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The Efficacy of *Enteromorpha intestinalis* and *Cladophora crispata* Extracts against Sarcoptic Mange *In Vitro* and *In Vivo*

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ABSTRACT

One of the important veterinary parasites is *Sarcoptes scabiei* which causes sarcoptic mange. The traditional treatment causes environmental hazards and might lead to resistance in the target species. The study was aimed to evaluate the efficacy of the methanol extracts of *Enteromorpha intestinalis* and *Cladophora crispata* algae against sarcoptic mange *in vitro* and *in vivo*. Forty-two rabbits infested with *S. scabiei* mites are used. LT_{50} and LC_{50} were calculated using probit analysis. The intensity and recovery of the lesion were described using grade codes and mites examination. All extracts concentrations have killed all mites *in vitro* at 2 h post-treatment, The LT_{50} values of *E. intestinalis* extract at 2, 1, and 0.5 g/mL were 0.5165, 0.7626, and 1.1982 h, respectively, the LT_{50} values of *C. crispata* extract at 2, 1, and 0.5 g/mL were 0.4656, 0.5165, and 0.6641 h, respectively. The LC_{50} values of *E. intestinalis* extract at 0.5 and 1 h post-treatment were 1.5869 and 0.3436 g/mL, respectively, the LC_{50} values of *C. crispata* extract at 0.5 and 1 h post-treatment were 1.9358 and 0.6252 g/mL, respectively. 2% of *E. intestinalis* and *C. crispata* is exhibited completely cleared infesting mites *in vivo* at 16 and 22 days post-treatment, respectively. The clinical sarcoptic mange was completely cured at 22 days post-treatment by the concentration of 20% of *C. crispata* extract, followed by 20% of *E. intestinalis* extract. The results indicate that the *E. intestinalis* and *C. crispata* extracts could be used for effective control of sarcoptic mange.

Keywords: *Cladophora crispata, Enteromorpha intestinalis*, Extract, Sarcoptic mange.

Introduction

Rabbits may be infested by a variety of mites, such as fur mites, ear mites, or burrowing mites. *Sarcoptes* sp. mites are deep burrowing mites that affect the head, feet, nose, and areas around the genitalia.[1] Sarcoptic mange in rabbits is a highly infectious, non-seasonal, pruritic skin disease and is caused by *Sarcoptes scabiei* mites. Overcrowded living conditions and poor hygiene are essential factors for *S. scabiei* mites infestation.[2] Mites widely spread from rabbit to another rabbit by direct contact or through connection with an infested environment.[3] They can also spread from rabbits to other animals like cats or other pet animals if they are kept on the same premises.[4]

One of the most important veterinary ectoparasites and cause greater weight loss, productivity, and quality of wool in rabbits are *S. scabiei* var. *cuniculi*. Previously, the possibility of zoonotic infection has been recorded.[5] It sparingly affects hairy rabbit parts such as the ears, nose, feet, and areas around the genitalia, resulting in hair loss, intense pruritus, hypertensive reaction, reduced body weight gain, and death.[6] Most of the sarcoptic mange is related to reduced feed consumption, digestibility, and rate of conversion with meningitis development.[7]

Recently, many chemical agents are commonly used in veterinary clinics for the treatment and control of sarcoptic mange, and with certain drugs like ivermectin and abamectin, relatively good treatment efficacy has been achieved. However, in addition to the toxicity and environmental hazards, the usage of chemicals might lead to resistance in the target species.[8] Other traditional sarcoptic mange treatment includes external application of pyrethroid compounds, organophosphates, or amitraz, but its use may be problematic in that it requires a frequent and cautious application and may have side effects.[9] Treatment of sarcoptic mange with various acaricides such as Diazinon, Fenvalerate, Deltamethrin, and Avermectin has been tried with varying success levels. The main difficulties associated with the use of artificial acaricides are the rapid development of resistance, environmental contamination, high cost, and health risks to humans during the treatment of an animal.[10] Given these difficulties, the use of algal or botanical acaricides against sarcoptic mange has been visualized.

Algae are important human nutrition compounds and hopeful sources of active metabolites for the advancement of the therapeutic industry.[11,12] In the latest decades, research has shown that algal species have anti-biological activities including anti-microbial, anti-allergic, anti-viral, anti-oxidant, anti-cancer, anti-inflammatory, and anti-obesity activities.[13,14]

Among the medicinally important algal species, the green algae *Enteromorpha intestinalis* and *Cladophora crispata*. The *E. intestinalis* has been used in many studies as an anti-tumours, [15,16,17] antibacterial, [18,19] antifungal, [20] antioxidant, [21,22] and antiparasite. [23,24] Whereas the *Cladophora crispata* has been used as antibacterial and antifungal, [25,26] and anti-parasite.[23,27]

So, as far as we know, the efficacy of *E. intestinalis* and *C. crispata* algae against sarcoptic mange has not been previously studied. The current study aimed to estimate the potential activity of *E. intestinalis* and *C. crispata* methanol extracts against sarcoptic mange in rabbits.

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Materials and Methods

Algal collection and extraction

Algal samples were collected in March 2019 using clean sample containers from the Al-Hartha area in Basrah / Iraq and transferred to the laboratory and identified by an algologist at the University of Basrah, Basrah, Iraq. Algal samples were carefully washed in distilled water and dried away from direct sunlight at room temperature. Dried samples were ground and preserved for further use at 18°C. According to, [28] the algal methanol extracts were prepared by dissolving 40 g of dried algae in 200 mL of methyl alcohol (95%). Then the mixture was left for 72 h in a flask and stirred every 24 h. It was subsequently filtered using Whatman filter paper No.1 and concentrated using a rotary evaporator at 40°C, then left to dry and preserved at 4°C in sterile vials.

Animals

Eighty *Oryctolagus cuniculus* healthy female rabbits of approximately the same age group and weight were naturally infected by close contact with other rabbits infected with sarcoptic mange, all of them curtaining in separate rabbit management cages at Animal House, Biology department, Education for Pure Sciences College, Basrah University, Basrah, Iraq. It was confirmed that the rabbits had a normal infection with *S. scabiei* var. *cuniculi* by a skin scraping test.

Ethics approval

The animal experimentation committee of the University of Basrah's Department of Biology approved the protocol for all animal experiments performed in this study (Ethical Approval Number: 204/2020).

The activity assay in vitro

The scabs were isolated from the infested rabbits and then placed in Petri dishes and incubated at 35˚C for 30 min in an incubator to collect the *S. scabiei* var. *cuniculi* adult mites for testing. To prepare 2, 1, and 0.5 g/ml concentrations, the algal methanol extract was diluted with distilled water containing glycerine (10%), 0.5 ml of the concentration was added to the Petri dishes (diameter 10 cm; height 2 cm) with filter paper chips to absorb the liquid. To evaluate the activity of the extracts, 10 samples of mites were placed on the filter paper in each Petri dish, then incubated for 0.5, 1, 2, 4, or 6 h under 25˚C and 75% relative humidity. [29,30] For each extract concentration, three replicates were performed. As an untreated control, three Petri dishes containing 0.5 ml of glycerol and distilled water were used, while 2% ivermectin was used as conventional treatment. The mites were frequently stimulated with a needle to evaluate their viability, and the mites were documented as dead if no response was detected.[31]

Animal treatment

A 22-day long experiment on forty-two rabbits selected from the infected groups was carried out. Rabbits were divided into seven groups (In each group were six animals) and classified into different treatments in a completely randomized design (Table 1). All the extracts were prepared for the treatment as an ointment (10% and 20%) by using Vaseline (w/w). Hair was clipped around the infected areas, the lesions were cleaned with warm water and left to dry for some time before the application of the ointment, the ointment was then added in adequate amounts to create a thin layer over the infected area. All groups were treated on alternative days (Day 0–14) seven times in a total, excluding the control group (the area of the lesion only cleaned with lukewarm water), and the ivermectin group was treated only once with 200 µg/kg (s/c inj.) of ivermectin at the beginning of the experiment (Day 0). On alternative days at the time of treatment application, response to treatment was observed in terms of mean recovery response and percentage decrease of mites.[10]

Clinical examination

The rabbit clinical examination was conducted on day 0 and then on alternate days up to day 22. The intensity of the lesion of an individual animal was described at the start of the experiment using grade codes from 1 to 4 indicating an increasing intensity of the skin reaction.

Table 1: Treated groups

Group no.	Treatment
1	*E. intestinalis* 10%
2	*E. intestinalis* 20%
3	*C. crispata* 10%
4	*C. crispata* 20%
5	Ivermectin 200 µg/Kg
6	Vaseline
7	Untreated (control)

E. intestinalis 10% and 20% = 10% and 20% ointment w/w of *E. intestinalis* methanol extracts; *C. crispata* 10% and 20% = 10% and 20% ointment w/w of *C. crispata* methanol extracts.

Following the treatment, (from day 0 to the end of the experiment) recovery in individual animals was described with grade codes from 0 to 4, and the mean recovery response of each group was calculated to compare the treatment effect. [32]

Mites examination in vivo

According to, [33] skin scrapings were taken from the areas of the lesions neighboring healthy tissue by scraping 1 cm^2 of the area from three different body sites. Samples were observed within 6 h of collection. Scrapings from three areas of the lesion were collected and placed in a tube containing distilled water and 10 % KOH (5 ml) then heated till epidermal scales and hair were liquified. Then they were centrifuged for 10 min at 10 g. The sediment was suspended in distilled water, and it has been re-centrifuged. Sediment was analyzed under a microscope to calculate the total number of the mites, the morphological keys were used to identify mites was according to. [34] The treatment efficacy was calculated by using the equation shown below. [35]

$$\text{Treatment efficacy \%} = \frac{\text{mites number before treatment} - \text{mites number after treatment}}{\text{mites number before treatment}} \times 100$$

Statistical analysis

The data were analyzed via SPSS ver. 19 software by using the ANOVA and the chi-square (χ^2) tests, the differences were considered statistically significant when $P \leq 0.05$. According to, [36] the probit analysis was used to calculate the median lethal concentration value (LC_{50}) and the median lethal time value (LT_{50}).

Results and Discussion

The algal methanol extracts demonstrated strong toxicity to the *S. scabiei* var. *cuniculi* mites, and their activity was dependent on time and concentration. Among all the treated groups, significant differences in mean mortality were observed at 0.5 h ($P = 0.005$) and 1 hour ($P = 0.000$) of the experiment. It has been observed that the mean mortality in the mites for the treated groups with the highest concentrations (2 g/ml) of both algae was approximately equal to the ivermectin group throughout the treatment period. The mean mortality at 0.5 and 1 h was 50% and 90% respectively of both 2 g/ml of *C. crispata* methanol extract and 2% of ivermectin groups, as for, the mean mortality of the treated group with 2 g/ml of *E. intestinalis* methanol extract at 0.5 and 1 hour was 50% and 80% respectively. Whereas the mean mortality in the mites for all treated groups was comparable (100%) at 2, 4, and 6 h. It is observed that the mites in the control group (treated with 10% glycerin) stayed alive after 6 h of treatment (Table 2).

The LT_{50} values of the algal methanol extracts against *S. scabiei* var. *cuniculi* mites are shown in Table 3. The current results indicate that algal methanol extracts at all concentrations were highly toxic to mites. The LT_{50} values of *E. intestinalis* methanol extract at the concentrations of 2, 1, and 0.5 g/mL were 0.5165, 0.7626, and 1.1982

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h, respectively, and the LT_{50} values of *C. crispata* methanol extract at the concentrations of 2, 1, and 0.5 g/mL were 0.4656, 0.5165, and 0.6641h, respectively. Whereas, the LT_{50} value of the ivermectin 2% was 0.5165 h. This indicates that the highest concentration of *C. crispata* methanol extract (2 g/mL) was highly toxic than ivermectin 2%, while LT_{50} values were comparable in both 2 g/mL of *E. intestinalis* methanol extract and 2% of ivermectin.

As shown in Tables 4 and 5. The LC_{50} values of the methanol extract of *E. intestinalis* at 0.5 and 1-hour post-treatment were 1.5869 and 0.3436 g/mL, respectively. Whereas the LC_{50} values of the methanol extract of *C. crispata* at 0.5 and 1 h post-treatment were 1.9358 and 0.6252 g/mL, respectively. While no LC_{50} values of all algal extract concentrations were recorded at 2, 4, and 6 h post-treatment due to the equal mean mortality (100%) of all algal extract concentrations.

The effect of different treatments on infected animals was assessed by estimating the mean recovery response (Table 6). There was a statistically significant difference in mean recovery response ($P \leq 0.05$) in all treated groups over the treatment periods, except for 2 and 14 days post-treatment, and it is also observable that algal methanol extracts were superior to ivermectin during the treatment periods. The highest mean recovery response was observed in animals treated with 20% of *C. crispata* methanol extract with a mean recovery response score of 4.00 at 22 days post-treatment. A high mean recovery response was also recorded with a score of 3.66 at 22 days post-treatment in animals treated with 20% of *E. intestinalis* methanol extract. Followed by the animal treated with 10% of the methanol extract of *C. crispata* with a mean recovery response of 3.66 at 22 days post-treatment. Whereas the mean recovery response was equal in animals treated with 10% of *E. intestinalis* methanol extract and with 200 mg/kg of ivermectin, it was 2.66 at 22 days post-treatment. While the animals that were treated with Vaseline showed no improvement and recovery.

The treatment efficacy of the algal methanol extracts was determined at 4, 10, 16, and 22 days post-treatment (Table 7). Although the treatment efficacy values of the different groups varied overtime periods, they were statistically non-significant ($P \leq 0.05$). The highest efficacy of treatment was detected in animals treated with 20% of the methanol extract of *C. crispata* at 4 days post-treatment with 87%, while the highest treatment efficacy was 96% and 100% at 10 and 16 days post-treatment respectively in animals treated with 20% of the methanol extract of *E. intestinalis*. The treatment efficacy of all animals in treated groups was 100% at 22 days post-treatment, it is evidence that the animals are free of the mites.

There are many disadvantages to the drugs presently used for sarcoptic mange, including resistance development, toxicity, and management difficulty, which increases the need for drugs that are safer and more effective. Further research on the treatment of sarcoptic mange using natural and herbal elements are required. [37] To our knowledge, no previous studies have used algal extracts for the management of sarcoptic mange and against *S. scabiei* var. *cuniculi* mites, although many studies have used plant materials for the management of sarcoptic mange in rabbits as an alternative treatment.

The methanol extracts of *E. intestinalis* and *C. crispata* exhibit strong toxic activity against sarcoptic mange. It was shown that the extract's toxicity was dependent on time and concentration. Similar influences have been seen *in vivo* for the crude aqueous and aqueous methanol extracts of neem seed kernel against sarcoptic mange,[10] as well in the *Eupatorium adenophorum* ethanolic extract against *S. scabiei*, [29,38] the neem aqueous leaf extract against *S. scabiei* var. *cuniculi in vivo* and *in vitro*, [39] also, for *Ligularia virgaurea* ethanolic extracts against the *S. scabiei* mite *in vitro*, [40] the oil of lemon against *S. scabiei* var. *cuniculi in vivo* and *in vitro*. [41]

The efficacy of *E. intestinalis* and *C. crispata* extracts may be due to their active compounds. *E. intestinalis* has many bioactive compounds such as carbohydrates, alkaloids, saponins, terpenes, sterols, and phenolics. [42,43] *C. crispata* may also possess alkaloids, tannins, fatty acids, polysaccharides, amino acids, proteins, terpenes, sterols, phenolics, flavonoids, aromatic organic acids, aldehydes, and ketones. [26] Active compounds can affect the parasite's body in several ways, for example, terpenes interact readily with biomembranes and membrane proteins, they could increase the fluidity and permeability of the membrane, which can lead to uncontrolled ion efflux and metabolites and even cell leakage, leading to cell necrotic or cell apoptotic, Also, the action of membrane proteins and receptors or ion channels can be modulated by them. Neuroreceptors are often alkaloid molecular targets or modulate other steps in the transduction of neuronal signals, including enzymes or ion channels that capture or metabolize second messengers or neurotransmitters, whereas certain alkaloids are mutagenic by alkylating or intercalating DNA. Some alkaloids interact with DNA, telomeres, telomerase, topoisomerase, cytoskeleton, or protein biosynthesis and induce apoptosis. Some types of saponins are amphiphilic compounds that bind to surface glycoproteins and glycolipids and can complicate cholesterol in biomembranes. This causes severe biomembrane tension and leakage. Whereas, there is a wide variety of medical and pharmacological properties of phenolics, such as anti-inflammatory, antioxidant, wound healing, sedating, antiviral and antimicrobial activities.[44]

Table 2: The mean mortality of *S. scabiei* var. *cuniculi* mites in treated groups

Treatment	Conc. g/ml	The mean mortality %				
		0.5 h	1 h	2 h	4 h	6 h
E. intestinalis	0.5	0	40	100	100	100
	1	20	70	100	100	100
	2	50	80	100	100	100
C. crispata	0.5	40	60	100	100	100
	1	50	80	100	100	100
	2	50	90	100	100	100
Ivermectin 2%		50	90	100	100	100
Control		0	0	0	0	0
χ^2		16.923	26.667	0.000	0.000	0.000
P-value		0.005	0.000	1.000	1.000	1.000

Table 3: The LT_{50} values of the algal methanol extracts and ivermectin

Treatment	Con. (g/ml)	Regression equation	LT_{50} (h)
E. intestinalis	0.5	Y = 6.5110x + 4.4886	1.1982
	1	Y = 4.6534x + 5.5476	0.7626
	2	Y = 3.2555x + 5.9339	0.5165
C. crispata	0.5	Y = 3.6763x + 5.6533	0.6641
	1	Y = 3.2555x + 5.9339	0.5165
	2	Y = 3.2555x + 6.0805	0.4656
Ivermectin 2%		Y = 3.2555x + 5.9339	0.5165

Table 4: The LC_{50} of the methanol extract of *C. crispata*

Time (h)	Regression equation	LC_{50} (g/ml)
0.5	Y = 0.4208x + 4.9156	1.5869
1	Y = 1.7078x + 5.7922	0.3436
2	-	-
4	-	-
6	-	-

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Table 5: The LC_{50} of the methanol extract of *E. intestinalis*

Time (h)	Regression equation	LC_{50} (g/ml)
0.5	Y = 3.2555x + 4.0661	1.9358
1	Y = 1.8187x + 5.3709	0.6252
2	-	-
4	-	-
6	-	-

Table 6: The mean recovery response of treated groups

Treatment	Mean recovery response ± SD										
	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14	Day 16	Day 18	Day 20	Day 22
E. intestinalis 10%	0 ± 0.00	1±0.00^{b}	1.33±0.51^{ab}	1.33±0.51^{a}	1.66±0.51^{b}	1.66±0.51^{b}	2±0.00	2±0.00^{d}	2.33±0.51^{b}	2.33±0.51^{b}	2.66±0.51^{b}
E. intestinalis 20%	0 ± 0.00	1.3± 0.51^{a}	1.66±0.51^{a}	1.66±0.51^{a}	2±0.00^{a}	2.66±0.51^{a}	3±0.00	3±0.00^{b}	3.33±0.51^{a}	3.33±0.51^{a}	3.66±0.51^{a}
C. crispata 10%	0 ± 0.00	1±0.00^{b}	1.33±0.51^{ab}	1.66±0.51^{a}	1.66±0.51^{b}	1.66±0.51^{b}	2±0.00	2.33±0.51^{c}	2.33±0.51^{b}	2.66±0.51^{b}	3±0.00^{b}
C. crispata 20%	0 ± 0.00	1.33±0.51^{a}	1.66±0.51^{a}	1.66±0.51^{a}	2.33±0.51^{a}	3±0.00^{a}	3±0.00	3.33±0.51^{a}	3.33±0.51^{a}	3.66±0.51^{a}	4±0.00^{a}
Ivermectin 200µg/Kg	0 ± 0.00	0±0.00^{c}	1±0.00^{b}	1.33±0.51^{a}	1.66± 0.51^{b}	1.66±0.51^{b}	2±0.00	2±0.00^{d}	2.33±0.51^{b}	2.66±0.51^{b}	2.66±0.51^{b}
Vaseline	0 ± 0.00	0±0.00^{c}	0±0.00^{c}	0±0.00^{b}	0±0.00^{c}	0±0.00^{c}	0±0.00	0±0.00^{e}	0±0.00^{c}	0±0.00^{c}	0±0.00^{c}
Control	0 ± 0.00	0±0.00^{c}	0±0.00^{c}	0±0.00^{b}	0±0.00^{c}	0±0.00^{c}	0±0.00	0±0.00^{e}	0±0.00^{c}	0±0.00^{c}	0±0.00^{c}
RLSD	n.s.	0.289	0.404	0.458	0.409	0.409	n.s.	0.289	0.458	0.458	0.354

Table 7: The treatment efficacy of algal methanol extracts and ivermectin

Treatment	The treatment efficacy %			
	Day 4	Day 10	Day 16	Day 22
E. intestinalis 10%	78	86	88	100
E. intestinalis 20%	83	96	100	100
C. crispata 10%	78	87	93	100
C. crispata 20%	87	90	91	100
Ivermectin 200µg/Kg	80	87	94	100
χ^2	0.724	0.749	0.845	0.000
P-value	0.948	0.945	0.932	1.000

Conclusion

The current study indicates that *E. intestinalis* and *C. crispata* methanol extracts have an active component(s), which suggests that *E. intestinalis* and *C. crispata* could be a source of new active compounds able to control pet mites effectively.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Kachhawa JP, Kachhawaha S, Srivastava M, Chahar A, Singh NK. Therapeutic management of scabies in rabbits. Intas Polivet. 2013; 14(2):306-308.
2. McCarthy JS, Kemp DJ, Walton SF, Currie BJ. Scabies: more than just an irritation. Postgrad med J. 2004; 80(945):382-387.
3. Panigrahi PN and Gupta AR. Therapeutic management of concurrent sarcoptic and psoroptic acariosis in rabbits. Intas Polivet. 2013; 14(2):319-321.
4. Sharma M, Jangir BL, Kumar T, Khurana R. Clinicopathological diagnosis and therapeutic management of sarcoptic mange in a rabbit and cat. Veterinarski arhiv. 2018; 88(6):863-869.
5. Harrenstien L, Gentz EJ, Carpenter JW. How to handle respiratory, ophthalmic, neurologic, and dermatologic problems in rabbits. Vet med. 1995; 90(4):373-380.
6. El-Sheshtawy EA. Survey on parasitic disease, of rabbits in Ismailia province., EVMSPJ. 2003; I(1):183-198.
7. Harkness JE and Wagner JE. Specific diseases and conditions. The Biology and Medicine of Rabbits and Rodents. USA: Williams & Wilkins. 1995. 172 p.
8. Borges FA, Almeida GD, Heckler RP, Lemes RT, Onizuka MK, Borges DG. Anthelmintic resistance impact on tropical beef cattle productivity: effect on weight gain of weaned calves. Trop animal health and prod. 2013; 45(3):723-727.
9. Kumar A, Kumar R, Archana KN. A successful treatment report on rabbits infected with sarcoptic mange. Pharma Innov J. 2018; 7(2):1-3.
10. Tabassam SM, Iqbal Z, Jabbar A, Chattha AI. Efficacy of crude neem seed kernel extracts against natural infestation

of Sarcoptes scabiei var. ovis. J ethnopharmacol. 2008; 115(2):284-287.
11. El-Gamal AA. Biological importance of marine algae. Saudi pharmaceut J. 2010; 18(1):1-25.
12. Gupta S and Abu-Ghannam N. Bioactive potential and possible health effects of edible brown seaweeds. Trends in Food Sci & Tech. 2011; 22(6):315-326.
13. Reichelt JL and Borowitzka MA. Antimicrobial activity from marine algae: results of a large-scale screening programme. InEleventh International Seaweed Symposium. Dordrecht: Springer; 1984; 116/117(1):158-168.
14. Pangestuti R and Kim SK. Biological activities and health benefit effects of natural pigments derived from marine algae. J functional foods. 2011; 3(4):255-266.
15. Wang X, Chen Y, Wang J, Liu Z, Zhao S. Antitumor activity of a sulfated polysaccharide from Enteromorpha intestinalis targeted against hepatoma through mitochondrial pathway. Tumor Biol. 2014; 35(2):1641-1647.
16. Kosanić M, Ranković B, Stanojković T. Biological activities of two macroalgae from Adriatic coast of Montenegro. Saudi J Bio Sci. 2015; 22(4):390-397.
17. Al-Jaber G, Al-Mayah S, Athbi A. Cytotoxic Activity of Enteromorpha intestinalis Extracts against Tumor Cell-Line HeLa. Technology (GC-mass), J Bio Agriculture and Healthcare. 2015; 5(24):17-21.
18. Saranya C, Parthiban C, Anantharaman P. Evaluation of antibacterial and antioxidant activities of seaweeds from Pondicherry coast. Advances in Appl Sci Res. 2014; 5(4):82-90.
19. Srikong W, Mittraparp-arthorn P, Rattanaporn O, Bovornreungroj N, Bovornreungroj P. Antimicrobial activity of seaweed extracts from Pattani, Southeast coast of Thailand. Food and Appl Biosci J. 2015; 3(1):39-49.
20. Hellio C, Bremer G, Pons AM, Le Gal Y, Bourgougnon N. Inhibition of the development of microorganisms (bacteria and fungi) by extracts of marine algae from Brittany, France. Appl Microbiol and Biotechnol. 2000; 54(4):543-549.
21. Farasat M, Khavari-Nejad RA, Nabavi SM, Namjooyan F. Antioxidant activity, total phenolics and flavonoid contents of some edible green seaweeds from northern coasts of the Persian Gulf. Iranian J pharmaceut res: IJPR. 2014; 13(1):163-170.
22. Chernane H, Mansori M, Latique S, El Kaoua M. Evaluation of antioxidant capacity of methanol extract and its solvent fractions obtained from four moroccan macro algae species. Euro Sci J. 2014; 10(15):35-40.
23. Spavieri J, Kaiser M, Casey R, Hingley-Wilson S, Lalvani A, Blunden G, Tasdemir D. Antiprotozoal, antimycobacterial and cytotoxic potential of some British green algae. Phytotherapy Res. 2010; 24(7):1095-1098.
24. Ravikumar S, Ramanathan G, Gnanadesigan M, Ramu A, Vijayakumar V. In vitro antiplasmodial activity of methanol extracts from seaweeds of South West Coast of India. Asian Pacific J Trop Med. 2011; 4(11):862-865.
25. Mahadik BB and Jadhav MJ. Antibacterial and Antifungal Activities of Green Alga Cladophora crispata. Indian J Appl Res. 2015; 5(3):37-39.
26. Hussein UA. Assessment of the antibacterial activity of macroalgae Cladophora crispata extract against extended spectrum beta-lactamase producing Escherichia coli isolated from diarrheic children. University of Thi-Qar J Sci. 2017; 6(3):3-11.
27. Athbi AM, Al-Mayah SH, Khalaf AK. Antiparasitic activity of the microalgae Cladophora crispata against the Protoscolices of hydatid cysts compared with albendazole drug. Afri J Biotech. 2014; 13(30):3068-3080.
28. Alade PI and Irobi ON. Antimicrobial activities of crude leaf extracts of Acalypha wilkesiana. J ethnopharmacol. 1993; 39(3):171-174.
29. Nong X, Ren YJ, Wang JH, Xie Y, Fang CL, Yang DY, Liu TF, Zhang RH, Chen L, Gu XB, Peng XR. Clinical efficacy of botanical extracts from Eupatorium adenophorum against the Sarcoptes scabiei (Sarcoptidae: Sarcoptes) in rabbits. Vet parasitology. 2013; 195(1-2):157-164.
30. Liao F, Hu Y, Tan H, Wu L, Wang Y, Huang Y, Mo Q, Wei Y. Acaricidal activity of 9-oxo-10, 11-dehydroageraphorone extracted from Eupatorium adenophorum in vitro. Exp parasitology. 2014; 140:8-11.
31. Pasay C, Mounsey K, Stevenson G, Davis R, Arlian L, Morgan M, Vyszenski-Moher D, Andrews K, McCarthy J. Acaricidal activity of eugenol based compounds against scabies mites. PloS one. 2010; 5(8):e12079.
32. Sharma DK, Saxena VK, Sanil NK, Singh N. Evaluation of oil of Cedrus deodara and benzyl benzoate in sarcoptic mange in sheep. Small Ruminant Res. 1997; 26(1-2):81-85.
33. Fthenakis GC, Papadopoulos E, Himonas C, Leontides L, Kritas S, Papatsas J. Efficacy of moxidectin against sarcoptic mange and effects on milk yield of ewes and growth of lambs. Vet parasitology. 2000; 87(2-3):207-216.
34. Soulsby EJ. Helminths, arthropods and protozoa of domesticated animals. London: Bailliere tindall, The English Language Book Society. 1982; 654p.
35. Khan MN, Hayat CS, Iqbal Z. Evaluation of acaricidal efficacy of Ivermectin, Diazinon, Permethrin and Coumaphos in cattle and buffaloes. Pakistan Entomologist. 1998; 19:58-60.
36. Miller LC and Tainter M. Estimation of the ED50 and its error by means of logarithmic-probit graph paper. Proceedings of the society for Experimental Biology and Medicine. 1944; 57(2):261-264.
37. Metwally DM. Investigating the anti-sarcoptic mange activity (in vivo) of propolis ointment in naturally infested rabbits. Biomed Res. 2017; 28(4):0976-1683.
38. Nong X, Fang CL, Wang JH, Gu XB, Yang DY, Liu TF, Fu Y, Zhang RH, Zheng WP, Peng XR, Wang SX. Acaricidal activity of extract from Eupatorium adenophorum against the Psoroptes cuniculi and Sarcoptes scabiei in vitro. Vet Parasitology. 2012; 187(1-2):345-349.
39. Seddiek SA, Khater HF, El-Shorbagy MM, Ali AM. The acaricidal efficacy of aqueous neem extract and ivermectin against Sarcoptes scabiei var. cuniculi in experimentally infested rabbits. Parasitology Res. 2013; 112(6):2319-2330.
40. Luo B, Liao F, Hu Y, Liu XI, He Y, Wu L, Tan H, Luo L, Zhou Y, Mo Q, Deng J. Acaricidal activity of extracts from Ligularia virgaurea against the Sarcoptes scabiei mite in vitro. Exp and Therapeut Med. 2015; 10(1):247-250.
41. Aboelhadid SM, Mahrous LN, Hashem SA, Abdel-Kafy EM, Miller RJ. In vitro and in vivo effect of Citrus limon essential oil against sarcoptic mange in rabbits. Parasitology Res. 2016; 115(8):3013-3020.
42. Manchu N, Melpha Y, Edwin James J. Phytochemical Investigation of Three Species of Ulva from Rasthacaud Coast, Tamil Nadu, India. J Chem Pharm Res. 2014; 6(8):570-574.
43. Abdel-Khaliq A, Hassan HM, Rateb ME, Hammouda O. Antimicrobial activity of three Ulva species collected from some Egyptian Mediterranean seashores. Inter J Eng Res and General Sci. 2014; 2(5):648-69.
44. Wink M. Modes of action of herbal medicines and plant secondary metabolites. Medicines 2015; 2(3):251-286.