elimination of motility or a reduction in parasite count/ mL taken as a significant activity of the extracts.

2.7. In vivo experiment: Nine groups of rats (n=10 each) were distributed into nine cages. Their description and status were represented in Table (1). They were intraperitoneally (IP) administered with 0.1 mL of *T. evansi* and each EO at its suitable *in vitro* concentration. DA was given IP 50 µl as chemical drug control, according to the manufacturer's instructions. Two healthy and two infected-untreated controlling groups were IP with PBS only. Parasitaemia was estimated every two days by wet preparation test and examined at 40 X magnification during the experiment. Every seven days, 10 ml blood was obtained from each group by heart puncture in two parts (with and without anticoagulant) on days: 7, 14, 21, 28, 35, and 42, Alterations of haematological, biochemical, immunological, and cytotoxic changeable were recorded. Also, growing legions and microscopically histopathological abnormalities were studied.

Table 1: Showing group status, treatment protocol, and the dose in healthy and different treated rat groups.

Group status (Code)	Treatment protocol	Dose
Healthy ♂ (HM)	Only PBS	100 µl/ 200 gm (4 times)
Healthy ♀ (HF)	Only PBS	100 µl/ 200 gm (4 times)
Infected untreated ♂ (IM)	Only PBS	100 µl/ 200 gm (4 times)
Infected untreated ♀ (IF)	Only PBS	100 µl/ 200 gm (4 times)
Infected ♂+ Treated (SM)	EO/ *S. fruticosa*	100 µl/ 200 gm (4 times)
Infected ♀+ Treated (SF)	EO/ *S. fruticosa*	100 µl/ 200 gm (4 times)
Infected ♂ + Treated (RM)	EO/ *R. officinalis* L.	100 µl/ 200 gm (4 times)
Infected ♀ + Treated (RF)	EO / *R. officinalis* L.	100 µl/ 200 gm (4 times)
Infected (♂+♀) + Treated (DA)	DA (3.5 mg/ k bwt)	50 µl after dilution/ two times (at days 1,15 pi)

2.8. Statistical analysis: Obtained data for haemogram, biochemical, and immunological parameters, and the significance of differences between groups were determined by analysis of variance (ANOVA) using the General Linear Model (GLM) procedure. [19] Means at the same column followed by different letters meant that were significantly differenced by the Least Significant Difference (LSD) test ($p \leq 0.05$) and a highly significant when $p<0.001$.

3. RESULTS

3.1. The yield and composition: In the present study, the oil of *S. fruticosa* produced yield more than *R. officinalis* L. oil. The GC/MS analysis shown in Table 2 revealed the presence of 27 compounds for *R. officinalis* L. and 12 compounds for *S. fruticosa*, mostly aromatic, represented 78.48% and 100% of the total essential oils (EOs), respectively. Eucalyptol (18.6%), Camphor (18.17%), and α-Pinene (14.4%) were the main compounds of the volatile oil extracted from *R. officinalis* L. fresh herb. Whereas the main components of *S. fruticosa* oil were; Eucalyptol (58.85%), Camphor (14.7%), α-Pinene (5.55%), 2-ß-Pinene (5.43%), and Camphene (5.28%) of the total volatile oil extracted fresh herb. Other components were found in less quantity.

Table 2: Constituents of *Rosmarinus officinalis* L essential oil and their percentages

Composition	%	Composition	%
α-Pinene	14.4	Tricyclene	0.31
Camphene	4.86	2- ß-Pinene	0.8
Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-	7.44	α-Terpinolene	0.48
Bicyclo[3.1.1]heptan-3-one, 2,6,6-trimethyl-, (1α,2ß,5α)	2.83	Linalyl-Acetate	5.02
Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, acetate, (1S-endo)-	0.48	Camphor	18.17
Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)- (1α,2α,5α)	1.22	(-)-Myrtenol	0.51
Bicyclo[3.1.0]hex-2-ene, 4-methylene-1-(1-methylethyl)-	1.2	ß-Myrcene	0.62
Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)	12.52	D-Verbenone	0.24
Cyclohexene, 1-methyl-4-(1-methylethylidene)-	0.29	3-Carene	0.22
CIS-D-Dihydrocarveol	0.66	Bornyl acetate	2.78
Benzene, methyl(1-methylethyl)-	1.08	Caryophyllene	1.0
δ-3-Carene	1.08	trans-Shisool	1.21
Eucalyptol	18.6	γ -Terpinene	0.42
α-Terpineol	1.59		

Table 3: Constituents of *Salvia fruticosa* essential oil and their percentages.

Composition	%	Composition	%
Camphor	14.7	α-Pinene	5.55
α-Terpineol	1.7	Camphene	5.28
Bornyl acetate	0.69	2- ß-Pinene	5.43
Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)	0.61	Eucalyptol	58.85
1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	1.0	Caryophyllene	3.63
Bicyclo[3.1.0]hexan-3-ol,4-methylene-1-(1-methylethyl)-,[1S-(1α,3ß-,5α)	1.39	ß-Myrcene	1.18

3.2. In vitro trypanocidal activity: After incubation with the infected blood, the different concentrations for each oil showed different responses against *T. evansi*, but EOs caused significantly higher trypanosome mortality than DA at three examined concentrations of 100 µg/mL, 200 µg/ mL, 400 µg/ mL. The activity increased with increasing incubation time and concentration as well. Whereas salvia killed 50% of trypanosomes after 3 minutes earlier than rosemary and DA. Rosemary was the more active after 10 min., and dissected trypanosomes before completely cleared it. The 100 µg/ mL concentration for EOs was preferred for submitting *in vivo* evaluation. The EOs of *R. officinalis* L. and *S. fruticosa* exhibited antitrypanosomal

activity against tested *T. evansi*, and the minimum inhibition concentrations (MIC) were in the range of 30–20 µg /mL, respectively.

3.3. Haematological, biochemical, and immunological parameters: The two EOs had shown comparable and stronger trypanosomal effects on *T. evansi* with significant increases or decreases in their clinical parameters compared to healthy and infected controls as shown in Tables (4-6) and illustrated in Fig. (1-3). The effect of *S. fruticosa* oil on both sexes demonstrated remarkable protective activity than the effects of *R. officinalis* L oil and DA drug. Also, salvia EO improved the blood constitute and was safer than rosemary. It caused significant increases in all parameters except for PLT that decreased compared to other groups. RBC, HGB, and HCT levels increased in the male-rats and WBC and Lymph% levels in female-rats. Whereas rosemary exhibits a slight changeable in most parameters, it increases MCH, MCHC, and PLT in male-rats and significantly decreased PLT in female-rats. DA treatment gave fluctuated results, within normal levels, except for increases of WBC count and significant decreases in HCT and PLT levels. These variations were recorded along with the experiment evolution or suppressed the infection after doses.

The biochemical results followed the type of treatment and the sex of the experimental rat group. The most common feature, hypoglycemia, was found in all infected and followed treated groups, and the other parameters have fluctuated increase or decrease. Whereas salvia EO showed significant constricts in Chol, Trigly, Urea, Creat, ALP, LDH, T. bilirubin levels, and increases in AST, ALT, Ca, and within the normal ranges in T. Protein in male-rats (SM). It exhibited increases in Ca, lipids, kidney functions, ALT, LDH, AST, Albumin, and T. Bilirubin levels in female-rats (SF). *R. officinalis* L. EO increased hypoproteinemia, hypocholesteremia, and kidney dysfunctions levels. Also, it decreased ALT, ALP, LDH liver enzymes, and Ca levels. The T. Bilirubin level was within the natural level in male-rats (RM). Whereas in the female-rats group (RF), T. protein level was within normal, and significant increases were observed in lipids, kidney functions, liver enzymes, and T. bilirubin. On the other hand, the oily treated groups exhibited a significant elevation in the immune-parameters (IgG, IgM). Also, the CRP level significantly increased along with the experiment than controls and noted that CRP was sharp increases in DA treated-group.

Table 4: Haemogram analysis of the infected and treated rats revealed a significant difference between groups until the end of the experimental period.

Group	WBC	Lymph	RBC	HGB	MCV	MCH	MCHC	HCT	PLT
	10^3/ µl	%	10^6/ µl	g/dL	fl	pg	g/dl	%	10^3/µl
HM	6.73^{b}	5.85^{b}	5.34^{a}	$7.1b^{c}$	39.93^{d}	13.35^{c}	33.53^{bc}	21.55^{ab}	679.3^{ab}
HF	10.6^{b}	9.15^{b}	4.88^{ac}	8.65^{b}	$53.9b^{c}$	17.55^{b}	32.33^{bc}	26.95^{a}	402^{bc}
IM	20.1^{a}	17.5^{a}	2.89^{d}	5.75^{c}	56.78^{ab}	19.93^{ab}	34.75^{b}	16.13^{bc}	468.3^{abc}
IF	8.95^{b}	6.95^{b}	2.53^{d}	5.05^{c}	63.4^{a}	23.25^{a}	35.78^{b}	15.08^{bc}	356.5^{bc}
SM	6.85^{b}	5.78^{b}	5.43^{a}	11.1^{a}	46.78^{dc}	18.65^{b}	29.9^{c}	27.33^{a}	525.3^{abc}
SF	24.94^{a}	19.2^{a}	3.94^{dc}	$6.65b^{c}$	53.8^{bc}	17.85^{b}	33.58^{bc}	20.75^{ab}	360.3^{bc}
RM	9.175^{b}	7.10^{b}	3.09^{dc}	5.05^{c}	49.93^{bc}	16.35^{bc}	33.98^{b}	14.65^{bc}	175.5^{c}
RF	10.9^{b}	9.04^{b}	2.96^{d}	4.625^{c}	55.8^{ab}	23.33^{a}	43.2^{a}	12.33^{c}	790.3^{a}
DA	11.45^{b}	9.13^{b}	5.98^{a}	$6.95b^{c}$	51.58^{bc}	19.18^{ab}	35.25^{b}	17.95^{bc}	$328b^{c}$
SE	2.578^{b}	1.10	0.59	0.182	2.509	1.353	1.23	2.37	117.46
Sig.	0.0015	0.002	0.0028	0.000	0.0002	0.0004	<0.000	0.0019	0.0457

Table 5: Showing lipid profile, kidney function, liver enzymes, and bile changeable in treated groups compared to controls.

Group	Lipid profile		Kidney function		Liver enzymes				Bile
	Chol.	Trigly.	Urea	Creat.	AST	ALT	ALP	LDH	T. Bili
	mg⁄ dl	mg⁄ dl	mg⁄ dl	mg⁄ dl	u/l	u/l	u/l	u/l	mg/dl
HM	114.75^{b}	176.0^{a}	102.75^{bc}	1.83^{b}	49.00^{c}	47.00^{ab}	169.5^{bc}	586.3^{b}	0.575^{b}
HF	49.75^{c}	123.3^{bcd}	90.75^{bcd}	0.75^{c}	31.63^{c}	33.45^{ab}	127.3^{cd}	158.8^{e}	0.500^{b}
IM	64.00^{c}	76.75^{bcd}	97.75^{bc}	1.16^{bc}	223.5^{a}	55.75^{a}	103.3^{de}	480.5^{bc}	1.825^{a}
IF	38.50^{c}	12.25^{d}	53.50^{d}	0.63^{c}	105.0^{b}	23.50^{b}	80.25^{de}	62.00^{e}	0.520^{b}
SM	36.75^{c}	25.25^{d}	75.25^{cd}	0.58^{c}	182.0^{a}	55.00^{a}	45.00^{e}	365.5^{cd}	0.265^{b}
SF	118.58^{b}	181.6^{abc}	105.63^{bc}	2.68^{a}	29.80^{c}	36.25^{ab}	129.5^{cd}	639.3^{b}	0.350^{b}
RM	37.925^{c}	219.3^{ab}	83.5^{bcd}	0.75^{c}	35.00^{c}	53.50^{a}	114.3^{cd}	201.8^{de}	0.525^{b}
RF	128.25^{ab}	295.75^{a}	175.33^{a}	0.97^{bc}	97.75^{b}	59.25^{a}	191.8^{ab}	401.5^{c}	1.638^{a}
DA	153.75^{a}	59.25^{cd}	116.0^{b}	$1.46b^{c}$	50.25^{c}	48.25^{ab}	235.5^{a}	977.3^{a}	0.378^{b}
SE	8.85	46.71	11.95	0.27	14.71	7.99	19.46	58.52	0.134
Sig.	<.0001	0.0047	<.0001	0.0007	<.0001	0.0513	<.0001	<.0001	<.0001

Table 6: Showing salts, proteins, and immunological parameters in treated groups compared to controls.

Group	Salts		Proteins				Immunological items		
	Ca	GLU	T. Pro	Alb	GLO	A/G	IgM	IgG	CRP
	mg/dl	mg/dl	g/dl	g/dl	g/dl	%	ng/ml	ng/ml	ng/ml
HM	8.5^{abc}	147.8^{a}	6.53^{ab}	3.50^{a}	3.03^{bc}	1.18^{bcd}	211^{bc}	697.5^{bc}	3.75^{b}
HF	5.85^{d}	123^{ab}	5.8^{bcd}	3.08^{ab}	2.53^{cde}	1.32^{bc}	173^{bcd}	661.0^{bc}	3.93^{b}
IM	6.78^{cd}	73.75^{b}	3.93^{e}	1.83^{d}	2.1^{def}	0.93^{cd}	233^{ab}	1021^{a}	4.80^{b}
IF	6.5^{d}	75.25^{b}	3.95^{e}	2.43^{c}	1.525^{f}	1.59^{b}	185^{bcd}	911.5^{ab}	5.38^{b}
SM	9.58^{ab}	71.75^{b}	5.43^{d}	3.63^{a}	1.8^{ef}	2.05^{a}	173^{bcd}	8356^{abc}	4.73^{b}
SF	6.33^{d}	87.50^{b}	5.53^{dc}	2.70^{bc}	2.83^{cd}	1.11^{cd}	169^{bcd}	685.0^{bc}	6.25^{b}
RM	7.7^{bcd}	82.25^{b}	5.63^{bcd}	3.05^{ab}	2.58^{cde}	1.23^{bcd}	160^{cd}	870.0^{ab}	7.03^{b}
RF	7.32^{cd}	128^{ab}	6.38^{abc}	3.10^{ab}	3.53^{ab}	0.89^{cd}	293.0^{a}	772^{abc}	5.50^{b}
DA	9.9^{a}	92.50^{b}	6.98^{a}	3.10^{ab}	3.88^{a}	0.80^{d}	137.0^{d}	531.8^{c}	20.0^{a}
SE	0.613	16.67	0.291	0.176	0.26	0.141	21.72	95.96	2.13
Sig.	0.002	0.0085	<.0001	<.0001	<.0001	<.0001	0.0042	0.041	0.001

3.4. Pathological changes in treated groups: Though this research is just beginning, rosemary EO usage treats hair follicles in laboratory experimental rat-groups. Gross examination of various tissues was observed from infected and treated rat groups showed mild splenomegaly, mild hepatomegaly, and congestion of lungs compared to control groups (HM, HF). The changes were more evident in DA than others. The treated groups were associated with no weight loss in rats opposite to the infected groups (IM, IF). Histopathological changes were seen microscopically in all tissues despite the short time of the experiment. It was consistent with trypanosome infection. Salvia-treatment showed normal brain tissues and only dilated and congested blood vessels in other organs (Figure 1). Rosemary-treatment causes perineural and perivascular brain edema, muscular hyalinosis with areas of hemorrhage in the heart, bronchopneumonia; bronchial hyperplasia, peribronchial, and interstitial leucocytic cells infiltrations, and thickened wall blood vessels in the lung. Also, congested hepatoportal blood vessels together with vacuolated hepatocytes and disorganized hepatic cords, interlobular dilated and congested blood vessels in the pancreas. The spleen shows congested blood vessels with a thickened wall and interstitial blood vessel congestion with a thickened wall in the kidney (Figure 2). Under the same conditions, DA-treatment showed perineural and perivascular edema, congested myocardial blood vessel, bronchopneumonia, mononuclear cell infiltration in the portal area, interlobular severely dilated and congested blood vessel in the pancreas, healthy lymphoid follicle in the spleen, and interstitial blood vessels dilatation and congestion in the kidney.

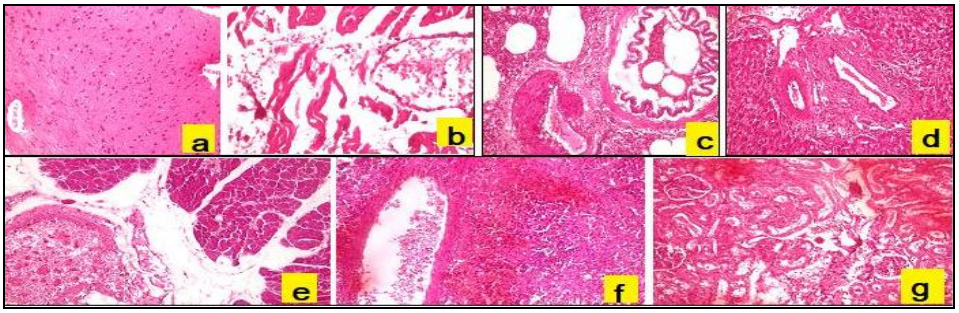

Fig. 1: Microphotograph for brain, heart, lung, liver, pancreas, spleen, and kidney sections in *salvia fruticosa*-treated groups represented as a, b, c, d, e, f, and g, respectively.

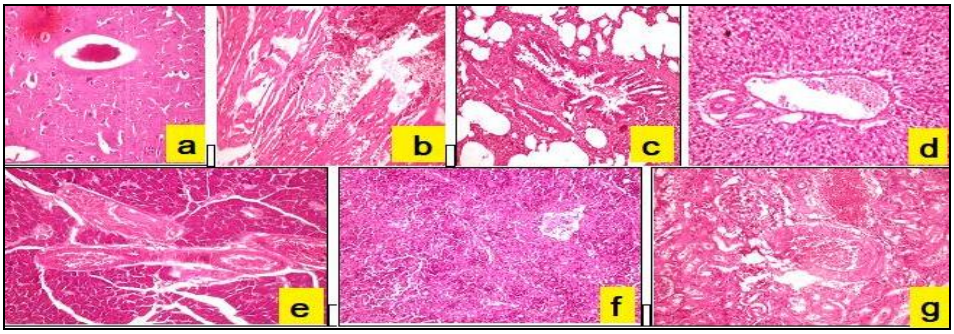

Fig. 2: Microphotograph for brain, heart, lung, liver, pancreas, spleen, and kidney sections in *Rosmarinus officinalis* L-treated groups represented as a, b, c, d, e, f, and g, respectively.

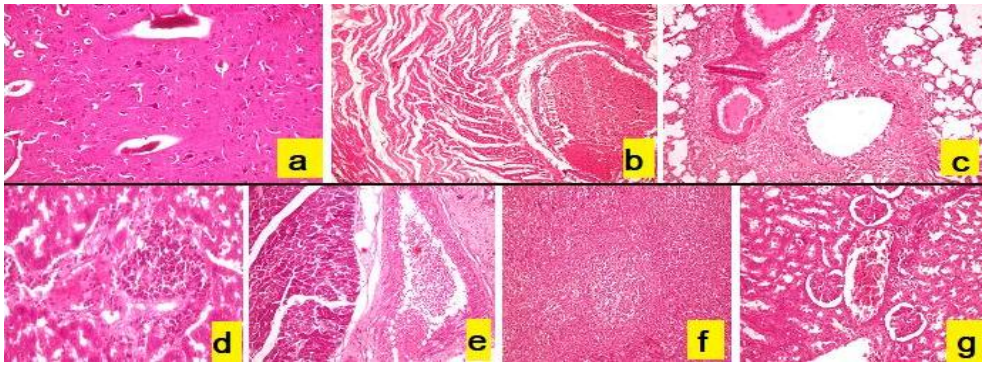

Fig. 3: Microphotograph for brain, heart, lung, liver, pancreas, spleen, and kidney sections in diminazene aceturate drug-treated group represented as a, b, c, d, e, f, and g, respectively.

4. DISCUSSION

Lamiaceae family oils are remarkable in pesticide, pharmaceutical, flavoring, perfumery, fragrance, and cosmetic industries.[20] Some essential oils (EOs) have significant antimicrobial potential against multidrug-resistant pathogens.[11,21] The therapeutic efficacy has described too many indigenous plants such as rosemary and salvia for various diseases,[22, 23] but the increasing interest for new and safe essential oils of salvia and rosemary have not been entirely investigated yet,[24-26] despite their use for folk medicine. In the present study, GC and GC/MS analysis of the two EOs showed significant variability in the chemical composition not depends on location and stages of development. It is related to the type of oil despite the same district and geographical region of plants. Rosemary oil produced compounds more than twice salvia oil and the main bioactivities of the two EOs are attributed mainly to Eucalyptol, Camphor, and α-Pinene. It may be offered as a means of therapy in the treatment of trypanosomiasis. Time of harvest, condition of leaves, and distillation equipment have no role in the quality and quantity of the oils as these parameters the same. These findings agree with the previous studies,[27,28] but with variable concentrations.

For treating trypanosomiasis, several medicinal plants were evaluated in a previous study.[23] Some of them are considered as having good *in vitro* and *in vivo* antitrypanosomal activities as *Salvia officinalis L., Camellia sinensis*, and *Thymus vulgaris.* Others have good *in vitro* but bad *in vivo* antitrypanosomal activity as *Mentha longifolia, Azadirachta indica*, and *Olea europoea*. The present study began with an *in vitro* study to evident the efficacy of the two oils and for preselection of suitable concentration. The higher activity of the two oils than DA, despite its pure nature as a chemical drug, evidenced that they exhibited high *in vitro* trypanocidal activity against *T. evansi* than DA in agreement with[23] comparable studies evaluated the activity, and IC50 of *R. officinalis* L. and *Salvia spp* EOs against some protozoon parasites: *Leishmania braziliensis, T. brucei, Plasmodium falciparum* with IC50 of 17.4 ± 0.43 µg/mL, IC50 = 19.1 µg/mL,[29] 6.4 ± 2.0 µg/mL and 4.8 ± 0.7 µg/mL,[30] IC50 = 17 µg/mL and 12 µg/mL respectively on D6 and W2).[31]

Clinically, EOs used in the present study gave direct evidence for their safety and selectivity the suitable concentration for *in vivo* study. In particular; no symptoms of toxicity have occurred for the two oils through the dosage administration used till 400 µg /ml. Although anemia is the main feature of the *T. evansi* infection course,[2,6] the treatments with salvia and somewhat rosemary EOs altered this with a slight decrease or increase in blood constitutes. The lower RBCs, HGB, and HCT levels in rosemary treated-groups agree with some previous studies that reported these parameters as characteristic features in trypanosomiasis in different animals.[32,33] It is attributed to the release of hemolytic factors into the rats' blood by dead trypanosomes which destroyed erythrocytes and HB and reduced HCH.[34] On contrary, the normalized value of HCH in salvia treated-groups may be due to their ability to eliminate parasites from the blood than rosemary oil and DA. Besides, the higher means of lymphocyte (%) in infected male-rats than others is an initial enhanced immunological response mostly followed by an immunosuppressive effect of trypanosome,[35] and changes in variable surface glycoprotein as reported before.[36] On the other hand, the increasing of the total leukocytes in the treated-groups may be due to the lytic effect of the newly developing *T. evansi* or multiple antigens resulting from more stimulation of the immune system, as a defense mechanism against the inflammatory processes produced in the body.[37]

In vivo course in the present study raised liver activity even at the safest doses administered in some oily and drug-treated rats. The increase in total bilirubin reflects severe damages in the liver with time in agreement with.[38,39] Hypoglycemia was observed in the infected controls, and the treated groups continued without abating as the infection progressed. That is due to consuming blood glucose during aerobic glycolysis leading to severe energy deficit in the host.[40] The elevation of creatinine levels during the experimental infection may be related to severe kidney dysfunction.[41] Another hand, the oily treated groups gave normal levels of total proteins, albumin and globulin resulted from decreases in tissue invasion by *T. evansi*.[42,43] Besides, the effect of *S. fruticosa* and *R. officinalis* oils on LDH did not exhibit a toxic effect on the treated rat

hepatocytes.[44] The highly significant difference in the serum albumin, globulin, T. bilirubin, and T. protein of infected and treated animals compared with the other controls could be due to the difference in the antitrypanosomal activities selected plant oil. The normalization in bilirubin level may be due to the ability of the liver to conjugate bilirubin. In contrast, the higher increase level observed only in female-rats treated with rosemary oil may result in stimulation of the release of bile, which is very important in fat digestion. [45] On the other hand, the normal urea and creatinine levels in the *T. evansi*-treated rats could be due to the ability of the kidneys to excrete creatinine during the disease is contrary to that previously reported.[46-48]

At the time, trypanosomes in infected-untreated animals caused death due to the failure or dysfunction of the spleen, lung, heart, kidney, liver, or brain in agreement with.[49] The histopathological changes in the lungs are in agreement with the reports of,[2] who observed similar changes in the lungs of *T. evansi* infected rats before and after treatments. The inflammatory response to the parasite led to exudation in the focal areas and increased the cellularity of the alveolar walls as reported by.[50] Also, it increased hyperplasia of the peri-bronchiolar lymphoid tissues and perivascular infiltration of lymphocytes around small blood vessels. The acute congestion along with segregation of lymphoid follicles and hyperplasia in the spleen agrees with the findings of[51] that attributed these changes to immediate hypersensitivity to *T. evansi*. The changes in the kidney and the brain are mainly due to toxins produced by the parasite. Besides, the accumulation of immune complexes impairs the structure and function of the kidney in agreement with the results.[52] The changes in the brain may result from the constant irritation caused by parasites as reported before.[53]

CONCLUSION AND RECOMMENDATION

The two essential oils and DA have different activities against trypanosomiasis. Whereas *R. officinalis* L oil has higher *in vitro* trypanocidal activity, *S. fruticosa* oil has higher *in vivo* antitrypanosomal activity. The clinical findings were improved in salvia treated animals than rosemary, then DA, and followed the type of treatment and the sex of the experimental animals. Further study on fractionating each component separately and validating the materials should carry out.

CONFLICT OF INTEREST STATEMENT

I declare that there is no conflict of interest.

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AUTHORS' CONTRIBUTIONS

The author planned, designed the work and collected field samples, carried out the experimental infection, and analyzed the clinical results. Also, drafted, wrote, revised, and approved the manuscript.

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