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Antioxidant, antimicrobial and antifeedant activity of phenolic compounds accumulated in *Hyoscyamus muticus* L.

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***Hyoscyamus muticus* is an endangered desert plant spread in the Arabian Peninsula and the Middle East deserts. The methanol extract of the aerial parts of *H. muticus* grown in the arid zones in the Northern regions of Saudi Arabia were subjected to primary phytochemical analysis which revealed the presence of phenolic compounds, flavonoids, tannins and sterols, the gas chromatography-mass spectrometry (GC-MS) analysis of the methanolic extract exhibited different types of phenolic compounds, including ferulic acid, 4-hydroxy-cinamic acid-ester, methyl salicylate and methyl ferulate. The accumulation of phenolic compounds supports the antioxidative properties of the plant against oxidative stress. The antioxidant testing showed that the methanolic extract of *H. muticus* has a noticeable antioxidant activity with an IC_{50} of 8.1±0.65 mg/ml and an EC_{50} of 12.74±1.12 mg/ml. The antimicrobial investigation on 11 microbial strains revealed that the methanolic extract of *H. muticus* areal parts showed average or weak antibacterial activity against gram-positive bacteria, weak antibacterial activity against gram-negative bacteria and no antifungal activity. Moreover, the investigations exhibited the presence of an antifeedant potential on the methanol extract of *H. muticus* on the 4th instar larvae of *Spodoptera littoralis*.**

Key words: *Hyoscyamus muticus*, phytochemical analysis, antioxidant capacity, antimicrobial activity, antifeedant assay *Spodoptera littoralis.*

INTRODUCTION

All living creatures in the animal kingdom are heterotrophic and life depends directly or indirectly on plants. Through the historical development of the human race, man has always depended on plants as a source of food,

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shelter, clothing medicine, cosmetics, ceremonies and even magic, until the industrial renaissance came and introduced the manufactured and synthesized products in the life of modern man with their enormous negative impacts on the environment and health. However, In recent years, the interest in medicinal plants is growing, the demand for medicinal plant products is increasing in the developing countries as well as in the developed countries, this is because they are inexpensive, have better acceptability and compatibility and have minimal negative effects (Pal and Shukla, 2003). Herbal drugs have many features which make them preferable to modern synthetic drugs, such as the ability of the plant compounds to interact together in harmony which decreases the possible negative impact; plant compounds can support official synthetic drugs in some difficult disease treatments like cancer; and frequent consumption of some medicinal plant products could prevent the appearance of some diseases and enhance the immune system (Rasool, 2012). Accordingly, it is worthy to explore the biological activities of plants. On the other side, some medicinal plants have some toxic symptoms and others might have antifeedant effects on insects. As an example, the cotton leafworm, *Spodoptera littoralis* (Lepidoptera: Noctuidae), is one of the most destructive pests in many countries in the Middle East. This insect causes critical injuries to a wide range of vegetables and crops including cotton, alfalfa, peanut, potato, pepper and tomato (Maged El - Din and El - Gengaihi, 2000; Kandil et al., 2003; Adham et al., 2009). Chemical synthetic pesticides have been used to control this pest and to reduce crop losses. This control strategy has potentially negative consequences on the environment and harmful effects on beneficial insects and natural enemies (Pavela et al., 2008). In this context, screening of botanical extracts against the target pest has been conducted by researchers in recent decades (Kebede et al., 2010; Kamaraj et al., 2010). Several studies have shown larvicidal, antifeeding and repellent activity of botanical extracts (Larocque et al., 1999; Gbolade, 2001).

Hyoscyamus muticus L., is a desert plant which grows in arid areas of Egypt, known in Egypt as Egyptian henbane and belongs to the family Solanaceae, a family rich in phytochemicals of pharmaceutical properties such as tropane alkaloids and hyoscyamine that has a direct effect on the central nervous system (Elmaksood et al., 2016). Phenolic acids have two main structures, hydroxycinnamic and hydroxybenzoic acids. The derivatives of the hydroxycinnamic acid include ferulic, caffeic, p-coumaric and sinapic acids, while the derivatives of hydroxybenzoic acid consist of gallic, vanillic, syringic and protocatechuic acids. Another major class of phenolic compounds is the cell wall phenolics, which is insoluble and found in complexes with other cell wall components. The two main groups of cell wall phenolics are lignins and hydroxycinnamic acids (Callemien et al., 2008). Phenolic compounds play a critical role in the cell wall during plant growth by protecting the plant against stresses such as infection, wounding and UV radiation (Santos et al., 2004). Moreover, the presence of phenolic compounds is the reason behind the formation of the blue fluorescence (Lichtenthaler and Schweiger, 1998). The use of *H. muticus* in medicine dates back to ancient Egypt; the plant has a hallucinogenic and poisonous properties, although it is used in medicine to relieve the symptoms of Parkinson's disease, to treat some gastric disorders, to induce smooth muscle relaxation and also for treatment of motion sickness (Sevon et al., 2001). The current study aimed to evaluate some of the biological activities of the areal parts of *H. muticus* such as phytochemical, antioxidant, antimicrobial and antifeedant activity.

MATERIALS AND METHODS

Collection of plant materials

The aerial parts of *H. muticus* grown in the arid zone at Wadi Arar, Arar region, Saudi Arabia, was collected during the summer season in 2016. Collected plants have been kindly verified and authenticated in the Desert Research Center; voucher specimens were deposited in the Herbarium of Desert Research lab, dried in shade and ground to fine powder.

Plant extraction

The dried powder of the areal parts of *H. muticus* (140 g) was extracted with 80% methanol (400 ml MeOH/100 H_2O) using Soxhlet extractor at 90°C for 16 h. The polar extract was evaporated at low pressure to obtain crude methanol extract. Then, the semi-solid crude extract was kept for further analysis.

Phytochemical screening

The previously prepared methanol extract was subjected to a qualitative chemical test to detect different classes of bioactive chemical constituents present in the plant using standard methods, as previously mentioned in some reports (Yusuf et al., 2014; Mujeeb et al., 2014).

GC-MS analysis

GC-MS analysis of crude methanol extract of *H. muticus* was performed on a Perkin Elmer Clarus® 600 GC System, fitted with a Rtx-5MS capillary column (30 m × 0.25 mm inner diameter × 0.25 µm film thickness; maximum temperature 350°C), coupled to a Perkin Elmer Clarus® 600C MS. Ultra-high purity helium (99.99%) was used as carrier gas at a constant flow rate of 1.0 ml/min. The injection, transfer line and ion source temperatures were all 290°C. The ionizing energy was 70 eV. Electron multiplier voltage was obtained from autotune. The oven temperature was programmed at 60°C (held for 2 min) to 280°C at a rate of 3°C/min. The crude samples were diluted with appropriate solvent (1/100, v/v) and filtered. Then, the particle-free diluted crude extracts (1 µl) were taken in a syringe and injected into injector with a split ratio 30:1. All the resulted data were obtained by collecting the full-scan mass spectra within the scan range 40 to 550 amu. The percentage

composition of the crude extract constituents was expressed as a percentage by peak area. Finally, the identification and characterization of the chemical compounds in the crude extract of *H. muticus* was based on GC retention time. The mass spectra were computer matched with those of standards available in mass spectrum libraries (Mooza et al., 2014; Admas, 1995).

Antioxidant testing

The antioxidant activity was evaluated using the ferric reducing power method (FRAP) as described by Abdallah et al. (2016), and 2,2-diphenyl-1- picrylhydrazyl (DPPH) radical scavenging method as described by El-Sharkawy et al. (2017). For the FRAP method, 1 ml of each sample concentration was mixed with 2.5 ml of potassium hexacyanoferrate $K_3Fe(CN)_6$ solution and 2.5 ml of phosphate buffer (0.2 mol/L, pH 7.0) and incubated at 50°C for 30 min. After, 2.5 ml of trichloroacetic acid (10%) was added to the mixture. Then, 2.5 ml of this solution was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%). The absorbance was measured at 700 nm and the concentration of the samples at which the absorbance of 0.5 (EC_{50}) was determined. Ascorbic acid and Quercetin were used as positive controls for comparison. For the DPPH scavenging method, 0.5 ml of each sample concentration was mixed with 0.5 ml of DPPH methanolic solution (0.04 g/l). The mixture was shaken vigorously and allowed standing for 30 min in darkness at 25°C. The absorbance of the resulting solution was measured at 517 nm with a spectrophotometer, and the percentage inhibition of activity was calculated as:

$$\% \text{ Inhibition} = \frac{(\mathbf{A}\text{blank} - \mathbf{A}\text{sample})}{\mathbf{A}\text{blank}} \times 100$$

The concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage plotted against the extract concentration. Ascorbic acid and quercetin were used as positive controls for this study.

Antimicrobial investigation

The antimicrobial activity of the methanol extract of *H. muticus* was evaluated against different gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 49461, *Bacillus cereus* ATCC 10876 and *Staphylococcus aureus* clinical isolate), gram-negative bacteria (*Escherichia coli* ATCC 35218, *Klebsiella pneumoniae* ATCC 700603, *Klebsiella pneumoniae* ATCC 27736 and clinical isolates of *Pseudomonas aeruginosa* and *Acinitobacter baumannii*) and fungi (*Aspergillus niger* ATCC 6275, *Candida albicans* ATCC 10231). Different strains from the same bacterial species were used to evaluate any potential variation in antibacterial susceptibility among them. The method used in this study was Kirby-Bauer disc diffusion test (NCCLS, 2002), with minor modification to fit with the plant extract. The crude methanol extract of *H. muticus* was reconstituted in 10% Di-methyl-sulphoxide (DMSO) to make 500 mg/ml. Microbial strains were sub-cultured in nutrient broth (for bacteria) or sabouraud dextrose broth (For fungi), samples from the broth cultures were pipette and diluted with sterile normal saline to make a suspension equivalent to the turbidity of the 0.5 McFarland standard. A sterile cotton swab was dipped in the adjusted suspension and smeared over previously prepared plates containing 20 ml Mueller Hinton agar for bacteria or sabouraud dextrose agar for fungi. Sterile paper discs, 6 mm in diameter were cut from No.1 Whatman filter paper and were immersed in the reconstituted extract (Absorb about 15 µl) and loaded over the seeded plates. Another two paper discs, one saturated with 10% DMSO while the other was erythromycin disc (15 µg) for bacteria or a disc saturated with clotrimazole (10 mg/ml) for fungi, were also used as a negative or positive control, respectively. Plates were incubated at 35 to 37°C for up to 24 h for bacteria or at 28 to 30°C for up to two days for fungi. Then, the diameter of inhibition zones (in mm) was measured and recorded.

Antifeedant activity and starvation percentage

A laboratory strain of *Spodoptera littoralis* was reared in the laboratory for more than 10 generations. Larvae were fed on fresh castor leaves, *Ricinus communius,* until pupation. Moths were fed on 10% sugar solution. Each jar was provided with branches of tafla, *Nerium oleander*, as an oviposition site. Insects were kept under controlled conditions at 26 ± 2°C and 65 ± 5 %R.H., with 8:16 L:D h photoperiod. The experiments were carried out on the 4th instar larvae. Series of ascending crude concentrations were prepared (5, 10, 20 and 40%) by dilution in 70% ethanol. Control discs were sprayed with the carrier solvent alone. 300 larvae were starved overnight, and then divided into 6 groups of 50 larvae each, four different concentrations (5, 10, 20 and 40%) of plant extract (*H. muticus)*, one group for the control and one group as starved larvae. Equal discs of fresh castor bean leaves were rinsed in each treatment and in the control, then treated and untreated leaves were shade-dried. All larvae of control and treated leaves were weighted before and after treatment for 3 days. The dried leaves were placed individually in plastic Petri-dishes. Ten larvae were transferred into each cup and allowed to feed on the treated and untreated leaves, the starved larvae were left without feeding for 24 h. Five replicates for each treatment were carried out. According to the equation of Mostafa (1969) and Abdel-Mageed et al. (1975), the starvation percentages of tested larvae were calculated as follows:

Starvation (%) = C – E/C – S × 100

Where:

C = Mean weight gain of untreated larvae after 24 h;
E = Mean weight gain of treated larvae for each concentration after 24 h; and
S = Mean weight gain of starved untreated larvae after 24 h.

The antifeedant index (AFI) was calculated according to Sadek (2003).

AFI (%) = [(C-T) / (C + T)] × 100

Where:

C: the amount of food consumed (leaves) in the control; and
T: the amount of food consumed (leaves) in the treatment.

Statistical analysis

The measurements were carried out in triplicate or in duplicate. The data obtained were presented as means ± standard error (S.E.) and the significant difference between groups was statistically analyzed using Student T-test or one-way ANOVA, as appropriate. A probability level of $P < 0.05$ was used in testing the statistical significance. The program used was SPSS-Statistical Package, version 11.

RESULTS AND DISCUSSION

The powder of dried aerial parts of *H. muticus* was

Table 1. The phytochemical analysis of 80% methanol extract of *Hyoscyamus muticus*.

Phytochemicals	Methanol extract (80% v/v)
Alkaloid	+++
Flavonoid	++
Tannin	++
Terpenoid	-
Sterol	+
Phenolic compounds	+++

+++ = Present in high amount, ++ = moderately present, + = Trace amounts, - = Absent.

extracted with 80% methanol in order to collect various non-polar and polar compounds, the obtained crude which was a brown sticky extract, was used for preliminary phytochemical investigation. Phytochemical testing revealed the presence of alkaloids, flavonoids, tannins, sterols and phenolic compounds. The major related compounds are the phenolic and alkaloid compounds, these results are shown in Table 1. The phenolic compounds of the aerial parts of *H. muticus* were analyzed by GC-MS, and the assay revealed the presence of ferulic acid, 4'-Hydroxy-3'-methylacetophenone, methyl isoferulat, methyl salicylate p-Cresol, 2,2'-methylenebis[6-tert-butyl, most of phenolic compounds found as ester–bound form, while only ferulic acid was found free as revealed in Table 2. The GC-MS assay is currently used to identify different classes of organic compounds especially phenolic compounds; these compounds were confirmed by reference sample on thin layer chromatography (TLC).

According to results represented in Tables 1 and 2, the aerial parts of *H. muticus* are rich in phenolic compounds, flavonoids, tannins and sterols, the GC-MS analysis of the methanolic extract exhibited different types of phenolic compounds, namely ferulic acid, 4-hydroxy–cinamic acid–ester, methyl silsilat and methyl ferulat. Many previous studies revealed the accumulation of phenolic compounds under environmental stress in some desert plants which grow in arid conditions under high temperature and water deficiency and exposed to different other environmental stress which affect the plant, and this may lead to the destruction of the plant cells, so plant adapt to these conditions by accumulating some antioxidant compounds to avoid these oxidative stresses. Amongst these compounds are the phenolic compounds which play an important role in protecting the plant cells from stresses. In addition, the area where *H. muticus* grows, is located in the arid zone, which is characterized by water deficiency and low levels of rainfall. Accordingly, compounds detected in the current investigations included ferulic acid, cinamic acid, benzoic acid, besides their salts; methyl ferulat, 4'-Hydroxy-3'-methylacetophenone and methyl salicylate are synthesized to support the antioxidative properties of the plant against oxidative stress. Most phenolic compounds are found in bounded ester form, the accumulation of phenolic compounds in ester form is considered as a mechanism of drought tolerance, this agrees with the report published by Stanlisla et al. (2009) as they have found the accumulation of ester bound to p-coumaric acid in *Vitis vinfiera* which grows under drought conditions in the green houses. The presence of ferulic acid also supports the role of phenolic compound in *H. muticus* as antioxidant, mainly ferulic acid belonging to biochemically active phenylpropanoids. By absorbing radiation, the phenolic compounds transform short-wave, high-energy and highly destructive radiation into the blue radiation of a longer wavelength and, therefore, it is less destructive to the cellular structures of the leaf, including the photosynthetic apparatus (Bilger et al., 2001).

Regarding the antioxidant evaluation, DPPH free radicals scavenging activity and the ferric reducing antioxidant power (FRAP) assay of methanol extract of aerial parts of *H. muticus* were carried out. The results showed that the methanolic extract of *H. muticus* has an important antioxidant activity with an IC_{50} of 8.1 ± 0.65 mg/ml and an EC_{50} of 12.74 ± 1.12 mg/ml (Table 3). The antioxidant capacity of the methanolic extract of *H. muticus*, based on the results obtained, is significantly lower than that of ascorbic acid (IC_{50}: 0.031 ± 0.001 mg/ml; EC_{50}: 0.095 ± 0.002 mg/ml) and quercetin (IC_{50}: 0.012 ± 0.002 mg/ml; EC_{50}: 0.019 ± 0.003 mg/ml) ($P < 0.05$). The antioxidant activity of this extract is due to its chemical composition, which showed the presence of different phytochemical groups that have an antioxidant activity such as alkaloids, flavonoids, tannins, sterols and phenolic compounds. Moreover, the chemical analysis using GC-MS allows identifying certain compounds which can be involved in the antioxidant mechanisms. Some studies have evaluated the efficacy of quinic acid as an antioxidant in the metabolization of tryptophan and nicotinamide (Pero et al., 2009). Also, Chuda and his group showed that quinic acid has a strong antioxidant activity (Chuda et al., 1996). Furthermore, other authors have shown the high antioxidant activity of Guaiacol (Brand-Williams et al., 1995), Cinnamic acid derivatives (Sharma, 2011), and Ferulic acid (Srinivasan et al., 2007;

Table 2. The analysis of phenolic compounds of 80% methanol extract of *Hyoscyamus muticus,* aerial parts.

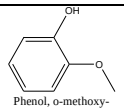
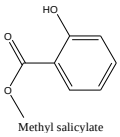
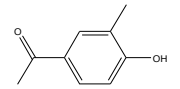
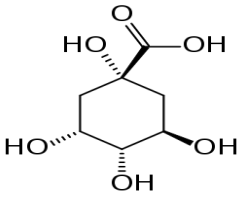
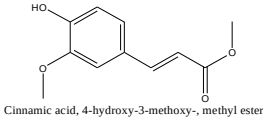
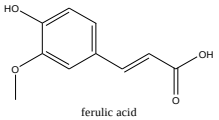
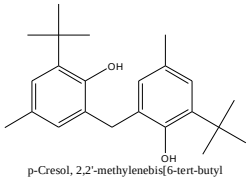
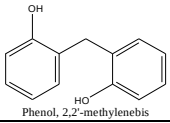
Compound	Rt	Structure	Chemical formula	Amount (%)
Phenol, o-methoxy- (Guaiacol)	1090	 Phenol, o-methoxy-	$C_7H_8O_2$	0.20
Methyl salicylate	1281	 Methyl salicylate	$C_8H_8O_3$	0.03
4'-Hydroxy-3'-methylacetophenone	1363	 4'-Hydroxy-3'-methylacetophenone	$C_9H_{10}O_2$	0.04
D-(-)-Quinic acid	1852		$C_7H_{12}O_6$	0.02
Cinnamic acid, 4-hydroxy-3-methoxy-, methyl ester (Methyl isoferulat)	1677	 Cinnamic acid, 4-hydroxy-3-methoxy-, methyl ester	$C_{11}H_{12}O_4$	0.52
Ferulic acid	1644	 ferulic acid	$C_{10}H_{10}O_4$	0.36
p-Cresol, 2,2'-methylenebis[6-tert-butyl	2788	 p-Cresol, 2,2'-methylenebis[6-tert-butyl	$C_{23}H_{32}O_2$	0.05
Phenol, 2,2'-methylenebis	2890	 Phenol, 2,2'-methylenebis	$C_{13}H_{12}O_2$	0.03

Table 3. The antioxidant capacity of methanol extract of *Hyoscyamus muticus*.

Antioxidant capacity (mg/ml)	Methanol extract	Ascorbic acid	Quercetin
IC_{50}	8.1 ± 0.65^a	0.031 ± 0.001^b	0.012 ± 0.002^c
EC_{50}	12.74 ± 1.12^a	0.095 ± 0.002^b	0.019 ± 0.003^c

Data are averages (± S.E.). Different letters stand for statistically significant differences between the results of each test at $P<0.05$ (Student T-test).

Table 4. The antimicrobial activity of the methanol extract of *Hyoscyamus muticus* areal parts against different microorganisms.

Tested compound	Mean zone of inhibition (mm)*										
	Gram-positive bacteria				Gram-negative bacteria					Fungi	
	Sa1	Sa2	Se	Bc	Ec	Pa	Ab	Kp1	Kp2	As	Ca
MeOH of *H. muticus*	10.5±0.5	13.0±1.0	11.75±0.25	11.5±0. 5	8.5±0.5	12.0±0.0	11.5±0.5	7.75±0.25	6.5±0.5	6.0±0.0	6.0±0.0
Erythromycin	18.5±0.5	25.5±0.5	31.0±1.0	29.5±0.5	12.5±1.5	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	-	-
Clotrimazole	-	-	-	-	-	-	-	-	-	19.0±1.0	27.5±0.5
10% DMSO	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0

*6.0 mm one of inhibition = no activity, Sa1= *Staphylococccus aureus* clinical isolate, Sa2=*Staphylococcus aureus* ATCC 25923, Se=*Staphylococcus epidermidis* ATCC 49461, Bc=*Bacillus cereus* ATCC 10876, Ec=*Escherichia coli* ATCC 35218, Pa=*Pseudomonas aeruginosa* clinical isolate, Ab=*Acinitobacter baumannii* clinical isolate, Kp1=*Klebsiella pneumoniae* ATCC 27736, Kp2=*Klebsiella pneumoniae* ATCC 700603, As=*Aspergillus niger* ATCC 6275, Ca=*Candida albicans* ATCC 10231

Mathew and Abraham, 2004). Our results disagree with the findings of Hajipoor et al. (2015), they reported that the antioxidant activity of *Hyoscyamus niger* collected from Iran has an EC_{50} of 377 ± 1.21 µg/ml, this differences may be related to the chemical composition, environmental conditions and/or the physiological system of this species.

The results of the antimicrobial testing are represented in Table 4 and Figures 1 to 4. Among all tested microorganisms, the gram-positive bacteria exhibited higher susceptibility towards the methanol extract of *H. muticus* areal parts which recorded 13.0 ± 1.0 mm for *S. aureus* ATCC 25923, 11.75 ± 0.25 mm for *S. epidermidis* ATCC 49461, 11.5 ± 0.5 mm for *B. cereus* ATCC 10876 and 10.5 ± 0.5 mm for *S. aureus* clinical isolate, respectively (Figure 1). However, all results of the gram-positive bacteria showed that they were most sensitive to the antibiotic (Erythromycin 15 µg/disc). 10% DMSO has no inhibitory effect on the growth of the gram-positive bacteria. The gram-negative bacteria showed average or weak antibacterial susceptibility towards the methanol extract of *H. muticus* areal parts. This recorded 12.0 ± 0.0 mm for *Pseudomonas aeruginosa* clinical isolate, 11.5 ± 0.5 mm for *Acinetobacter baumannii* clinical isolate, 8.5 ± 0.5 mm for *Escherichia coli* ATCC 35218, 7.75 ± 0.25 mm for *Klebsiella pneumonia* ATCC 27736, and 6.5 ± 0.5 mm for *Klebsiella pneumonia* ATCC 700603, respectively (Figure 2). However, the tested antibiotic (Erythromycin 15 µg/disc) showed weak or no activity against the gram-negative bacteria. Moreover, there was no statistical significance between the susceptibility of different strains from the same bacterial species (*K. pneumonia* and *S. aureus*). However, the clinical isolate of *S. aureus* was more resistant to erythromycin compared to *S. aureus* ATCC 25923 (Table 4 and Figures 1 and 2). Regarding the antifungal potential, neither *Aspergillus niger* ATCC 6275 nor *Candida albicans* ATCC 10231 revealed any susceptibility against the methanol extract of *H. muticus* areal parts, concluding that the studied plant extract has no inhibitory effect on the tested fungal strains, compared with clotrimazole 10 mg/ml (Figure 3).

Based on the above-mentioned results, the methanolic extract of *H. muticus* areal parts showed average or weak antibacterial activity against the gram-positive bacteria, weak antibacterial activity against the gram-negative bacteria and no antifungal activity. Moreover, in the current study, the gram-positive bacteria were more susceptible than the gram negative bacteria, which are attributed to the structure of the cell wall layers. In general, since the studies on antimicrobial activities of the areal parts of *H. muticus* are scanty, it would be valuable to compare our results on *H. muticus* with other available reports on different *Hyoscyamus* spp., which surprisingly showed that findings of the current study are generally in harmony with some previous studies on varied *Hyoscyamus* spp. The ethanol extract of *H. albus* showed no inhibitory effect on different bacterial strains; however, it was published that the alkaloid fraction revealed some degrees of antibacterial effects that ranged from 14.0 to 7.0 mm zone of inhibition (Kadi et al., 2013). Methanol extracts of stem, leaves and seeds of *H. niger* were investigated for antibacterial properties against some gram-positive and gram-negative bacteria, seeds showed antibacterial effects much better than leaves and stem (Snigh and Pandey, 2009). Accordingly, it is recommended to investigate the antibacterial potential of the seeds of *H. muticus*. The findings of Almalki (2017) supports our

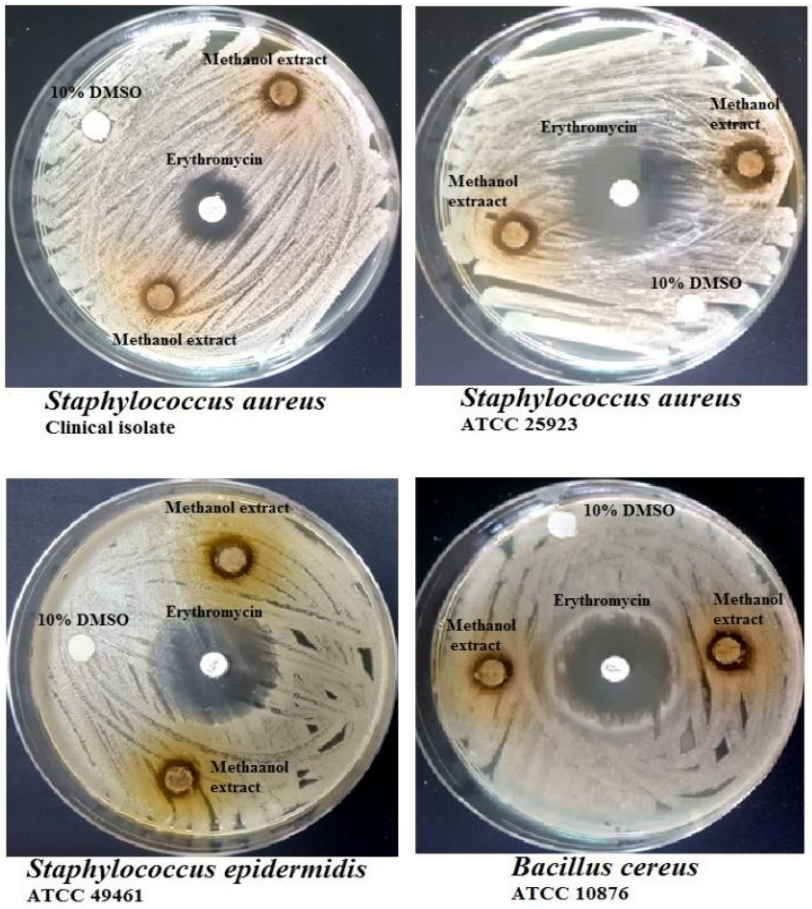


Figure 1. Susceptibility of gram-positive bacteria to methanol extract of *Hyoscyamus muticus* compared to erythromycin

recommendation, he studied the antibacterial and antifungal activity of *H. muticus* among other plants; seeds of *H. muticus* showed varying degrees of antibacterial and antifungal activities. On the other hand, in the current study, the absence of antifungal activity in H. muticus extract is a reasonable result (Figures 3 and 4). This is because; it was found that in nature, many fungal species, including endophytic fungi reside in H. muticus (El-Zayat et al., 2008). The results of the antifeedant potential of the methanol extract of *H. muticus* are tabulated in Table 5, showing that the methanolic extract of *H.* muticus exhibited antifeedant effect on the 4th instar larvae of *S. littoralis*. The antifeedant activity ranged from 86.38, 78.77, 73.81 to 73.47% at concentrations 40, 20, 10 and 5%, respectively. It was observed that the antifeedant activity increased with time in all concentrations after treatment. Data in Table 6 shows the starvation percentage of the 4th instar larvae of *S. littoralis* treated with the methanolic extract of *H. muticus*. The starvation percentage as well as the antifeedant activity increased with increasing concentration and time of exposure. Moreover, the average of the starvation percentage ranged from 98.50 to 87.18% at high concentration, whereas at lower concentration, the repellence effect ranged from 78.74 to 73.79%. This can be explained based on the phytochemical analysis of *H. muticus* which showed that this plant has special alkaloid compounds such as hyoscyamine and scopolamine that are anti-cholinergic and anti-spasmodic drugs. Moreover, this plant has anti-spasmodic, analgesic and sedative properties (Alaghemand et al., 2013). In literature, it was reported that *H. niger* was used to control the larvae of Anopheles (Mahmoodreza et al., 2017). Various botanical extracts contain a complex of chemicals with a unique biological activity (Farnsworth and Bingel, 1977). Finally, the current findings can further illustrate the perspective to control larvae of *S. littoralis* without imposing environmental damage.

Conclusion

Medicinal plants are rich sources of bioactive compounds

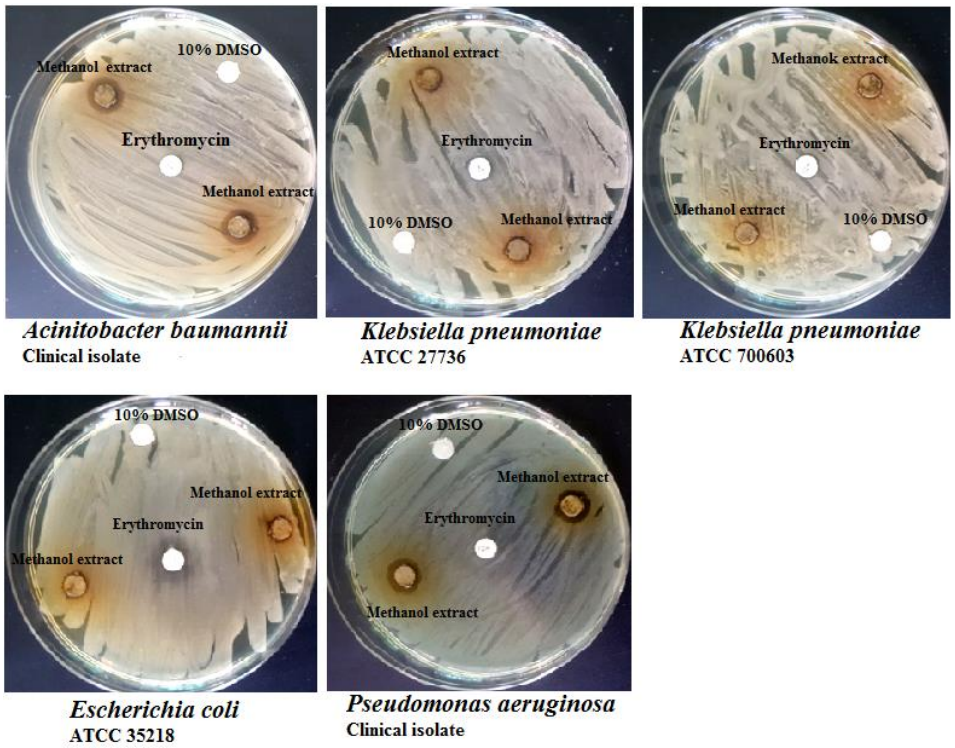
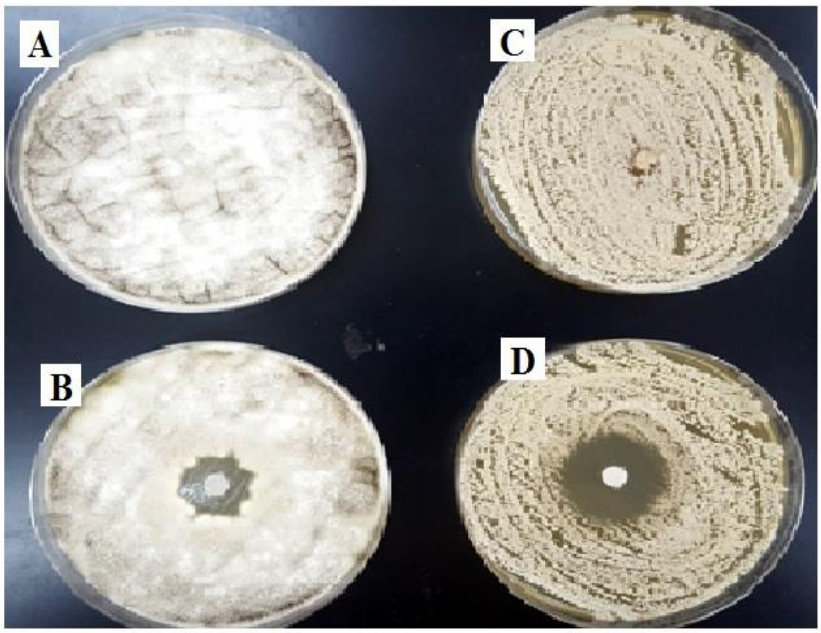


Figure 2. Susceptibility of gram-negative bacteria to methanol extract of *Hyoscyamus muticus* compared to erythromycin.



A= *Aspergillus niger* + the extract disc in the middle (No inhibition zone)
B= *Aspergillus niger* + the clotrimazole disc (Obvious inhibition zone)
C= *Candida albicans* + the extract disc in the middle (No inhibition zone)
D= *Candida albicans* + the clotrimazole disc (Obvious inhibition zone)

Figure 3. Susceptibility of some fungal strains to methanol extract of *Hyoscyamus muticus* compared to clotrimazole

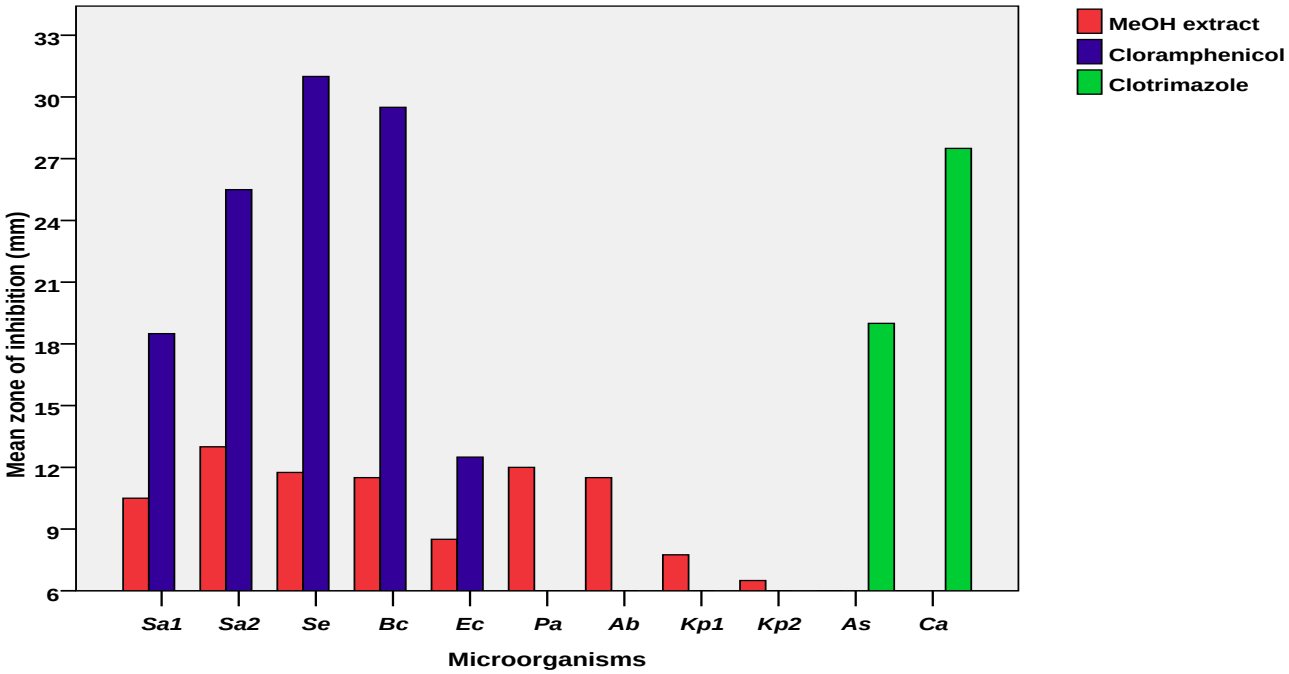


Figure 4. Mean zone of inhibitions of different microorganisms due to the effect methanol extract of *Hyoscyamus muticus* compared with antimicrobial drugs.

Table 5. Antifeedant activity of the methanolic extract of *Hyoscyamus muticus* against 4th instar larvae of *S. littoralis*.

Conc. (%)	Antifeedant index (%) post-treatment			Mean*
	1st day	2nd day	3rd day	
5	62.16	69.68	88.57	73.47
10	64.58	70.87	85.99	73.81
20	76.20	74.32	85.79	78.77
40	83.65	89.04	86.45	86.38

Data are expressed as mean ± SE (n=5), * total mean of each treatment at different time intervals, values were analyzed by one-way ANOVA, where means within each column followed by different letters are significantly different (P< 0.05 by LSD).

Table 6. Starvation percentage (%) of the 4th instar larvae of *S. littoralis* treated with the methanolic extract of *Hyoscyamus muticus.*

Treatment	Time	Average weight (mg/larva)	Difference* (mg/larva)	Starvation (%)	Average
5%	0 min	56.50	-	-	73.79%
	24 h	63.40	+6.90	65.20	
	48 h	65.60	+9.10	71.91	
	72 h	67.90	+11.4	84.28	
10%	0 min	56.01	-	-	78.74%
	24 h	59.60	+3.60	74.62	
	48 h	62.30	+6.30	75.90	
	72 h	65.01	+9.00	85.70	

Table 6, Contd.

20%	0 min	60.20	-	-	87.18%
	24 h	61.40	+1.20	81.45	
	48 h	59.01	-1.20	86.58	
	72 h	56.00	-1.40	93.52	
40%	0 min	59.02	-	-	98.50%
	24 h	54.01	-5.01	99.14	
	48 h	50.02	-9.01	97.69	
	72 h	46.12	-12.9	98.67	
Control	0 min	61.10	-	-	-
	24 h	90.09	+29.8	-	
	48 h	120.7	+59.6	-	
	72 h	214.8	+153.7	-	
Starved larvae	0 min	67.90	-	-	-
	24 h	65.60	-5.310	-	
	48 h	63.40	-10.62	-	
	72 h	56.50	-15.14	-	

with varied effects on human, animals, plants, insects and microorganism. Plants that survive under environmental stresses could produce important unique phytochemicals. *H. muticus* revealed the presence of phenolic compounds, flavonoids, tannins and sterols. The methanolic extract was found to be rich in phenolic compounds, ferulic acid, 4-hydroxy–cinamic acid–ester, methyl salicylate and methyl ferulate; these compounds support the antioxidative properties of the plant againstoxidative stress, as seen in the antioxidant evaluation. The antimicrobial investigation exhibited moderate or weak antibacterial effects, perhaps these moderate efficacy particularly against the gram-positive bacteria, which are enough to control these bacteria prevalent in soil, or may have some sort of associations with this plant. The absence of antifungal compounds may allow the endophytic fungi to grow in/on the tissues of *H. muticus*. Moreover, this plant protects itself from insects by means of some antifeedant compounds as revealed from the findings of present study. Accordingly, the results generated from the current study provides the bases for further future investigations to isolate some important compounds of bioactive properties which could be useful for biocontrol and pharmaceutical industries.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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