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Assessment of phytochemicals, antioxidant, antimicrobial and anthelmintic activities of *Balanites aegyptiaca* from semi-arid regions of Rajasthan, India

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ABSTRACT

The majority of medications used to treat nematodiasis in animals have become ineffective as a result of helminth drug resistance. Additionally, going back to medicinal plants and their substances is an environmentally beneficial option to cure nematodiasis since anthelmintics, like many medications, have been designated as hazardous environmental contaminants. The current study was carried out to evaluate phytochemicals, antioxidant, antibacterial and anthelmintic properties from locally available plant *Balanites aegyptiaca* (*B. aegyptiaca*). An *in vitro* free radical scavenging activity of the different extracts was determined by 2,2-azino bis (3-ethylbenzothiazoline-6-sulfonate) method. The antibacterial test was performed by agar well diffusion method using crude aqueous and hydro alcohol extracts of immature and mature fruits. The egg hatch assay and larval mortality assay were used to evaluate the extracts' in vitro effectiveness. 25% crude extracts (aqueous, methanol, ethanol, hydro alcohol and acetone) were used at different concentrations (10 mg/ml to 0.01 mg/ml). Thiabendazole was employed as a reference standard for EHA at a dose of 10 mg/ml. For LMA levamisole was used @ 10 mg/ml as reference standard. In both the assays' distilled water was used as negative control. In methanol extract of immature fruits Geraniol was the main phytochemicals while in its ethanol extract 4-O-Methylmannose was predominant. Pentanoic acid, 3-methyl- (Valeric acid) and Hexanoic acid (Butyric acid) are predominantly present in mature fruits. In both methanol and ethanol extracts of bark 1,4- Dimethyl-7-(prop-1-en-2-yl) decahydroazulen-4-ol (Pogostole) was predominant. Leaves, bark and mature fruits showed remarkable antioxidant activity. Hydro alcohol extract of immaturte fruit showed marked antimicrobial activity against *E. coli*. At 200 and 300 mg/g of faeces, adding powder to the faecal culture reduced larval growth by more than 97%. All the extracts of studied plant parts were very effective (>95%) in preventing hatching of eggs and killing the larvae (L1 and L2). The *in vivo* study with ethanol extract of immature fruit (@ 0.5 g per kg body weight once) showed non-significant variation in faecal egg counts in extract treated and infected untreated control groups, however, compared to the control group, the extract-treated group's numerical mean intensity of infection was lower on the majority of days. Nevertheless, the reduction in infective larvae observed in faecal cultures from extract treated lambs was highly significant from day 3 to end of the study. In conclusion the crude extracts of *B. aegyptiaca* showed terpenoids, flavonoids, fatty acids and carbohydrates and possessed significant antibacterial, ovicidal and larvicidal properties against *Haemonchus contortus* (*H. contortus)*.

Keywords: *Balanites aegyptiaca*, Disc Diffusion Technique, Egg Hatch Assay, 2,2-azino bis (3-ethylbenzothiazoline-6-sulfonate), *Haemonchus contortus*, Larval mortality Assay.

INTRODUCTION

One of the most dangerous and widespread infectious diseases that significantly impacts livestock productivity and performance is Haemonchosis [1, 2]. The disease's primary cause, *Haemonchus contortus* (*H. contortus*), is considered to be the most pathogenic abomasal nematode that affects ruminants globally [3, 4]. Animal health is significantly impacted by the infection, with the primary symptoms being anaemia, inappetence to off-food, and acute infection-related mortality, especially in young animals [5]. Chemotherapeutics were frequently employed for decades to manage parasite infections in farm animals. Multi-drug-resistant gastrointestinal nematodes (GINs) have developed as a result of the prolonged, excessive, and improper use of anthelmintic medications [6]. Furthermore, the need to find effective substitutes for anthelmintics in order to maintain parasite control has been spurred by the dangers associated with medication residues in food animals as well as their affordability [7]. *H. contortus* was reported to have been the first helminth to develop multi-drug resistance to a variety of anthelmintic classes, including benzimidazole, levamisole, and macrocyclic lactones [8]. Medicinal plants with potentially beneficial bioactive components could offer substantial, environmentally friendly, and cost-effective treatments for animal parasite infection [9]. Plant treatments for endoparasites may be single oral dose, daily doses mixed with feed, and through forage crops rich in secondary metabolites [10].

The detection of unexpected bioactivities that could not have been predicted based on the available data may be made possible by the random selection of plant species. In order to assess the anthelmintic effects of plant extracts against *H. contortus*, we have mostly researched species from the semi-arid ICAR-CSWRI farm. In the present study 25% aqueous, methanol, ethanol, hydro alcohol and acetone extracts of leaves, immature and mature fruits (both epi carp and meso carp) and bark of *Balanites aegyptiaca* (*B. aegyptiaca*) were studied. *B. aegyptiaca*, a desert spiny tree that may grow up to 10 mm in height and is found throughout Africa and South Asia, is a member of the Balanitaceae family and is also referred to as "desert date" [11]. Fruits have long been used to treat syphilis, fever, gastrointestinal disorders, and parasite infections [12,13]. The fruits have been shown to be efficient against *Fasciola gigantica*, *Schistosoma mansoni* and *Trichinella spiralis*, and are frequently used to remove intestinal parasites [14, 15, 16]. Additionally, fruits have a strong anti-parasitic effect on *H. contortus* in lambs [7, 17].

MATERIAL AND METHODS

Plant materials

The leaves, bark, immature fruits and mature fruits of *B. aegyptiaca*, commonly known as hingota in Rajasthan (India) were collected from grazing areas of ICAR-Central Sheep and Wool Research Institute, Avikanagar, Rajasthan, India located in the semi-arid regions of Rajasthan, India (26°17′ N, 75°28′ E, and 320 m altitude). (Figure 1 and 2). Leaves, bark and immature fruits were collected in the month of June while mature fruits in the month of November and December. The materials were cleaned under running water, allowed to air dry at room temperature, and then ground into a powder using an electric mill.

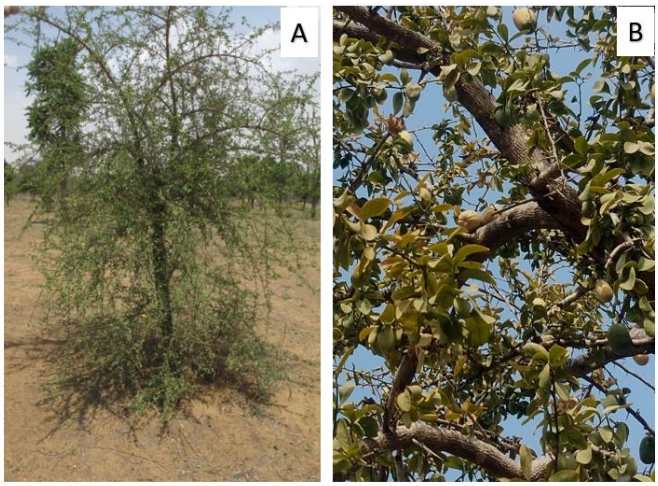


Figure 1: *Balanites aegyptiaca* plant, **A**- Whole plant, **B**- Plant with mature fruit

Figure 2: Parts of *Balanites aegyptiaca* plant, A- Leaves, B- Immature fruit, C-Mature fruit, D- Bark

Extraction Procedures for the Plant Materials

After being ground, the 250g of powdered plant material was suspended in 1000 ml of solvents (acetone, methanol, ethanol, hydro alcohol, and water) in an orbital shaker for the duration of the night. The hydro alcohol was made up of 20% distilled water and 80% methanol. On the subsequent day, Whatman filter paper No. 1 was used to filter the extracts. After first being evaporated in an evaporator, the solvents were heated to 45–50 °C in a hot air oven. Ultimately, dry crude extracts devoid of solvent were produced and kept at -40°C until they were needed for in vitro bioassays.

Detection of the extract's bioactive ingredients

The Folin-Ciocalteu method was used to measure the concentrations of condensed tannins, total phenols, flavonoids, and total saponins [18], aluminium trichloride method using quercetin as standard [19], and as per [20], respectively. GC-MS analyses of methanol and ethanol extracts were carried out on a Shimadzu GC-MS-QP2010 Ultra system [21]. The constituent compounds of the chromatograms were identified by analysing their retention index (RI), retention time (RT), mass spectra comparison with those listed in the NISTMS (National Institute of Standards and Technology), mass spectral library of the GC-MS data system, and co-injection with genuine compounds. Using 2, 2-azino bis (3-ethylbenzothiazoline-6-sulfonate) (ABTS) radical scavenging techniques, antioxidant activity has been examined [22].

Harvesting Haemonchus contortus eggs

After being collected from the abomasi of sheep that were necropsied at ICAR-CSWRI, adult female *H. contortus* worms were crushed to release their eggs. For six to seven days, the eggs were in vitro cultured on petri plates at a temperature of 28±2°C. Infectious larvae were collected on the seventh day. Three 7-8 months old donor lambs who were naive to parasites were experimentally infected with larvae (L3) at a rate of 350 L3/kg of body weight. The donor lambs' eggs were collected, counted, and adjusted to 20–40 eggs per 100 µl of solution.

In vitro antibacterial evaluation of extracts

For the antibacterial activity against bacterium *Escherichia coli* different concentrations of the aqueous extract of leaves and fruits were prepared and tested by the disc diffusion method [23]. Using the spread plate approach, the test microorganisms were seeded into the appropriate medium using 24-hour cultures of bacteria cultivated in nutrient broth. Following solidification, test organism-seeded plates were covered with filter paper discs (10 mm in diameter) coated with the extract. The antibacterial assay plates were incubated at 37°C for 24 hours, and the widths of the inhibition zones were determined in millimetres. Ciprofloxacin (5 µg/ml) discs were utilised as a positive control.

In vitro anthelmintic evaluation of extracts

The egg hatch assay (EHA) and larval mortality assay (LMA) were used to evaluate the extracts' in vitro effectiveness. Using a saturated salt solution, fresh eggs were isolated from newly collected faeces (via rectum) from donor lambs infected predominantly with *H. contortus* for *in vitro* tests. After counting, the quantity of eggs was adjusted to 20–40 eggs per 100 µl of solution. To make all of the dried ethanol and methanol extracts more soluble in water, 0.5 millilitres of DMSO was used as the minimal solution. After then, various concentrations were obtained by serially diluting the solutions. Each well of a 48-well multi-well plate held 0.5 ml of egg suspension, which was then combined with 0.5 ml of crude extracts at various concentrations (5.0, 5.0, 2.5, 1.25, 0.625, 0.312, 0.156, 0.078, and 0.039 mg/ml). In a 48-well multiwell plate, 0.5 ml of egg suspension was added to each well for the LMA experiment. Following 24 to 48 hours, wells were combined with the same volume of crude extracts in six duplicates at varying concentrations (10.0, 5.0, 2.5, 1.25, 0.625, 0.312, 0.156, 0.078, and 0.039 mg/ml). Thiabendazole and levamisole were utilized as a reference standard for EHA and LMA, respectively at a dose of 10 mg/ml, with distilled water serving as the control. Subsequently, the plates were incubated for 48 hours at 28± 2°C in a

BOD incubator. To prevent additional hatching, one drop of Lugol's iodine was applied to each well of the EHA plate. Larvae and unhatched eggs were counted.

***In vivo* anthelmintic evaluation of extract**

For evaluating *in vivo* activity of the plant crude ethanol extract of immature fruit of was used. 21 male lambs (7-8 months of age, 17-25 kg body weight) were challenged with predominantly *H. contortus* larvae. These lambs were assigned to different treatment groups as Gr-1 (treated on 21st day post challenge with single dose of crude ethanol extract @ 0.5 g per kg body weight once), Gr-2 (treated on 21st day post challenge with single dose of levamisole @ 15.0 mg/kg body weight) and Gr-3 (infected and untreated control). To determine the faecal egg count reductions of *H. contortus*, faecal samples of each animal in the respective treatment groups were collected directly from the rectum in the morning daily starting from 0 day of treatment to 11 days and thereafter at every five days interval up to 40 days post treatment. Blood samples for packed cell volume (PCV) and haemoglobin (Hb) estimation, body weights and pooled faecal samples for determining larval count were collected at weekly interval.

Ethical Approval

The ICAR-Central Sheep and Wool Research Institute, Avikanagar's Ethics Committee offered its approval (IAEC/353//E10).

Statistical analysis

A one-way ANOVA was used to compare the mean inhibition percentage of egg embryonation, mean inhibition percentage of egg hatching, and mean percentage of larval mortality at various doses with the control. DMRT was used to compare the means for statistical significance [24]. Probit analysis was used to determine the 50% inhibitory concentration (IC_{50}) and lethal concentration (LC_{50}) for each experiment [25].

RESULTS AND DISCUSSION

The primary goal of this study was to compare the in vitro anthelmintic potency of crude extracts from different parts of native *B. aegyptiaca* plant with common medications Nilverm and Thiabendazole. Also, it aimed to detect the phytochemicals present in different parts, to study the antibacterial potential and lastly to *in vivo* test the most effective extract in *in vitro* tests.

Yield of different extracts of *Balanites aegyptiaca*

The recovered percentage yield of all extracts was maximum from mature fruits and minimum from bark.

Phytochemical analysis

Preliminary phytochemical analysis is shown in Table 1. The leaves, bark and mature fruits had highest concentration of phenol which resulted into higher antioxidant activity in these parts as compared to immature fruits. Saponin was more in mature fruits, bark and leaves as compared to immature fruit. Flavonoids were more in bark and immature fruit. These results are comparable to those of other workers who have used this plant [26, 27, 28]. With regards to antioxidant ability leaves, bark and mature fruits showed an average antioxidant activity. [29] also found average antioxidant activity of mesocarp of the fruit of *B. aegyptiaca*. The findings of phytochemical studies demonstrate the presence of flavonoids, tannins and polyphenol compounds along with other chemical constituents. [30] presented the similar studies on different medicinal plants exhibiting the presence of similar kind of phytoconstituents. [31] found higher quantity of flavonoid and polyphenol in *Cyclocarya paliurus* leaves and expressed its maximum antioxidant capacity. It suggested that the presence of large quantity of flavonoid and polyphenol might be interconnected with greater antioxidant property of the plant [31]. The findings of antioxidant activity of *Cassia tora, Portulaca oleracea, Alternanthera sessilis, Ipomoea aquatica, Basella alba, Digera muricata, Leucas cephalotes* and *Solanum nigrum* indicates that with higher phenolic and flavonoid contents could be a significant source of natural antioxidants [32].

Results of Gas chromatography (GC) coupled to a mass spectrophotometer detector (GC-MS) are shown in Table 2-4, Figure 3-9). Six and 7 compounds were detected in methanol and ethanol extract of leaves, respectively. In methanol extract the major component was Diethyl Phthalate followed by (Z)-Tetradec-11-en-1-yl 2,2,2-trifluoroacetate, Butanoic acid, 4-(2-hydroxycyclohexyl)-, methyl ester, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (DMPP), 1,2,3,4,7,7a-Hexahydro-2,4,7-trimethyl-6H-2-pyrindin-6-one (Tecomine) and Hexadecanoic acid, methyl ester.However, in ethanol extractButanoic acid, 4-(2-hydroxycyclohexyl)-, methyl ester was major component followed by Diethyl Phthalate, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (DMPP), Hexadecanoic acid, methyl ester, 1-3 Prpanedeol,2-dodecyl and Undecane, 4,7-dimethyl.

The methanol extract of bark contained only 6 compounds with major components of 1,4- Dimethyl-7-(prop-1-en-2-yl) decahydroazulen-4-ol or progostole, 3-O-Methyl-d-glucose and Cryptomeridiol. The ethanol extract had only 5 compounds with major components of 1,4-Dimethyl-7-(prop-1-en-2-yl)decahydroazulen-4-ol or progostole, Propane,1-1- diethoxy-2 methyl-,Cryptomeridiol and (4aS,7R)-7-(2-Hydroxypropan-2-yl)-1,4a-dimethyl-4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-one.

The methanol extract of immature fruit was characterized by the presence of 22 compounds. The major components identified were-Geraniol, 1'-Hydroxy-4,3'-dimethyl- icyclohexyl-3,3'-dien-2-one, 6-Ethoxy-6-methyl-2-cyclohexenone, Diethyl phthalate, Succinic acid, 4-methoxy-2-methylbutyl penta, 1-(+)-Ascorbic acid 2,6-dihexadecanoate and DMPP, a flavonoid. All other components were present in amount less than 4.0 %. In ethanol extract 4-O-Methylmannose was the predominant compound followed by Propane,1-1- diethoxy-2 methyl-. In hydro alcohol extract also4-O-Methylmannose predominated.

The methanol extract of mature fruit was characterized by 32 compounds. The major components were Pentanoic acid, 3-methyl- or Valeric acid, Hexanoic acid or Butyric acid a saturated fatty acid and Geraniol.

Antimicrobial activity

In vitro antimicrobial activity (Plate 1) results revealed high antibacterial activity of hydro alcohol extract of immature fruit against *E. coli* at a concentration of 400 mg/ml with an inhibition zone of 28.2±0.6. The aqueous extract of immature fruit had lower activity at the same concentration (23.8±0.4). Positive control ciprofloxacin (5 µg/ml) caused an inhibition zone of 32.0±0.57. A high antibacterial activity of hydro alcohol extract of immature fruit against *E. coli* may be attributed to the predominance of a polysaccharide, 4-O-Methylmannose. This phyto compound has been shown to possess strong antibacterial activity [33]. Several studies revealed antibacterial activity of *B. aegyptica* against most strains of bacteria [34, 35, 36].

***In vitro* Egg hatch assay**

The standard reference compound Thiabendazole showed 97.08± 0.47 and 89.75z0.50% reduction in egg embryonation and hatching at 10 mg/ml and 0.04 mg/ml concentration, respectively while in control (distilled water) reduction was only 3.08±0.57%.

Leave extracts

A poor embryonicidal effect was observed on *H. contortus* eggs by aqueous, ethanol and hydroalcohol extracts, while moderate

embryonicidal activities (>60%) by both methanol and acetone extracts (@ 2.5 to 10.0 mg/ml, Plate 2). All the extracts of leaves showed excellent inhibition of egg hatching. Among these extracts, aqueous extract showed >99% egg hatch inhibition with minimum concentration of 0.63 mg/ml, followed by hydro alcohol and ethanol (> 98%) with minimum concentration of 0.31 mg/ml. Being the lowest IC_{50} of aqueous extract (0.031±0.001 mg/ml) for inhibition of egg hatching this extract had the remarkable effect on egg hatching.

Bark extracts

A moderate embryonicidal effect (>85%) was observed on *H. contortus* eggs by methanol from 10 to 1.25 mg/ml concentration (Plate 3). However, remaining extracts did not show much embryocidal effect. All the extracts of *Balanites* bark except ethanol showed excellent inhibition of egg hatching (>98%) up to 0.31 mg/ml concentration. Being the lowest IC_{50} of methanol extract for inhibition of egg embryonation (0.214±0.12 mg/ml) and ethanol (0.008±0.001 mg/ml) and hydro alcohol (0.037±0.001) extracts for egg hatching, it can be inferred that respective extracts presented the highest embryonicidal and ovicidal activities.

Immature fruit extracts

An excellent embryonicidal effect (>93%) was observed on *H. contortus* eggs by methanol extract with minimum concentration of 1.25 mg/ml and hydro alcohol (>96%) at minimum concentration of 5.0 mg/ml (Plate 4). The pattern of embryonicidal effect was found uniform across replicates. All extracts of *Balanites* immature fruits showed excellent inhibition of egg hatching. Among these extracts, ethanol extract showed >99% egg hatch inhibition with minimum concentration of 0.63 mg/ml. The hydro alcohol extract showed >97% inhibition with minimum concentration of 1.25 mg/ml. Being the lowest IC_{50} of methanol extract for inhibition of egg embryonation (0.188±0.010 mg/ml) and egg hatching (0.021±0.005 mg/ml) both these extracts had the highest embryocidal and ovicidal potential.

Mature fruit extracts

An excellent embryonicidal effect (>97 to 99%) was observed on *H. contortus* eggs by acetone, hydro alcohol and ethanol only on the highest concentration used i.e. 10.0 mg/ml. All the extracts of *Balanite* mature fruits showed excellent inhibition of egg hatching. Among these extracts, 100% egg hatch inhibition was shown by acetone extract with minimum concentration of 1.25 mg/ml followed by >95% inhibition by hydro alcohol and ethanol extracts at minimum concentration of 2.50 mg/ml. As acetone extract showed the lowest IC_{50} for both inhibition of egg embryonation (0.396±0.033 mg/ml) and egg hatching (0.057±0.001 mg/ml) it had highest embryocidal and ovicidal activities. The eggs in treated wells showed damage of egg shell and dead and morbid larvae inside the embryonated eggs) Plate 5).

In vitro larval mortality assay

The standard reference compound levamisole showed 100 % larval mortality at concentrations from 10 to 0.08 mg/ml while in control (distilled water) reduction varied from 15.05±1.10%.

Leave extracts

All the extracts showed 100% larvicidal activity at concentration of 10.00 to 0.31 mg/ml (Plate 6). Being the lowest IC_{50} of aqueous extract (0.031±0.001 mg/ml) for inhibition of egg hatching and IC_{50} value of ethanol extract (0.025±0.001 mg/ml) for larval mortality, it can be inferred that respective extracts presented the highest ovicidal and larvicidal activities.

Bark extracts

All the extracts showed excellent (>99%) larvicidal activity at minimum concentration of 0.15 mg/ml (Plate 6). The IC_{50} values for larval mortality was lowest in aqueous (0.015±0.005) followed by hydro alcohol (0.024±0.001) these extracts had highest larvicidal activity.

Immature fruit extracts

All the extracts showed 100% larvicidal activity at minimum concentration of 0.15 mg/ml (Plate 6). and IC_{50} value of acetone (0.056±0.001 mg/ml) and hydro alcohol extract (0.055±0.001 mg/ml) for larval mortality, it can be inferred that methanol extract presented the highest embryonicidal and ovicidal activities and hydro alcohol and acetone showed highest larvicidal activity. The larvae were found moribund with vacuolation and damaged internal organs and inflated cuticle (Pate 7).

Mature fruit extracts

All the extracts killed 100% larvae at minimum concentration of 0.08 to 0.15 mg/ml (Plate 6). The lowest IC_{50} value of aqueous (0.020±0.001 mg/ml) and acetone extract (0.035±0.001 mg/ml) for larval mortality showed that both extracts had the highest larvicidal activity.

The *in vitro* tests using free living stages of parasitic nematodesare considered as the best means of screening the anthelmintic activity of new plant compounds [37]. All extracts obtained from the *B. aegyptiaca* in the current study have remarkable egg hatching inhibition as compared to some plants studied previously [38.39,37,40]. The ethyl acetate extract of *Spigelia anthlmia* required concentration of 50 mg/ml to induce 100% egg hatch inhibition [39]. The ethanol extracts from all plant parts studied except mature fruit induced greater than 98% inhibition of egg hatching at as lower as 0.31 mg/ml concentration in a dose dependent manner compared with negative control and drug standard group. This conforms the finding of [2] who found significant anthelmintic activity of crude ethanol extract of *B. aegyptiaca* fruits on adult *H. contortus*. The ethanol extract of mature fruit caused 100% inhibition at higher concentrations (i.e. from 10.0 to 2.5 mg/ml) only. In addition to inhibition of egg hatching, the larvae of those eggs which were able to hatch at lower concentrations such as 0.08 and o.04 mg/ml were found dead indicative of the larvicidal property of the extracts. Our observation from this study confirms the traditional applications of this plant against internal parasites.

Based on the concentration required to produce 50% egg hatch inhibition (IC_{50}), the most potent extracts were the ethanol extract of bark and immature fruit and the least potent extracts were aqueous, methanol and ethanol extracts of mature fruit. Based on larval development inhibition, the aqueous and ethanol extract of leaves, aqueous extract of bark, ethanol, hydro alcohol and acetone extracts of immature fruit, aqueous and ethanol extract of mature fruit of *B. aegyptiaca* was the most potent one inducing 100% larval mortality at the minimum concentration of 0.15 mg/ml. Previous study on other plant, *Azadirachta indica* showed that at 50 mg/ml, Ethyl acetate and ethanol extracts induced only 68.1 and 87.1% larval development inhibition of *H. contortus*, respectively [40].

In vivo evaluation of ethanolic extract of *B. aegyptiaca* immature fruits

The reduction in infective larvae observed in faecal cultures from lambs treated with *B. aegyptiaca* immature fruit ethanol extract was highly significant from day 3 to end of the trial. There was more than 90% reduction in infective larvae.

A non-significant variation in FECs was observed in both extract treated and infected untreated control groups, however on majority of days numerically mean intensity of infection remained low in extract treated group as compared to control group (Table 5). This might be

because of low dose of extract. Reduction in faecal egg count recorded was maximum (>55%) on day 2 and 3 post treatment. [41] found maximum reduction of 69.6% in FEC on day 14 post treatment with *B. aegyptiaca* fruit mesocarp @ 9g/kg BW. Similarly, [42] found excellent faecal egg count reduction (100%) at the 7th day post treatment with crude ethanol extract of fruit when given (@ 3 g/kg BW) for 3 successive days. The difference might be because of low dose of extract (@ 0.5 g/kg BW once) in the present trial. A non-significant variation was observed for weekly haemoglobin, packed cell volume and body weights in all the groups during the period of observations.

The anthelmintic activity might be because of presence of phytochemicals like fatty acids, terpenoids, alkaloids and flavonoids. These bioactive compounds work separately or in combination to alter the membrane permeability of different stages of parasite or binding to a specific glycoprotein of the cuticle of the parasite [43].

Table 1: Phytochemicals present in different parts of *Balanites aegyptiaca*

Part of plant	Phenol (mg/g)	Total tannin(mg/g)	Flavonoids (mg/g)	Saponins (mg/g)	Antioxidant activity (%RSA)
Leaves	2.78	1.370	0.87	34.80	40.97
Bark	1.42	0.57	4.80	45.44	29.23
Immature fruit	0.63	0.39	2.83	17.12	14.70
Mature fruit	1.04	0.18	1.30	47.79	29.35

Table 2: Chemical compounds identified in methanol extract of different parts of *Balanites aegyptiaca*

Peak No	Compound	Molecular formula	% area	Chemical class
A. Leaves				
1	Diethyl Phthalate	$C_{12}H_{14}O_4$	71.44	Phthalate ester
2	(Z)-Tetradec-11-en-1-yl 2,2,2-trifluoroacetate	$C_{16}H_{27}F_3O_2$	8.86	Fatty acid
3	Butanoic acid, 4-(2-hydroxycyclohexyl)-, methyl ester	$C_{11}H_{20}O_3$	8.26	Fatty acid
4	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (DMPP)	$C_6H_8O_4$	5.14	Flavonoid
5	1,2,3,4,7,7a-Hexahydro-2,4,7-trimethyl-6H-2-pyrindin-6-one or Tecomine	$C_{11}H_{17}NO$	3.75	Alkaloid
6	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	2.56	Fatty acid
B. Bark				
1	1,4- Dimethyl-7-(prop-1-en-2-yl) decahydroazulen-4-ol or Pogostole	$C_{15}H_{26}O$	63.29	Sesquiterpenoid
2	3-O-Methyl-d-glucose or -O-Methylhexose	$C_7H_{14}O_6$	12.04	Carbohydrate
3	Cryptomeridiol	$C_{15}H_{28}O_2$	10.06	sesquiterpenoid
C. Immature fruits				
1	Geraniol	$C_{10}H_{18}O$	29.87	Terpenoid
2	1'-Hydroxy-4,3'-dimethyl-bicyclohexyl-3,3'-dien-2-one	$C_{14}H_{20}O_2$	12.69	Terpenoid
3	6-Ethoxy-6-methyl-2-cyclohexenone	$C_9H_{14}O_2$	9.55	Alkane
4	Diethyl phthalate	$C_{12}H_{14}O_4$	7.00	Phthalate ester
5	Succinic acid, 4-methoxy-2-methylbutyl penta	$C_{24}H_{46}O_5$	6.84	Fatty acid
6	1-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	6.64	Vitamin
7	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (DMPP)	$C_6H_8O_4$	4.29	Flavonoid
D. Mature fruits				
1	Pentanoic acid, 3-methyl- or Valeric acid	$C_6H_{12}O_2$	61.06	Fatty acid
2	Hexanoic acid or Butyric acid	$C_4H_8O_2$	10.18	Saturated fatty acid
3	Geraniol	$C_{10}H_{18}O$	6.19	Terpenoid
4	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	$C_6H_8O_4$	4.34	Flavonoid
5	1-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	3.02	Vitamin

Table 3: Chemical compounds identified in ethanol extract of different parts of *Balanites aegyptiaca*

Peak No	Compound	Molecular formula	% area	Chemical class
A. Leaves				
1	Butanoic acid, 4-(2-hydroxycyclohexyl)-, methyl ester	$C_{11}H_{20}O_3$	23.32	Fatty acid
2	Diethyl phthalate	$C_{12}H_{14}O_4$	17.23	Phthalate ester
3	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (DMPP)	$C_6H_8O_4$	16.43	Flavonoid
4	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	14.28	Fatty acid
5	1-3 Prpanedeol,2-dodecyl	$C_{17}H_{34}O_2$	12.71	Fatty acid
6	1,2,3,4,7,7a-Hexahydro-2,4,7-trimethyl-6H-2-pyrindin-6-one or Tecomine	$C_{11}H_{17}NO$	11.19	Alkaloid
7	Undecane, 4,7-dimethyl	$C_{13}H_{28}$	4.84	Alkane
B. Bark				
1	1,4- Dimethyl-7-(prop-1-en-2-yl)decahydroazulen-4-ol or Pogostole	$C_{15}H_{26}O$	59.71	Terpenoid
2	Propane,1-1- diethoxy-2 methyl	$C_8H_{18}O_2$	23.16	Ether
3	Cryptomeridiol	$C_{15}H_{28}O_2$	10.39	Sesquiterpenoid
4	(4aS,7R)-7-(2-Hydroxypropan-2-yl)-1, 4a-dimethyl-4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-one	$C_{15}H_{24}O_2$	6.73	Not identified
C. Immature fruits				
1	4-O-Methylmannose	$C_7H_{14}O_6$	83.14	Polysaccharide
2	Propane,1-1- diethoxy-2 methyl	$C_8H_{18}O_2$	9.52	Ether compound
3	Di-hydro citronellyl angelate	$C_{15}H_{28}O_2$	2.28	Terpenoid
4	Eicosanoic acid	$C_{20}H_{40}O_2$	1.20	Fatty acid

Table 4: Chemical compounds identified in hydroalcohol extract of different parts of *Balanites aegyptiaca*

Peak No	Compound	Molecular formula	% area	Chemical class
A. Immature fruits				
1	4-O-Methylmannose	$C_7H_{14}O_6$	99.80	Polysaccharide
2	1-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	0.20	Vitamin
B. Mature fruits				
1	4-O-Methylmannose	$C_7H_{14}O_6$	93.10	Polysaccharide
2	2-Azabicyclo[3.3.0]octane, 3-(hydroxydiphenylmethyl)-, cis-	$C_4H_8O_2$	2.83	-?
3	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (DMPP)	$C_6H_8O_4$	1.76	Flavonoid

Table 5: Mean (±S.E.) faecal egg counts in different groups of sheep

Days post treatment	Extract treated	Levamisole treated	Control
0	7800.0±3109.4	7800.0 ± 4852.1^{b}	7800.0±3365.4
1	7300.0 ± 2961.1^{B}	333.3 ± 152.0^{aA}	5783.3 ± 2235.2^{AB}
2	5033.3 ± 1960.7^{AB}	100.0 ± 51.6^{aA}	8350.0 ± 3473.8^{B}
3	4833.3 ± 2270.0^{AB}	50.0 ± 34.2^{aA}	6083.3 ± 1846.7^{B}
4	4783.3 ± 1910.0^{AB}	33.3 ± 21.1^{aA}	10566.7 ± 4334.5^{B}
5	5583.3 ± 1922.01^{AB}	116.7 ± 83.3^{aA}	8550.0 ± 3154.7^{B}
6	8266.7 ± 1907.1^{B}	33.3 ± 21.1^{aA}	10383.3 ± 3268.9^{B}
7	4233.3 ± 1034.3^{AB}	33.3 ± 21.1^{aA}	7900.0 ± 2376.7^{B}
8	7600.0±3252.7	116.7 ± 47.7^{a}	9583.3±4211.6
9	5550.0 ± 2273.3^{AB}	33.3 ± 21.1^{aA}	8616.7 ± 2699.6^{B}
10	4000.0 ± 1097.9^{AB}	66.7 ± 21.1^{aA}	9400.0 ± 3048.6^{B}
11	3833.3 ± 896.4^{AB}	150.0 ± 56.3^{aA}	10700.0 ± 3373.0^{B}

14	3150.0±994.2^{AB}	116.7±60.1^{aA}	10116.7±2777.6^{B}
19	3250.0±1005.6^{AB}	100.0±36.5^{Aa}	10550.0±3556.8^{B}
24	4016.7±1398.0^{AB}	100.0±25.8^{aA}	11116.7±2998.0^{B}
29	4650.0±1573.1^{AB}	133.3±76.0^{aA}	12000.0±3676.1^{B}
34	3850.0±1973.4^{AB}	116.7±40.1^{aA}	12066.7±3222.5^{B}
39	3833.3±2056.6^{AB}	216.7±122.2^{aA}	12116.7±3730.0^{B}

Lower case- Comparison within group; Upper case- Comparison between groups

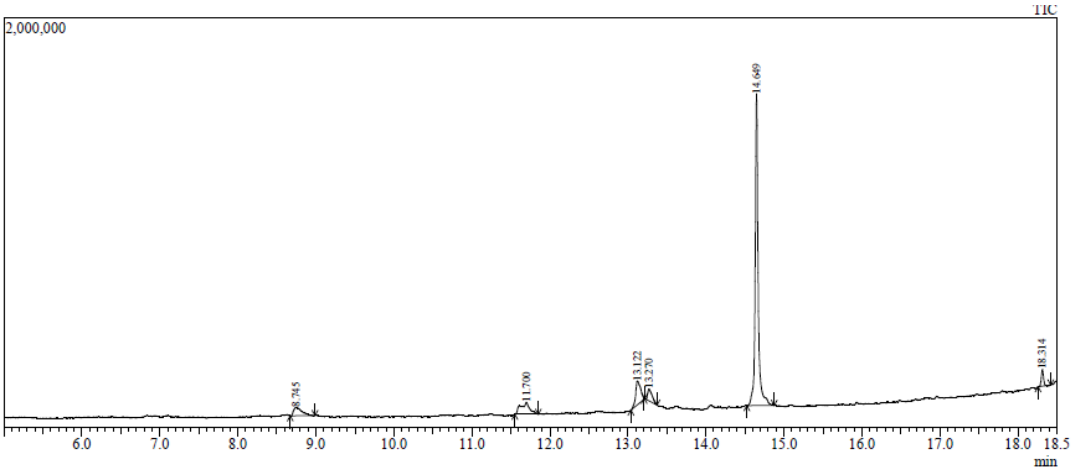


Figure 3: Chromatograph of methanol extract of *B. aegyptiaca* leaves on GC-MS

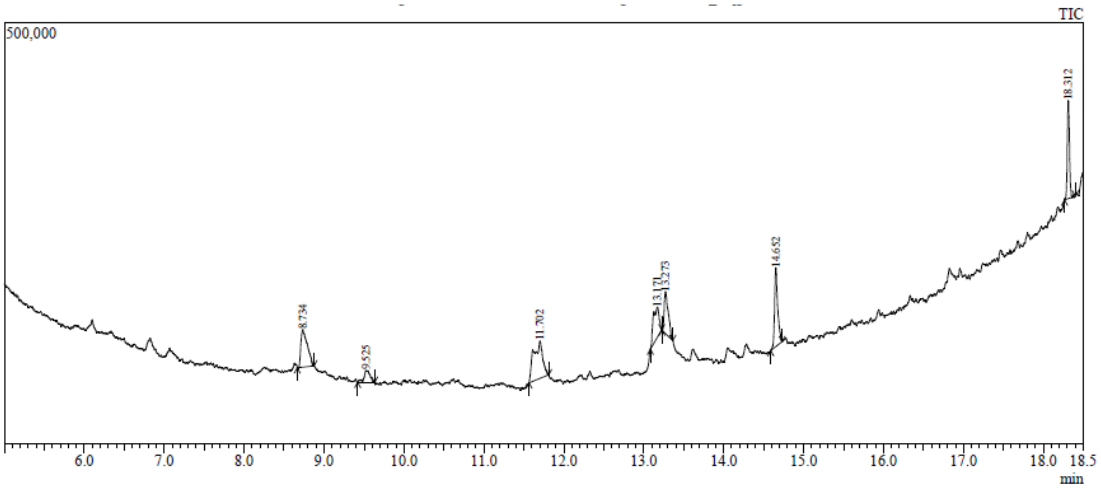


Figure 4: Chromatograph of ethanol extract of *B. aegyptiaca* leaves on GC-MS

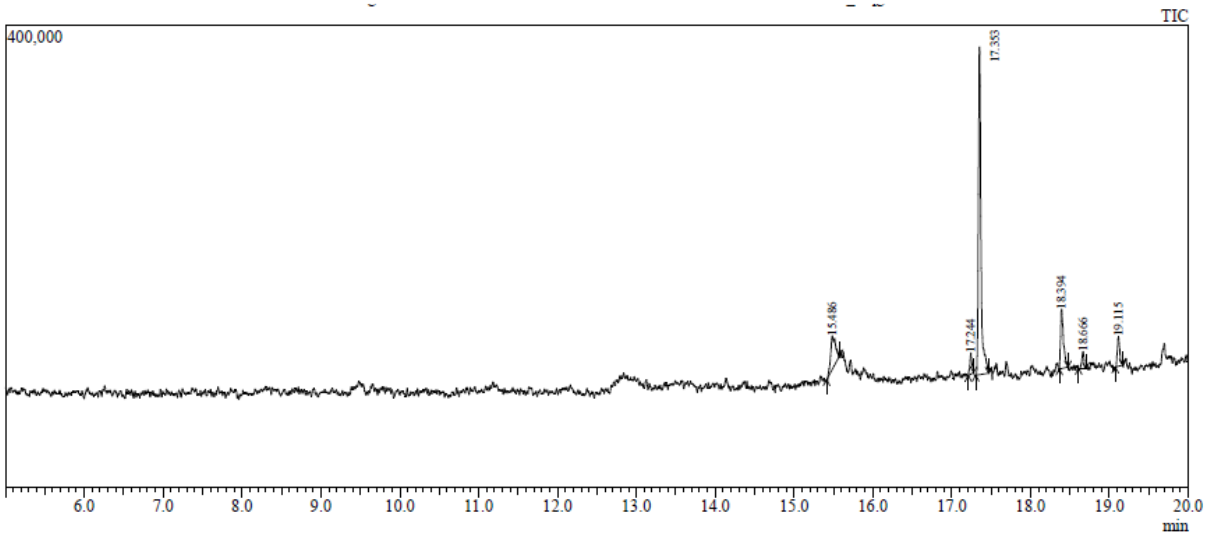


Figure 5: Chromatograph of methanol extract of *B. aegyptiaca* bark on GC-MS

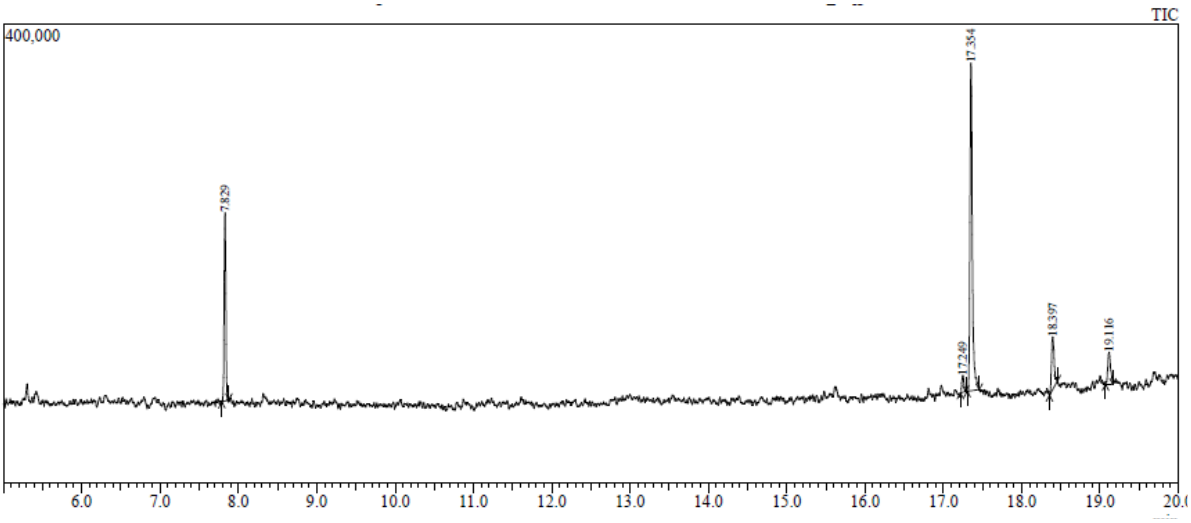


Figure 6: Chromatograph of ethanol extract of *B. aegyptiaca* bark on GC-MS

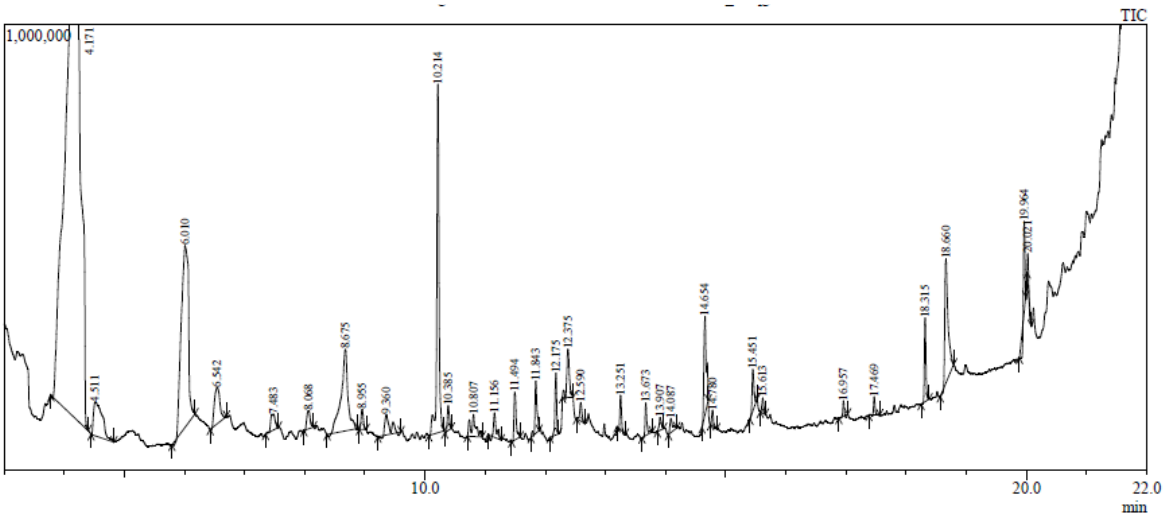


Figure 7: Chromatograph of methanol extract of *B. aegyptiaca* immature fruits on GC-MS

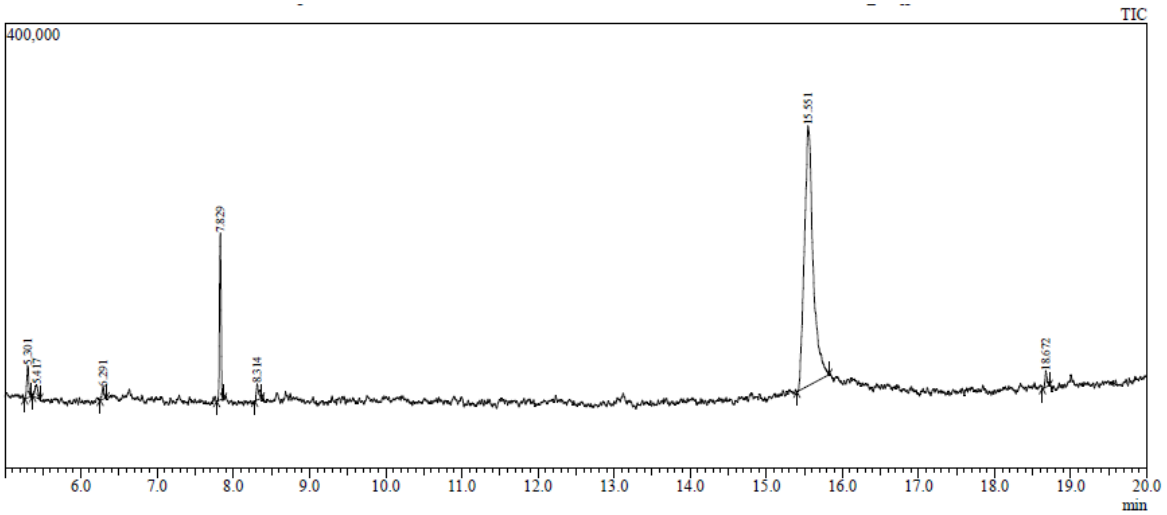


Figure 8: Chromatograph of ethanol extract of *B. aegyptiaca* immature fruits on GC-MS

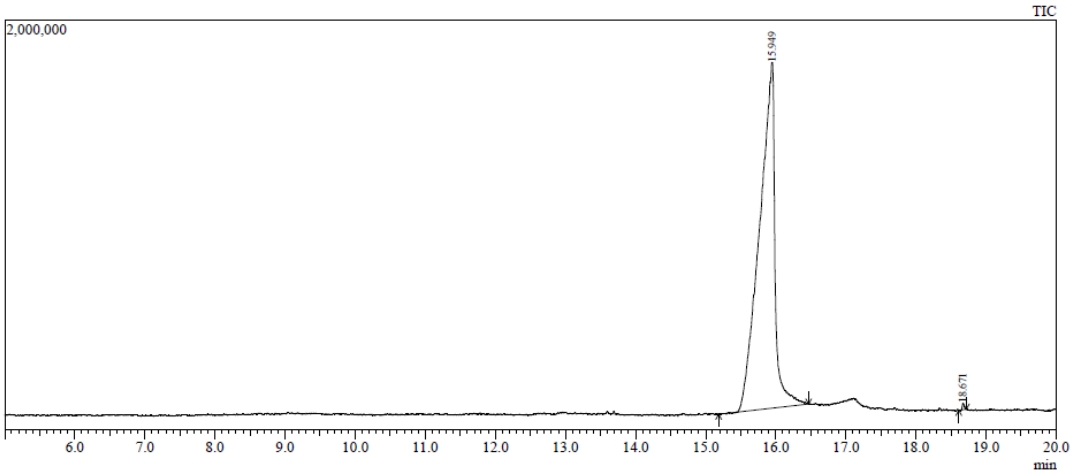
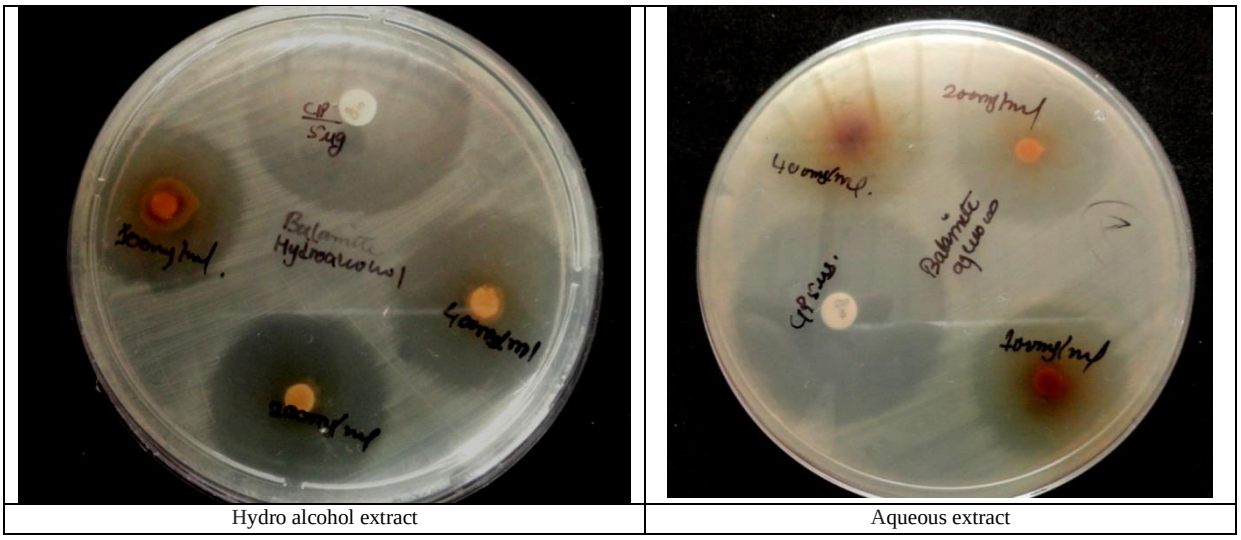
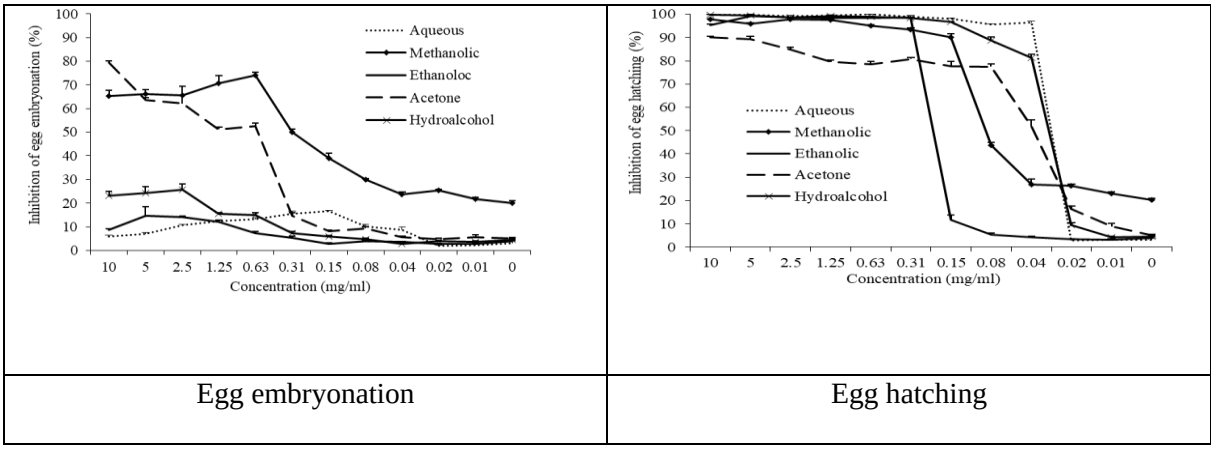


Figure 9: Chromatograph of hydro alcohol extract of *B. aegyptiaca* immature fruits on GC-MS



Hydro alcohol extract	Aqueous extract

Plate 1: Bacteriostatic zone of extracts of *B. aegyptiaca* against *E. coli* at different concentrations



Egg embryonation	Egg hatching

Plate 2: Effect of extracts of *Balanites aegyptiaca* leaves

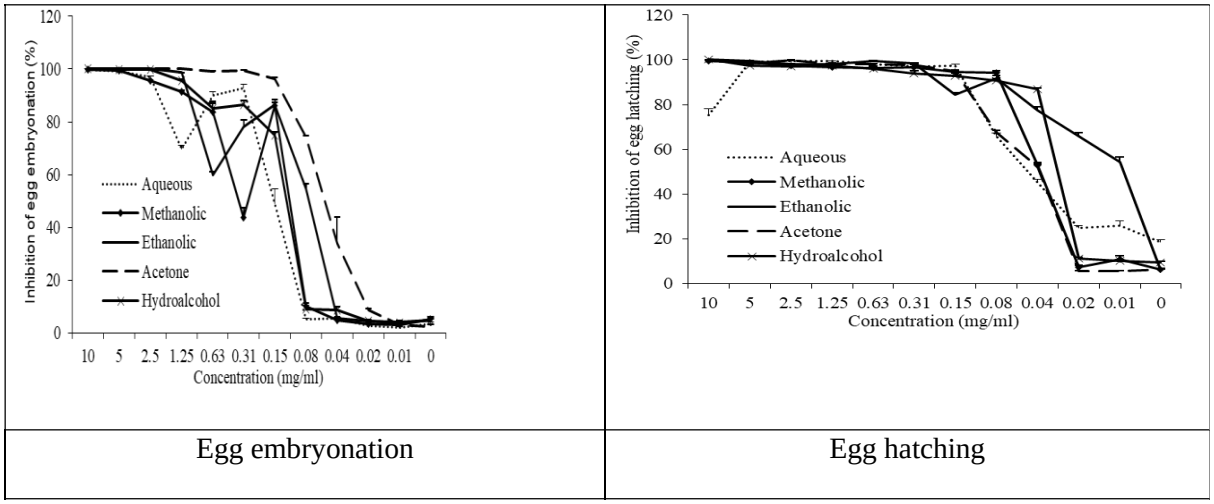


Plate 3: Effect of extracts of *Balanites aegyptiaca* bark

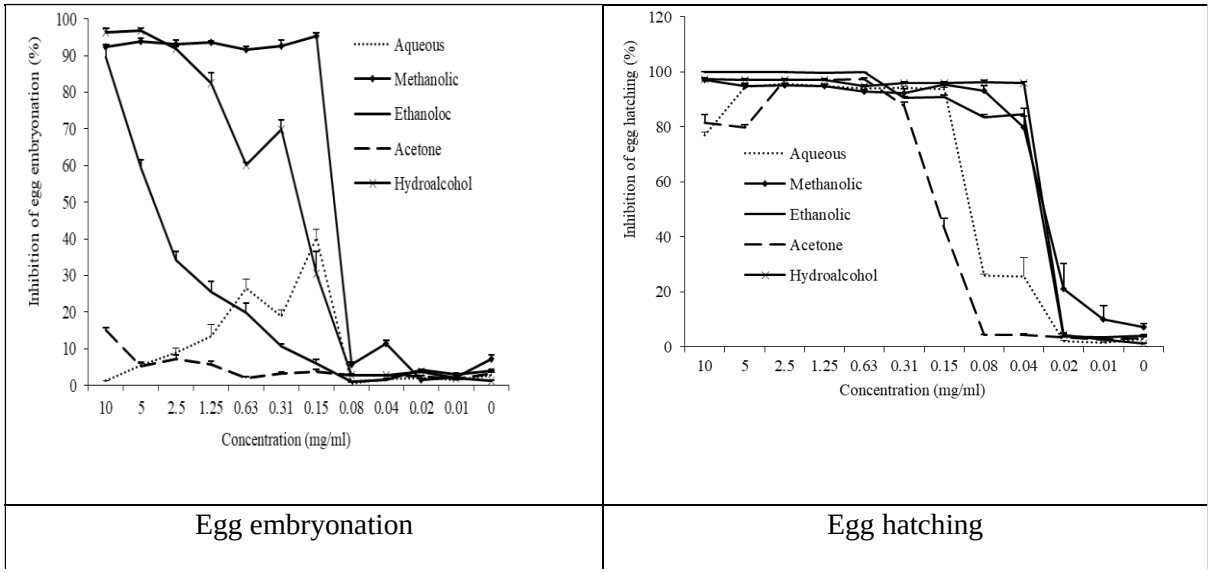


Plate 4: Effect of extracts of *Balanites aegyptiaca* immature fruits

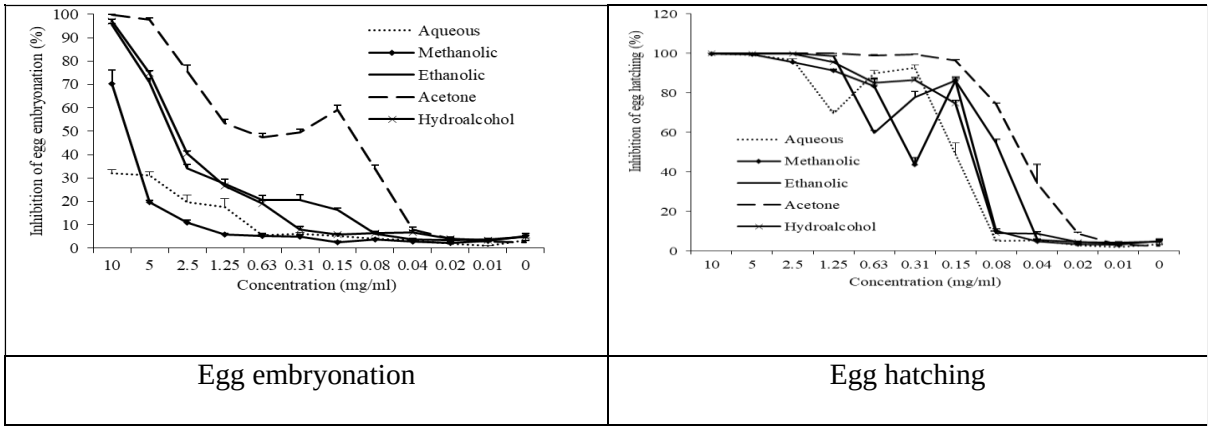


Plate 5: Effect of extracts of *Balanites aegyptiaca* mature fruits

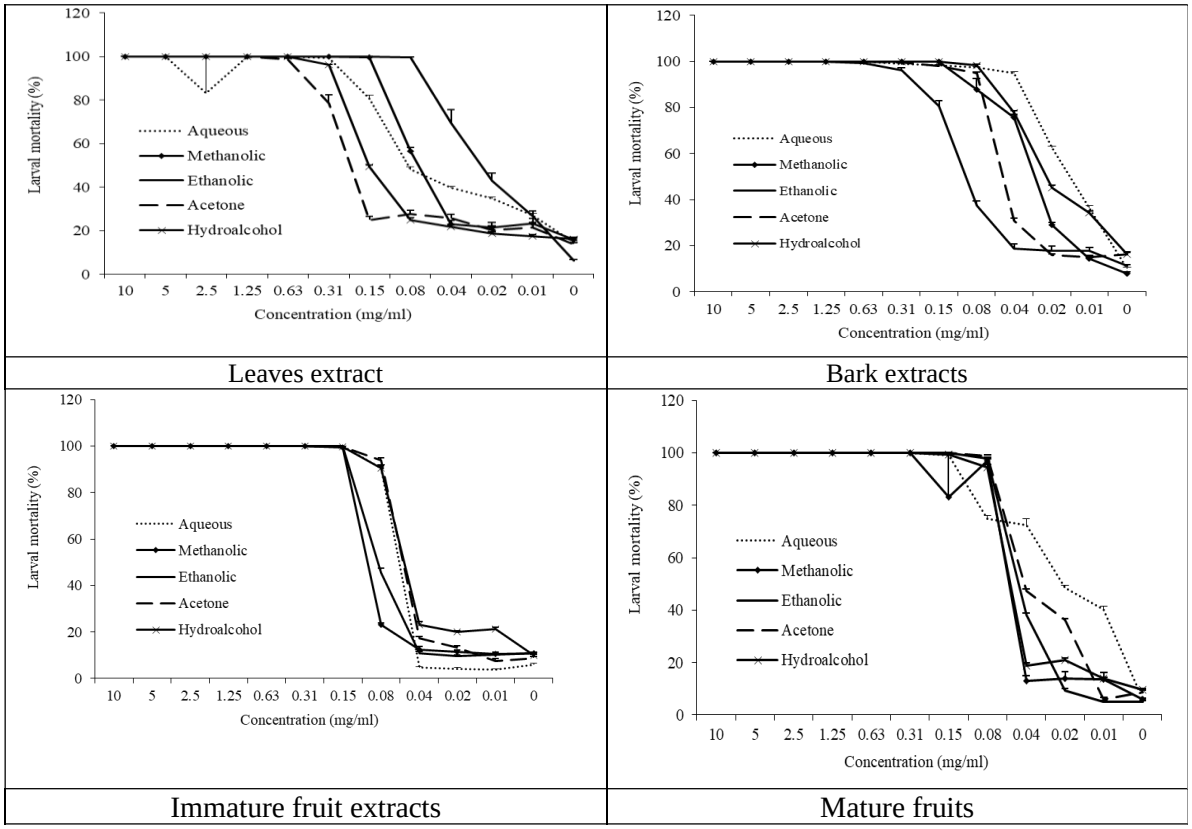


Plate 6: Effect of extracts of *Balanites aegyptiaca* immature fruit on larval mortality

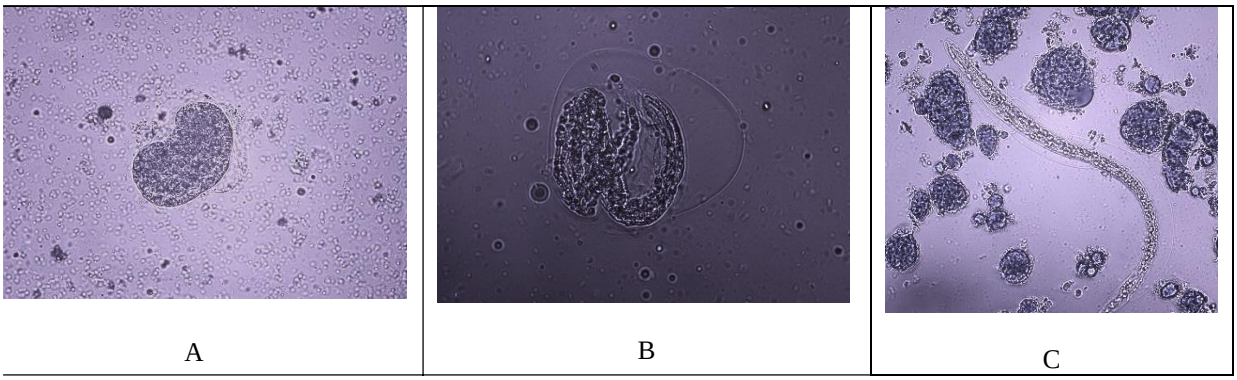


Plate 7: Developmental stages of *Haemonchus contortus* treated with crude ethanol extract of immature fruit of *Balanites aegyptiaca*, A: unembryonated egg with damaged egg shell in EHA (X40), B: Embryonated eggs with moribund and vacuolated embryos in EHA (X40), C: Larva (L2) with damaged internal organs and inflated cuticle in LDA (X40).

CONCLUSION

Studying plants, one of the most abundant sources of potentially useful, diverse chemical compounds, has become increasingly popular over the past few years. Finding new resources to tackle the problems presented by emerging and modern diseases may be easier with plants' aid. The presence of all these phytochemicals and bioactive compounds in *B. aegyptiaca* may make it an entirely novel source of pharmaceuticals, the study's results indicate. However, it requires more research to elucidate the exact chemical compound responsible for its anti-*H. contortus* activity, so as to isolate and extract it to potentiate its action. Isolation of individual phytochemical constituents may proceed to find a novel drug.

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Conflict of interest

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