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RESEARCH ARTICLE

The Efficacy of *Saussurea costus* Extracts against Hematophagous Arthropods of Camel and Cattle

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A B S T R A C T

Plant extracts are becoming an increasingly precious source of eco-friendly pest control tools. This work investigated for the first time the validity of hexane and methanol extracts of *Saussurea costus* against four cattle and camel ectoparasites through envelop treatments. For phytochemical analyses, Gas Chromatography-Mass Spectrometry was used. All mortalities were significantly diverse from the controls (P>0.05). The mortality (MO)% of *Hyalomma dromedarii* seven days after treatment (AT) with 12.5 and 25 mg/ml of methanol and hexane extracts was 100 and 90% and LC_{50} values were 1.37 and 2.33 mg/ml, respectively. Meanwhile, such values against *Rhipicephalus* (*Boophilus*) *annulatus* were 100 and 93.33% plus 1.23 and 1.95 mg/ml, respectively. Both extracts completely killed the cattle lice, *Haematopinus eurysternus*, one and three days AT with 6.3 mg/ml and LC_{50} values were 0.31 plus 0.57 mg/ml, respectively. The MO% seven days after treatment of the louse fly, *Hippobosca maculata*, with extracts of methanol and hexane (12.5 mg/ml) was 100% and LC_{50} values were 1.26 and 0.63 mg/ml, respectively. *S. costus* extracts had mainly sesquiterpene, fatty acid esters, phenols, and acyclic hydrocarbons. This study proved the innovative use of *S. costus* extracts against hematophagous arthropods of camel and cattle. The eco-friendly use of methanol extract would be a helpful approach to prevent vector- borne diseases infecting large animals. Future studies could be directed to studying the safety profile of *S. costus* against non- target organisms.

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INTRODUCTION

Different arthropods, such as ticks, mosquitoes, flies, and lice, have the potential to spread infectious diseases thus, they play an important role in affecting human and animal health and affect the production of farm animals (Ali *et al.,* 2020; Peter, 2020; Ceylan *et al.,* 2021). For example, body lice and louse-borne illnesses have plagued humans for millennia. Body lice, *Pediculus humanus* Linnaeus, and the short-nosed cow louse, *Haematopinus* (*Ha.*) *eurysternus,* continue to be two arthropods of public and veterinary health concern in Egypt (Reeves *et al.,* 2006).

For control of insects and arachnids of medical and veterinary significance, synthetic repellents, insecticides and acaricides have been used worldwide for decades. Widespread use of such control agents has resulted in issues like resistance, contaminated environment, and negative influences on non-target creatures, including humans (Khater, 2012; Khater *et al.,* 2019; Ahmed *et al.,* 2021). Due to these constraints, scientists are working on alternate control methods. Natural pest control based on medicinal plants could safely prevent vector bites and their related diseases. For years, medicinal herbs have been used to combat parasitism, and this practice continues even today. The efficacy of different plant extracts against pests has been widely studied (Khater *et al.,* 2009, 2011, 2014; Baz *et al.,* 2021; 2022a, b; Radwan *et al.,* 2022). During their life, plants produce a wide

range of secondary metabolites, including terpenoids, acting as insecticides and insect repellents; botanicals could also affect insect growth, reproduction, life span, and oviposition (Khater and Geden, 2018,2019; Ahmed *et al.,* 2021; Eltaly *et al.,* 2022).

Saussurea (*S.*) *costus* (Falc.) Lipschitz (*S. lappa*) (Asterales: Asteraceae) is called Al-Kost Al-Hindi or Al-Kust Al Bahri in Arabic and distributed worldwide. This plant is being used in the Arab countries due to its medicinal properties (Ahmad *et al*., 2009). It has antimicrobial (Abdallah *et al*., 2017), anticancerous, anti-inflammatory, antimicrobial, antiulcer, anticonvulsant, hepatoprotective, gastro-protective effect, spasmolytic, hypoglycaemic and immunomodulatory activity (Kamalpreet *et al*., 2019). Plant-based insecticides/ acaricides are considered eco-friendly and safe for beneficial insects (Murugan *et al*., 2015; Radwan *et al*. 2022) as they decay faster than synthetic products (Khater, 2012, Ahmed, 2021). Accordingly, it is hypothesized that *S. costus* possesses secondary metabolites that could control insects/arachnids and could be used as a valuable alternative to industrial insecticide and acaricide. This study was planned to explore the efficacy of the methanol and hexane extracts of *S. costus* against hematophagous arthropods of cattle and camel and determine its lethal concentrations and phytochemical analysis.

MATERIALS AND METHODS

Collection of parasites: Ticks including *Hyalomma* (*H.*) *dromedarii* (Koch, 1844), and *Rhipicephalus* (*R.*) *annulatus* (Say, 1821) were collected from areas around infested camel and cattle, respectively, brought for slaughtering at Benha abattoir, Qalyubiya governorate, Egypt. The cattle louse, *Ha. eurysternus*, and louse fly*, Hippobosca* (*Hi.*) *maculata*, were also picked from infested cattle in the same place.

Preparation of plant extracts: Dry roots of *S. costus* were purchased from Pure Life Company, Cairo, Egypt. Plant material was identified and authenticated at the Herbarium of the Faculty of Science, Cairo University. The purchased plant material (50g) was properly cleaned and ground into powder in an electric mixer. Plant extract with both solvents, i.e. methanol and hexane (200 ml), was prepared in the Soxhlet apparatus. After extraction, filtration of the solution was done via a Buchner funnel and the extract was dried at 50°C for 6 h (Tankeu *et al.,* 2016). To achieve total solubility of the extract in water, different quantities of plant extracts (0.8, 1.6, 3.1, 6.3 and 12.5 mg/ml, and 1.6, 3.1, 6.3, 12.5, and 25 mg/ml) were made with the addition of 1 ml of tween 80 as an emulsifier.

Toxicity Bioassays: The antiparasitic activity of both extracts of *S. costus* against the four selected parasites was evaluated through the treated envelope method reported by Zahir *et al.* (2010) with a little modification. Briefly, adult parasites were treated with five concentrations, i.e. 1.6-25 mg/ml for *H. dromedarii* and *R. annulatus* and 0.8 - 12.5 mg/ml for *Hi. maculata* and *Ha. eurysternus*. Three replicates (ten adults/ each) were managed for each concentration.

Each pest group was put into an envelope made from a Whatman filter paper No.1 (125 mm in diameter). The inner surface of each bag was treated with a 3-ml test solution of each concentration of the extracts, whereas each control group was treated with distilled water and tween 80. The envelope was closed using a metallic clip with a label of the pest type and tested solution and concentration.

Treated groups were transported to Petri dishes containing filter papers and held in reserve at 80±5% relative humidity and 28±2°C. Lethal effect of each concentration was recorded at 1, 3 and 7 days after treatment (AT).

Biochemical assessments: Gas Chromatography-Mass Spectrometry (GC-MS) was performed to analyze the components of *S. costus*. Thermo Scientific Trace GC Ultra/ISQ Single Quadrupole MS, TG-5MS fused silica capillary column (0.1 mm, 0.251 mm, plus 30 m thick) was utilized for the GC/MS biochemical analyses. Analyses were done according to the previously described protocols (Ashmawy *et al*., 2018; El-Hefny *et al*., 2018) at the Pesticide Laboratory, Cairo governorate, Egypt. An electronic ionizer, 70 eV ionization energy, was operated and Helium gas was used as a carrier (flow rate= 1 ml/min). At 280°C, the MS transmission line and injector were set. Starting at 50°C, the oven temperature was escalated to 150°C, (7 °C per minute); subsequently to 270°C (5°C / min); pause for two minutes; and lastly at 310°C (3.5°C/min for 10 min). The relative peak area was utilized to inspect the quantification of all constituents. Willy Library data from the GC-MS instrument was used to compare the mass spectra of the chemicals and retention periods to those of NIST. The chemicals were marked through the collective spectra of the user-generated reference libraries; Single-ion chromatographic reconstructions were performed to assess the peak homogeneity. To validate the GC retention times, Co-chromatographic analyses of the reference compounds were applied.

Data analyses: Statistical analysis was done via SPSS V23 (IBM, USA); the one-way analysis of variance (ANOVA) using the Post Hoc/Turkey's HSD test at $P< 0.05$. The Probit analyses were performed for the calculation of the lethal concentration (LC) values.

RESULTS

Pesticidal effects: The data indicated that all *S. costus* extracts induced significant mortalities than those of the control groups ($P>0.05$). The extracts showed significant ($P>0.05$) toxic effects against pests 24 h AT with the higher concentrations.

Seven days AT of *H. dromedarii* by 12.5 mg/ml of the methanol extract, the mortality% and LC_{50} value were 100% and 1.37 mg/ml compared to the matching values for the hexane extract, which were 80% and 2.32 mg/ml, respectively (Table 1 and 2). The extracts were also effective on the cattle tick *R. annulatus*. After treatment with the methanol and hexane extracts (12.5 mg/ml) for seven days, the MO% were 100 and 83.33%; whereas the

LC_{50} values were 1.23 and 1.95 mg/ml, respectively (Table 1 and 2).

Data showed that the methanol plus hexane extricates of *S. costus* adversely affected (P>0.05) *H. eurysternus*. Subsequent to treatment with 6.3 mg/ml, MO% was 100% and their LC_{50} values, seven days AT, were 0.31 and 0.57 mg/ml, respectively (Table 3 and 4). *Hi. maculata* was substantially controlled (P>0.05) AT with methanol together with hexane extracts of *S. costus* as complete mortalities were reported seven days AT with 12.5 mg/ml and the LC_{50} values were 1.26 and 0.63 mg/ml, respectively (Tables 3 and 4).

Table 1: Efficacy of the plant extract, *Saussurea costus* against ticks

Species	Conc. (mg/ml)	Methanol extract			Hexane extract		
		1st day	3rd day	7th day	1st day	3rd day	7th day
Hyalomma dromedarii	0	0.0±0.0fC	3.33±3.33fB	6.67±3.33fA	0.0±0.0fC	3.33±3.33fB	6.67±3.33fA
	1.6	13.33±3.33eC	30.00±5.77eB	60.00±5.77eA	10.00±5.77eC	26.67±3.33eB	50.00±5.77eA
	3.1	23.33±3.33dC	56.67±3.33dB	76.67±6.67dA	20.00±5.77dC	36.67±8.82dB	56.67±3.33dA
	6.3	43.33±3.33cC	66.67±6.67cB	83.33±8.82cA	33.33±3.33cC	50.00±5.77cB	66.67±3.33cA
	12.5	53.33±6.67bC	73.33±6.67bB	100±0.00bA	43.33±3.33bC	66.67±8.82bB	80.00±5.77bA
	25	73.33±6.67aC	86.67±3.33aB	100±0.00aA	60.00±5.77aC	73.33±3.33aB	90.00±5.77aA
Rhipicephalus (Boophilus) annulatus	0	0.0±0.0fC	3.33±3.33fB	6.67±3.33eA	0.0±0.0fC	3.33±3.33fB	6.67±3.33fA
	1.6	16.67±3.33eC	33.33±6.67eB	63.33±6.67dA	13.33±3.33eC	30.00±5.77eB	53.33±8.82eA
	3.1	26.67±3.33dC	60.00±5.77dB	80.00±5.77cA	23.33±8.82dC	40.00±10.00dB	60.00±5.77dA
	6.3	46.67±3.33cC	70.00±10.00cB	83.33±8.82bA	36.67±3.33cC	53.33±3.33cB	70.00±0.00cA
	12.5	56.67±3.33bC	76.67±3.33bB	100±0.00aA	46.67±6.67bC	70.00±10.00bB	83.33±6.67bA
	25	76.67±3.33aC	90.00±5.77aB	100±0.00aA	63.33±3.33aC	76.67±3.33aB	93.33±3.33aA

a, b & c: There is no significant difference (P>0.05) flanked by any two means, within the same column have the same letter: A, B & C: There is no significant difference (P>0.05) among any two means within the same row with the same letter.

Table 2: Lethal concentrations of plant extract, *Saussurea costus* against *H. dromedarii* and *B. annulatus*

Species	Days	Solvents	LC_{50} (95%CL) *	LC_{90} (95%CL)	LC_{95} (95%CL)	Slope ±SD	X^2
Hyalomma dromedarii	1	Methanol	9.55 (7.87-11.91)	75.84 (48.61-146.37)	136.46 (79.64-304.81)	1.424±0.150	1.048*
		Hexane	15.67 (12.17-22.08)	171.17 (90.38-475.23)	337.12 (157.01-1152.60)	1.234±0.151	0487
	3	Methanol	3.48 (2.66-4.34)	35.34 (23.87-63.98)	68.18 (41.28-147.95)	1.273±0.147	4.786*
		Hexane	6.66 (5.25-8.49)	89.50 (51.02-220.44)	186.92 (92.94-579.99)	1.136±0.142	0.872
	7	Methanol	1.37 (2.66-4.34)	6.77 (23.87-63.98)	10.64 (41.28-147.95)	1.850±0.254	8.773
		Hexane	2.32 (1.53-3.10)	34.57 (22.07-71.72)	74.31 (41.07-199.91)	1.0932±0.148	0.616
Rhipicephalus (Boophilus) annulatus	1	Methanol	8.13 (6.70-10.05)	67.66 (43.71-129.23)	123.33 (72.37-273.84)	1.393±0.148	1.082
		Hexane	13.28 (10.37-18.40)	162.61 (85.03-463.10)	330.78 (151.35-1178.61)	1.178±0.147	0.342
	3	Methanol	2.92 (2.19-3.66)	27.07 (19.01-45.82)	50.86 (32.27-101.86)	1.326±0.151	4.559
		Hexane	5.47 (4.27-6.91)	73.18 (43.04-170.87)	152.62 (78.39-448.19)	1.138±0.142	0.661
	7	Methanol	1.23 (0.88- 1.61)	6.14 (4.20-8.10)	9.67 (7.17-12.15)	1.840±0.228	11.891
		Hexane	1.95 (1.27-2.63)	24.52 (16.67-45.01)	50.20 (30.04-115.87)	1.167±0.153	2.677

* $LC_{50, 90,}$ and $_{95}$ values= lethal concentration that kills 50, 90, and 95% of the exposed ectoparasite; X^2= chi-square; Significant at P < 0.05 level.

Table 3: Efficacy of the plant extract, *Saussurea costus* on the cattle lice and fly

Species	Conc. (mg/ml)	Methanol extract			Hexane extract		
		1st day	3rd day	7th day	1st day	3rd day	7th day
Haematopinus eurysternus	0	0.0±0.0fC	3.33±3.33eB	6.67±3.33dA	0.0±0.0fC	3.33±3.33fB	6.67±3.33eA
	0.8	33.33±8.82eC	56.67±3.33dB	83.33±6.67cA	26.67±3.33eC	43.33±8.82eB	63.33±6.67dA
	1.6	60.00±10.00dC	83.33±8.82cB	93.33±3.33bA	50.00±5.77dC	66.67±3.33dB	86.67±6.67cA
	3.1	83.33±3.33cC	93.33±6.67bB	100±0.00aA	66.67±6.67cC	76.67±8.82cB	90.00±10.00bA
	6.3	96.67±3.33bC	100±0.00aB	100±0.00aA	76.67±6.67bC	86.67±6.67bB	100±0.00aA
	12.5	100±0.00aC	100±0.00aB	100±0.00aA	93.33±3.33aC	100±0.00aB	100±0.00aA
Hippobosca maculata	0	0.0±0.0fC	3.33±3.33fB	6.67±3.33fA	0.0±0.0fC	3.33±3.33fB	6.67±3.33fA
	0.8	16.67±3.33eC	23.33±3.33eB	46.67±3.33eA	20.00±5.77eC	36.67±3.33eB	60.00±5.77deA
	1.6	23.33±3.33dC	40.00±10.00dB	60.00±5.77dA	43.33±8.82dC	60.00±5.77dB	86.67±6.67cA
	3.1	40.00±5.77cC	56.67±3.33cB	66.67±8.82cA	60.00±11.55cC	70.00±5.77cB	93.33±6.67bA
	6.3	56.67±3.33bC	70.00±10.00bB	83.33±6.67bA	80.00±5.77bC	80.00±11.55bB	100±0.00aA
	12.5	70.00±5.77aC	83.33±3.33aB	100±0.00aA	96.67±3.33aC	100±0.00aB	100±0.00aA

There is no significant difference (P>0.05) in the middle of any two means inside the same column contain the same small letter: There is no significant difference (P>0.05) among any two means inside the same row contain the same capital letter.

Table 4: Lethal concentrations of *Saussurea costus* against the cattle lice and fly

Species	Days	Solvents	LC_{50} (95%CL) *	LC_{90} (95%CL)	LC_{95} (95%CL)	Slope ±SD	X^2
Haematopinus eurysternus	1	Methanol	1.23 (1.04-1.41)	3.93 (3.31-4.92)	5.47 (4.44-7.26)	2.539±0.233	0.694
		Hexane	1.78 (1.45-2.13)	10.99 (8.31-16.21)	18.38 (12.95-30.28)	1.625±0.159	2.501
	3	Methanol	0.70 (0.53-0.85)	2.30 (1.97-2.80)	3.23 (2.67-4.20)	2.480±0.273	0.814
		Hexane	1.07 (0.82-1.31)	5.97 (4.71-8.25)	9.71 (7.19-14.94)	1.718±0.179	6.702
	7	Methanol	0.31 (0.14-0.46)	1.20 (0.95-1.48)	1.76 (1.44-2.31)	2.175±0.359	0.916
		Hexane	0.57 (4.21-7.01)	2.55 (1.75-3.36)	3.89 (2.88-4.78)	1.981±0.275	10.448
Hippobosca maculata	1	Methanol	4.91 (3.99-6.28)	46.97 (28.23-102.33)	89.07 (47.99-231.25)	1.307±0.147	0.663
		Hexane	2.17 (1.82-2.55)	11.09 (8.48-16.03)	17.60 (12.65-27.96)	1.811±0.170	0.536
	3	Methanol	2.72 (2.23-3.28)	20.99 (14.52-35.81)	37.45 (23.72-73.34)	1.445±0.149	0.375
		Hexane	1.39 (1.09-1.68)	8.59 (6.76-11.85)	14.40 (10.62-21.96)	1..621±0.151	6.501
	7	Methanol	1.26 (0.39-1.83)	8.53 (6.77-43.37)	14.67 (12.93-124.91)	1.544±0.158	10.296
		Hexane	0.63 (0.47-0.79)	2.29 (1.95-2.76)	3.28 (2.72-4.23)	2.312±0.248	1.532

* $LC_{50, 90,}$ and $_{95}$ values= lethal concentration that kills 50, 90, and 95% of the exposed ectoparasite; X^2= chi-square; Significant at P<0.05 level.

Table 5: The major chemical ingredients of the methanol extracts of *Saussurea costus*

No.	Molecular formula	Chemicals (100%)	Area (%)	RT	Nature of compound
1	$C_{15}H_{24}$	CYCLOHEXANE, 1-ETHENYL-1-METHYL-2,4-BIS(1-METHYLETHENYL)-, [1S-(1à,2á,4á)]-	2.22	10.51	phenol
2	$C_{15}H_{24}$	BICYCLO[7.2.0]UNDEC-4-ENE, 4,11,11-TRIMETHYL-8-METHYLENE-, [1R-(1R*,4E,9S*)]-	1.76	11.01	fatty acid esters
3	$C_{13}H_{20}O$	3-BUTEN-2-ONE, 4-(2,6,6-TRIMETHYL-2-CYCLOHEXEN-1-YL)-	0.34	11.36	carboxylic acid
4	$C_{15}H_{24}$	Tricyclo[5.4.0.0(2,8)]undec-9-ene, 2,6,6,9-tetramethyl-, (1R,2S,7R,8R)-	0.27	12.30	fatty acid esters
5	$C_{15}H_{24}$	Aromandendrene	0.69	12.38	fatty acid esters
6	$C_{15}H_{24}$	á-Longipinene	0.41	12.56	fatty acid ester
7	$C_{17}H_{26}O_2$	Methyl 4,7,10,13-hexadecatetraenoate	0.15	12.65	acid hexadecyl ester
8	$C_{15}H_{24}$	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2à,4aà,8aá)]-	0.28	12.74	sesquiterpene
9	$C_{15}H_{26}O$	2,6,10-DODECATRIEN-1-OL, 3,7,11-TRIMETHYL-	0.17	12.99	sesquiterpene alcohol
10	$C_{15}H_{24}O$	Caryophyllene oxide	1.02	14.27	phenol
11	$C_{17}H_{28}$	1,8,11,14-Heptadecatetraene, (Z,Z,Z)-	16.27	16.02	acyclic hydrocarbons
12	$C_{15}H_{24}O$	2-((2R,4aR,8aS)-4a-Methyl-8-methylenedecahydronaphthalen-2-yl)prop-2-en-1-ol	2.49	17.87	sesquiterpene
13	$C_{15}H_{24}O$	Aromadendrene oxide-(2)	0.29	18.52	sesquiterpene
14	$C_{15}H_{20}O_2$	2(3H)-Benzofuranone, 6-ethenylhexahydro-6-methyl-3-methylene-7-(1-methylethenyl)-, [3aS-(3aà,6à,7á,7aá)]-	22.67	19.29	sesquiterpene
15	$C_{15}H_{20}O_2$	Dihydrodehydrocostus lactone	2.29	20.87	sesquiterpene
16	$C_{15}H_{18}O_2$	Azuleno[4,5-b]furan-2(3H)-one, decahydro-3,6,9-tris(methylene)-, [3aS-(3aà,6aà,9aà,9bá)]-	47.28	22.10	phenol
17	$C_{15}H_{20}O_2$	2(3H)-Benzofuranone, 6-ethenylhexahydro-6-methyl-3-methylene-7-(1-methylethenyl)-, [3aS-(3aà,6à,7á,7aá)]-	1.40	23.07	sesquiterpene

Table 6: The foremost phytochemical constituents of the hexane extracts of *Saussurea costus*

No.	Molecular formula	Chemicals (100%)	Area (%)	RT	Nature of compound
1	$C_6H_{14}O_2$	HYDROPEROXIDE, HEXYL	0.27	5.03	secondary alcohol
2	C_8H_{16}	1-Hexene, 3,4-dimethyl-	0.38	6.25	terpenes
3	$C_{15}H_{24}$	CYCLOHEXANE, 1-ETHENYL-1-METHYL-2,4-BIS(1-METHYLETHENYL)-, [1S-(1à,2á,4á)]-	0.49	14.57	fatty acid esters
4	$C_{15}H_{24}$	BICYCLO[7.2.0]UNDEC-4-ENE, 4,11,11-TRIMETHYL-8-METHYLENE-, [1R-(1R*,4E,9S*)]-	0.33	15.17	fatty acid
5	$C_{17}H_{28}$	1,8,11,14-Heptadecatetraene, (Z,Z,Z)-	7.43	21.13	sesquiterpene lactone
6	$C_{15}H_{24}O$	Caryophyllene oxide	0.25	23.38	phenol
7	$C_{15}H_{24}O$	2-((2R,4aR,8aS)-4a-Methyl-8-methylenedecahydronaphthalen-2-yl)prop-2-en-1-ol	0.33	23.43	sesquiterpene lactone
8	$C_{15}H_{20}O_2$	2(3H)-Benzofuranone, 6-ethenylhexahydro-6-methyl-3-methylene-7-(1-methylethenyl)-, [3aS-(3aà,6à,7á,7aá)]-	19.90	25.09	fatty acid esters
9	$C_{15}H_{20}O_2$	Dihydrodehydrocostus lactone	1.61	26.96	fatty acid esters
10	$C_{15}H_{18}O_2$	Azuleno[4,5-b]furan-2(3H)-one, decahydro-3,6,9-tris(methylene)-, [3aS-(3aà,6aà,9aà,9bá)]-	1.11	28.07	fatty acid
11	$C_{15}H_{18}O_2$	ETHANONE, 1-(7,8-DIHYDRO-3-HYDROXY-4-PROPYL-2-NAPHTHALENYL)-	67.27	28.67	sesquiterpene
12	$C_{15}H_{20}O_2$	2(3H)-Benzofuranone, 6-ethenylhexahydro -6-methyl-3-methylene-7-(1-methylethenyl)-, [3aS-(3aà,6à,7á,7aá)]-	0.63	29.65	Phenol

Biochemical analysis: The phytochemical elements of *S. costus* extracts were detected by GC–MS analysis and illustrated by GC chromatogram (Table 5 and 6 and Fig. 1 and 2) indicating that the main chemical compounds of *S. costus* extracts belonged to sesquiterpene, fatty acid esters, phenols, and acyclic hydrocarbons.

DISCUSSION

Hematophagous arthropods transmit debilitating or lethal pathogens to humans and livestock all over the world (McIntyre, 2000). *Saussurea* spp. had been evaluated against the selected hematophagous arthropods for the first time in this study.

The data of this investigation showed that methanol and hexane extracts of *S. costus* extracts induced a clear efficiency in killing *H. dromedarii* and *R. annulatus* ticks. A similar finding was recorded in our previous and recent work as *Commiphora molmol* and *Araucaria heterophylla* extracts effectively controlled the same ectoparasites using the same extracts and technique (Baz *et al.,* 2022b).

The present study showed that plant extracts of *S. costus* induced high mortalities against the lice, *Ha. eurysternus,* and fly, *Hi. maculata,* at 12.5 mg/ml after 24 hours of exposure and up to seven days. Likewise, Baz *et al*. (2022b) reported similar finding AT with methanol and hexane extracts using the same concentration and pests.

Analogous studies proved that plant extracts had an acaricidal effect against ticks such as *Artemisia herba-alba* against *H. dromedarii* (Abdel-Ghany *et al.,* 2021); *Melia azedarach* and *Azadirachta indica* leaves as well as their combination after application on the cattle tick, *H. anatolicum* (Hatzade *et al.,* 2022) and *Protium spruceanum* against a resistant strain of *R. annulatus* AT with the ethanol and ethyl acetate extracts, respectively (Figueiredo *et al.,* 2019).

There is a lack of natural alternatives to insecticidal treatments for managing cattle lice, but parallel studies against the other species of lice indicated that the volatile oils of peppermint, onion, rosemary, and chamomile (*Cinnamomum camphora*, *Mentha piperita*, *Allium cepa*, *Rosmarinus officinalis*, and *Matricaria chamomilla*, respectively) showed *in vitro* and *in vivo* anti-lice and ovicidal effects after treatment of the buffalo infested with *Ha. Tuberculatus* (Khater *et al*. 2009). Moreover, pumpkin, clove, garlic, marjoram, and onion essential oils *in vitro* controlled the louse of dogs*, Trichodectes canis* (Abdel-Meguid *et al.,* 2022) and camphor oil was highly effective *in vitro* and *in vivo* against the louse of pigeons*, Columbicola columbae* (Khater *et al.,* 2014).

Similar findings AT of *Hi. maculata* were also reported as the leaves of *Gloriosa superba*, *malabarica malabarica,* and *Ricinus communis* (chloroform, chloroform, and ethanol extracts, respectively) controlled *Hi. maculata* as well as *Haemaphysalis bispinosa* (Zahir *et al.,* 2010). *Catharanthus roseus* as aqueous crude leaf extract had toxic effect against the adult stages of *Hi. maculata* and *Bovicola ovis*, the biting louse of sheep (Velayutham *et al.,* 2012).

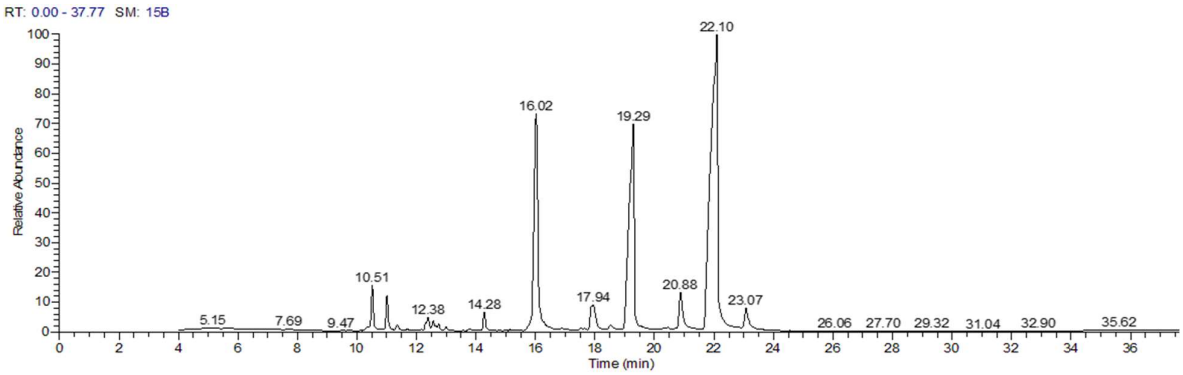

Fig. 1: GC chromatogram of *Saussurea costus* methanol extracts.

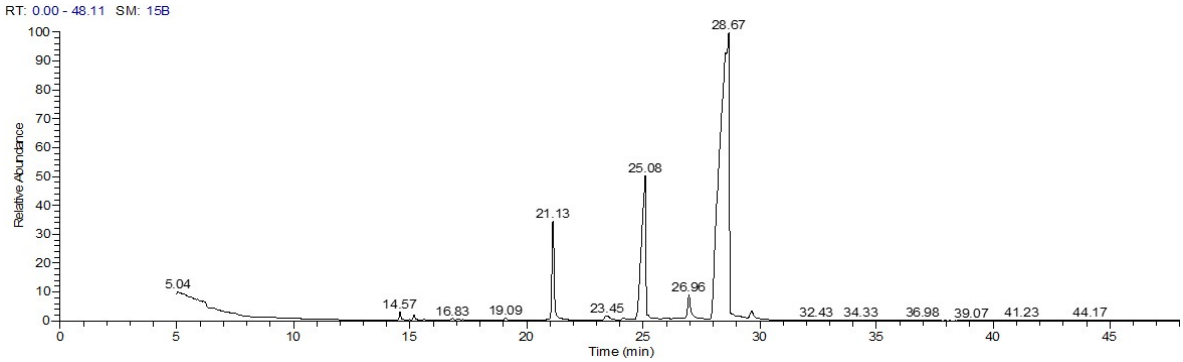

Fig. 2: GC chromatogram of *Saussurea costus* hexane extracts.

A parallel research indicated that the cattle tick larvae of *R. (Boophilus) microplus* and *Hi. maculata* were highly susceptible to the aqueous extract, synthesized Ag NPs and $AgNO_3$ solution of *Cissus quadrangularis* (Santhoshkumar *et al.,* 2012).

Alike study showed the larvicidal capability of *S. costus* 24 h AT of 4th instar larvae of *Anopheles stephensi, Culex quinquefasciatus*, and *Ae. aegypti* and the methanol extract of the roots was the most effective against *An. stephensi* (Ali and Venkatesalu, 2020). An analogous study screened insecticidal activity of 57 plants against *Aedes aegypti* (the yellow fever mosquito) and recorded that *Saussurea lappa*, exhibited high mortality against females at 5 μg/mosquito, whereas its ethanol extract was efficient larvicide (Al-Massarani *et al.,* 2019).

Saussurea spp. induced anitfeedant effect like the ethanol extract of *S. costus* against larvae of the Egyptian cotton leafworm, *Spodoptera littoralis* (Abdallah *et al.,* 2017). Costunolide is a product of *S. lappa* was effective against larvae of the lime butterfly*, Papilio demoleus* L., Lepidoptera: Papilionidae (Vattikonda *et al.,* 2015). In contrast to our funding, acetone extract of *S. costus*, was less effective as insect growth regulator against the red flour beetle *Tribolium castaneum* (Sagheer *et al.,* 2014). Such dissimilar result could be attributed to using different plant extracts and pest species.

Our data for GC–MS analysis showed that *S. costus* extracts contained chemical compounds belonging to Sesquiterpene, fatty acid esters, phenols, and acyclic hydrocarbons. Similarly, the methanol extract of *S. costus* roots is abundant in some phytochemical compounds like flavonoids, alkaloids, phenols/polyphenols, terpenoids, quinines, tannins, steroids, coumarins, cardiac glycosides, and resins (Abdallah *et al.,* 2017).

A comparable finding recorded that *S. lappa* has expected flavonoids, phytosterols, lignans, terpenes possessing a wide range of biological activity like anti-inflammatory, anticancer, anti-viral, anti-hepatotoxic, etc. The main chemical constituents are sesquiterpenes, dihydrocostunolide, dehydrocostuslactone, Lappadilactone and costunolide (Hassan and Masoodi, 2020).

The presence of phenols, flavonoids, and tannins could explain the high efficacy of the plant extracts used in this study against cattle and camel pests. Phenolics are related to insecticidal efficacy as they play a role in plant-herbivore and pathogen interactions. Moreover, the antioxidant properties of phenolics are presumed to be the main activate in the pesticide effect (Ukoroije and Otayor, 2020).

Analogous study indicated that extracts (hexane and methanol) of *C. molmol* are rich in sesquiterpene, phenols, and fatty acid esters; but those of *A. heterophylla* contained phenols, sesquiterpene, terpene, monoterpene, alcohols, and fatty acid (Baz *et al*., 2022b). Different findings may be attributed to using plant or pest species, locality, and the extraction procedure.

The reported active components in this work included phenols, flavonoids, and tannins could explain the high insecticidal efficacy of the plant extracts against cattle and camel ectoparasites. Similar finding was recorded (Abdallah *et al*., 2017; Baz *et al.,* 2021; 2022b).

Conclusions: It is vital to protect domestic animals against blood feeding ectoparasites and their related

diseases. For control of resistant pests, natural products are used as eco-friendly insecticides and acaricides and this study revealed the efficacy of *S. costus* extracts against ectoparasites of large animals for the first time. The methanol extracts were more potent in killing treated pests than hexane extracts. Future studies should be carried out to evaluate the on farm efficacy of these extracts and also to check the safety profile of *S. costus* against non-target organisms.

Authors contribution: Conceptualization, MMB, MMH, and HFK; methodology, MMB, MMH, RMM, NME and HFK; software, YAE, MMB and NME; validation, AS, HFK, and YAE; formal analysis, MMB, AS, and HFK; investigation, MMB, AS, and HFK: data analysis, MMB, and HFK; writing—original draft preparation, MMB, MMH, NME, YAE and HFK.; writing—review and editing, MMB, AS, HFK, RMM and YAE; supervision, MMB, RMM, AS, and HFK; All authors had red and agreed about this version of the manuscript.

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