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# ANTIBACTERIAL ACTIVITY OF METHANOL EXTRACT AND ESSENTIAL OIL OF *HYOSCYAMUS NIGER* AGAINST SELECTED PATHOGENIC BACTERIA

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## ABSTRACT

Evaluation of medicinal plants has shown potential antimicrobial effects which introduced many drugs with antibiotic properties. *Hyoscyamus niger*, has been used as a medicine since last centuries and has been described in all traditional medicines. This study evaluates the antibacterial effect of methanol extract and essential oil of *Hyoscyamus niger* against different pathogenic microorganisms including 2 Gram positive bacteria and 2 Gram negative bacteria by three methods such as disc diffusion, well diffusion and micro dilution methods. The results showed inhibition zone in well diffusion method was larger than disk diffusion method and this method is more sensitive than disc diffusion method. The results showed Gram positive bacteria were more sensitive than Gram negative ones. Among Gram positive bacteria *Bacillus cereus* and among Gram positive *Salmonella enterica* was more sensitive than others and the zone of inhibition for essential oil was more than methanol extract in well diffusion. In well and disk diffusion, methanol extract had comparable antibacterial activity to Gentamicyn but in MIC this plant hadn't good activity so the results showed there wasn't any correlation to these methods. The results concluded that the methanol extract and essential oil of *Hyoscyamus niger* is a potential natural antibacterial agent.

**Keywords: *Hyoscyamus niger*, antimicrobial, methanol extract, essential oil**

## INTRODUCTION

Microbial infections cause systemic diseases and researchers are trying to discover plant antimicrobials because of antimicrobial resistance and toxicity of synthetic drugs [1]. Medicinal plants produce bioactive compounds that can inhibit the growth of pathogenic bacteria [2-3] and they are considered as being good candidates for production of new antimicrobial drugs [4] and there are many studies about antimicrobial activity on medicinal plants [5]. *Hyoscyamus niger* is from Solanaceae family and it is commonly known as black henbane and other synonyms for this plant are henbane, devil's eye, foetid nightshade, hogs bean, Jupiter's bean, poison tobacco and stinking nightshade [6]. This plant used for the treatment of inflammation, rheumatism, cough, fevers, motion sickness, bronchitis and spasms [7-8] and it has antispasmodic, sedative and analgesic properties [9-10]. In ancient Iranian medicine, *Hyoscyamus niger* has been used for diarrhea, stomach pain and some central nervous system disorders such as parkinsonism and hysteric patients [11]. It is widely distributed in Europe and Asia and 18 species have been reported from Flora Iranica and it can be found at slopes, roadsides, sandy bank rivers [11]. Hyoscyamus species especially *H. niger* contain tropane alkaloids such as hyoscyamine and scopolamine and they are synthesized in the roots of this plant [12]. hyoscyamine and scopolamine are widely used for antispasmodic, anticholinergic and analgesic [13]. hyoscyamine, hyoscine, skimmianine, apohyoscine, apoatropine, tropine and belladonines are extracted from aerial parts of *Hyoscyamus niger* and major chemical compounds of this plant are Apoatropine, Cuscohygrine, Daturamine, Hyoscine (Scopalimine), tropine, phytin, choline, hyoscypicrin, ,hyoscine, aphoyoscine, alpha and beeta belladonine [14]. The methanol extract from seeds of *H. niger* showed strong antibacterial activity against Staphylococcus aureus [15]. So, the aim of this study was to evaluate the antimicrobial activity of essential oil and methanol extract from *Hyoscyamus niger* that extracted from aerial part of plant against four microorganisms including Gram positive and Gram negative bacteria.

## MATERIALS AND METHODS

### Plant material

The aerial parts of *Hyoscyamus niger* were collected in 2014 from North khorasan. The leaves were air-dried at room temperature and were stored for later analysis.

**Preparation of the methanol extract**

The air dried plant extracted with methanol (Merck) using the maceration method. The extractive solution obtained was filtered and the solvent was removed with a rotary evaporator at 40◦C and then kept in small bottles under refrigerated conditions until used [16].

**Isolation of essential oil**

The samples were subjected to hydro distillation using a Clevenger for 4 h to obtain the essential oil. It was dried with sodium sulfate and stored at 4°C in darkness [17].

**Microbial Cultures and Inoculation Conditions**

Methanol extract and essential oil of *Hyoscyamus niger* were assayed for antimicrobial activity against four species of bacteria. The bacterial strains included Gram-positive *Staphylococcus Aureus* (PTCC 1431) and *Bacillus cereus* (PTCC 1015); Gram-negative *Salmonella Entrica* (PTCC 1709), *Escherichia coli* (PTCC 1399). All microorganisms were clinical isolates, obtained from the Persian Type Culture Collection (PTCC). Mueller-Hinton broth was applied for growing and diluting the microorganism suspensions. Bacterial strains were grown in Mueller-Hinton broth at 37 °C for 18 h and adjusted to a final density of $10^8$ CFU/ml by diluting fresh cultures and comparing with McFarland density. In this study, antibacterial activity was assayed by three methods such as Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC), Disk and well diffusion methods.

**Disk-diffusion method**

The discs with 6 mm diameter were placed on the plates with 80 mm diameters containing Mueller Hinton agar (MHA) with the $1.5\times10^8$ bacterial cells. Methanolic extract and essential oil were dissolved in dimethyl Sulfoxide (DMSO) to give 500 mg $ml^{-1}$ concentration of them. 20 µl of samples placed on the discs and then they were incubated at 37°C for 24 h. Antimicrobial activity was evaluated by measuring the zone of inhibition surrounding the discs. Gentamycin was positive control and DMSO used as negative control [18-19].

**Well diffusion method:**

Wells with 6 mm diameter were punched with the help of sterilized cork borer (6

mm), into the agar plates of the appropriate media, which had been surface spread with bacteria at a $10^8$ CFU/ml density. Stock solution of plant extract was prepared at a concentration of 500 mg/ml and then 50 µl of them were added sterile syringe into the wells and allowed to diffuse at room temperature for 2hrs. The plates were incubated at 37 °C for 24h. Antibacterial activity was evaluated by measuring the zone of inhibition (mm). DMSO was negative control and Gentamycin used as positive control [20- 21].

**MIC and MBC Method**

MIC was determined by micro-dilution method using serially diluted plant extracts according to the National Committee for Clinical Laboratory Standards. MIC was determined by the broth micro-dilution method in a 96-wells micro-plate. The final concentration of microorganism inoculum was $1.5\times10^6$ CFU $ml^{-1}$ [22]. The extract and essential oil were diluted in dimethyl Sulfoxide (DMSO) and Mueller Hinton Broth (MHB) to give a concentration of 200, 100, 50, 25, 12.5, 6.25 and 3.125 mg $ml^{-1}$ [23]. Then, 100 µl of each concentration was added in a well into 96-well micro plate containing of $1.5\times10^6$ CFU $ml^{-1}$. The micro plate was incubated at 37°C for 24 h. One well included antibiotic control and one well included organism control. The lowest concentration of extract that produced no visible bacterial growth when compared with the control well was considered as MIC [24]. To determine MBC, 10 µl was taken from each well and inoculated in MHB for 24 h at 37ºC. The highest dilution that yielded no signal bacterial colony on the solid medium was taken as MBC [25].

**RESULTS**

The antibacterial activity from aerial parts of *Hyoscyamus niger* was done with three methods; Disc and well diffusion and MIC-MBC methods. The antibacterial activity against four selected pathogens is shown in **Tables 1-3**.

**Table 1: The quantitation of antimicrobial activity for essential oil and methanol extract of *H. niger* measured by Disc diffusion method. The effectiveness of samples is demonstrated by the size of the microorganism growth inhibition zone around the filter paper disc, which is typically expressed as the diameter of the zone in mm.**

| | Disc diffusion (mm) | | |
|---|---|---|---|
| **Microorganisms** | **Methanol extract** | **Essential oil** | **Gentamicyn** |
| ***S. aureus*** | **15** | **6** | **29** |
| ***B. cereus*** | **14** | **6** | **32** |
| ***S. enterica*** | **13** | **6** | **27** |
| ***E. coli*** | **13** | **6** | **31** |

**Table 2: The quantitation of antimicrobial activity for essential oil and methanol extract of *H. niger* measured by Well diffusion method. The effectiveness of samples is demonstrated by the size of the microorganism growth inhibition zone, which is typically expressed as the diameter of the zone in mm**

| | Well diffusion (mm) | | |
|---|---|---|---|
| **Microorganisms** | **Methanol extract** | **Essential oil** | **Gentamicyn** |
| ***S. aureus*** | **24** | **27** | **32** |
| ***B. cereus*** | **26** | **29** | **33** |
| ***S. enterica*** | **22** | **23** | **31** |
| ***E. coli*** | **22** | **13** | **32** |

**Table 3: Minimum inhibitory concentrations (MIC, mg/ml) and Minimum bactericidal concentrations (MBC, mg/ml) from the methanol extract and essential oil of *H. niger***

| **Microorganisms** | **Methanol extract** | | **Essential oil** | |
|---|---|---|---|---|
| | **MIC (mg/ml)** | **MBC (mg/ml)** | **MIC (mg/ml)** | **MBC (mg/ml)** |
| ***S. aureus*** | **50** | **100** | **200** | **>200** |
| ***B. cereus*** | **50** | **>100** | **200** | **>200** |
| ***S. enterica*** | **100** | **>100** | **>200** | **>200** |
| ***E. coli*** | **100** | **>100** | **200** | **>200** |

## DISCUSSION

In general, screening of medicinal plants for antimicrobial activities is important for finding new compounds for medicinal purposes. Interests on antimicrobial properties of extracts from plants are very important, because some pathogenic bacteria are commonly resistant to many antibiotics. As shown in **Tables 1 and 2**, the methanol extract of the plant was found to be more effective on microorganisms in comparison to the essential oil. In disc diffusion method the methanol extract of *Hyoscyamus niger* exhibited antibacterial activity against all investigated microorganisms with a 13-15 mm zone of inhibition. The inhibition zone of the oil in disc diffusion was 6 mm because the essential oil of plant hasn't any Power for outing from paper disc diffusion and spread in the environment and then it had no inhibition zone. While for the positive control it was in the range of 27-32 mm but in well diffusion method, the essential oil was effective on microorganisms and its zone of inhibition was more than methanol extract on 3 bacteria. The inhibition zone of the methanol extract in well diffusion method was in range of 22-26 mm and it was in range of 13-29 mm for essential oil and it was in the range of 31-33 for positive control. The results showed inhibition zone in well diffusion method was larger than disk diffusion method and this method is more sensitive than disc diffusion method, because in well diffusion

method, the antimicrobials present in the plant extract are allowed to diffuse out into the medium and interact with the test organisms. In well diffusion method polar compounds would not be influenced by the hydroxyls on the surface of the paper and would diffuse easily. Thus well diffusion method is more convenient than the disc diffusion [26]. The disk diffusion method allows for the simultaneous testing of a large number of antimicrobials in a relatively easy and flexible manner and this method isn't quantitative and it is the major disadvantages of this method [27] and microdiution method is found to overcome these limitations of the disk diffusion method [28]. Among all four microorganisms included in the present study, both Gram-positive bacteria exhibited the lowest MIC values and Gram-negative bacteria are resistant to antimicrobials due to the hydrophilic surface of their outer membrane rich in lipopolysaccharide molecules, which acts as a protective barrier. Moreover, the enzymes in the periplasmic space are capable of breaking down the antimicrobials [29-30]. However, in our study the essential oil and methanol extract of *Hyoscyamus niger* have significant antibacterial activity against the Gram-negative pathogens. The oil of plant had some antimicrobial activity against different microorganisms and varied according to the type of pathogen. Gram positive bacteria were more sensitive than Gram negative bacteria. The antimicrobial activity of oil was comparable with antibiotics. B. cereus and S.aureus were sensitive (Table 3). Among Gram positive bacteria *Bacillus cereus* and among Gram positive *Salmonella enterica* was more sensitive than others.

A correlation between MIC values and inhibition diameter wasn't found, because essential oil had good antibacterial activity in well diffusion method and it hadn't any antibacterial activity in MIC method. Inhibition diameter of Gram positive bacteria is larger than Gram negative bacteria and the MIC values of Gram positive bacteria were smaller than Gram negative bacteria. These results show that these three methods are necessarily comparable. The MIC of the essential oil and methanol extract were within concentration ranges from 50 to 200 mg/ml, and the respective MBC were >100 and >200 mg/ml. The essential oils of some populations with the highest percentage of monoterpenes had relatively higher inhibitory activity against bacteria,

than the essential oils from the other populations. The antimicrobial activity of the essential oils can be explained by the lipophilic nature of the monoterpenes contained in the oils [31].

The occurrence of alkaloids, tyramine derivative, withanolides, lignanamides and flavonoids in *H. niger* have confirmed [32-35]. Flavonoids are responsible for antibacterial activity of plant [36]. Flavonoids in *H. niger* are rutin and spiraeoside [37]. Hyoscyamus species are rich sources of tropane alkaloids such as hyoscyamine and scopolamine [38] and all parts of this plant contain tropane alkaloids [39]. The major alkaloids in *H. niger* are hyocyamine, atropine and scopolamine [40]. Hyoscyamine and scopolamine are widely used in medicine for their mydriatic, antispasmodic, anticholinergic, analgesic, antibacterial and sedative properties and they are synthesized in roots and then transported to the aerial parts of the plant [41]. In one study, the aqueous 60 % methanol extract from seeds of *H. niger* showed strong antibacterial activity against *Staphylococcus aureus*, with inhibition zones of 25.0 mm and this extract from the seeds of *H. niger* has significant activity and suggest that it may be useful in the treatment of infections [15].

## CONCLUSION

The present study shows that the compounds from *H. niger* possess potent antimicrobial activity and its essential oil and methanol extract contains the effective compounds responsible for eliminating the bacterial pathogens. The results of this work indicate that this plant should be studied for treatment of many infectious diseases.

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## REFERENCES

**[1]** Alzoreky NS, Nakahara K. Antimicrobial activity of extracts from some edible plants commonly consumed in Asia. International Journal of Food Microbiology, 80, 2003, 223–230.

**[2]** Chopra RN, Nayer SL, Chopra IC. Glossary of Indian Medicinal Plants. 3rd ed. Council of Scientific and Industrial Research, New Delhi. 1992, pp 7-246.

**[3]** Bruneton J. Pharmacognosy, Phytochemistry, Medicinal Plants. Lavoisiler Publishing Co, France. 1995, pp 265-380.

**[4]** Zaika LL. Spices and herbs: Their antimicrobial activity and its determination. Journal of Food Safety, 9, 1988, 97-118.

**[5]** Silva NCC, Fernandes, JA. Biological properties of medicinal plants:a review of their antimicrobial activity. J. Venomous Animals Toxins TropicalDiseases, 16 (3), 2010, 402–413.

**[6]** Schönbeck-Temesy, E. In Rechinger, K. H. (ed.) Flora Iranica, Solanaceae. 100(1), 1972, pp 12

**[7]** Ramoutsaki IA, Askitopoulout H, Konsolaki E. Pain relief and sedation in roman byzantine texts: *Mandragoras officinarum, Hyoscyamos niger* and *Atropa belladonna*. International Congress Series, 1242, 2002, 43-50

***[8]*** Harrison AP, Bartels EM. A modern appraisal of ancient etruscan herbal practices. American Journal of Pharmacology and Toxicology, 1, 2006, 26-29.

**[9]** Zargari A. Herbal plants. Third volume, Tehran university pub, 1989, 335-346

**[10]** Mirheydrar H. Ma`arefe giahi, Plants applications in prevention and cure of the disesases, fifth volume, theran farhang eslami pub, 1996, 43-46

**[11]** Zargari A. Medicinal Plants. 5th ed. Tehran, Tehran University Publications, 1990, pp.570-575.

**[12]** Hashimoto T, Yukimune Y, Yamada Y. Tropane alkaloid production in Hyoscyamus root cultures. Journal of Plant Physiology, 124, 1986, 61–75.

**[13]** Hashimoto T ,Hayashi A, Amano Y, Kohno J, Iwanari H, Usuda S, Yamadn Y. Hyoscyamine 6 betahydroxylase, an enzyme involved in tropane alkaloid biosynthesis is localized at the pericycle of the root. Biological Chemistry, 266(7), 1991, 4648-53.

**[14]** Aparna K, Joshi-Abhishek J, Mahesh V. Phyto-chemical and pharmacological profiles of hyoscyamus niger linn (parasika yavani)- a review. Pharma science monitor 6(1), 2015, 153-158.

**[15]** Dulger B, Goncu BS, Gucin F. Antibacterial Activity of the Seeds of *Hyoscyamus niger* L.

(Henbane). Asian Journal of Chemistry, 22, 2010, 6879-6883.

**[16]** Seethalaxmi MS, Shubharani R, Nagananda GS, Sivaram V. Phytochemical analysis and free radical scavenging potential of Baliospremum montanum (Willd.) Muell. leaf. Asian Journal of Pharmaceutical and Clinical Research, 5(2), 2012, 135-137.

**[17]** Ben Hsouna A, Hamdi N. Phytochemical composition and antimicrobial activities of the essential oils and organic extracts from Pelargonium graveolens growing in Tunisia: BioMed Central Ltd. Lipids in Health and Disease, 11(167), 2012, 1-7.

**[18]** Billah MM, Islam R, Khatun H, Parvin S, Islam E, Islam SA, Mia AA. Antibacterial, antidiarrhoeal, and cytotoxic activities of methanol extract and its fractions of Caesalpinia bonducella (L.) Roxb leaves. BMC Complementary and Alternative Medicine 13(101), 2013, 1-7.

**[19]** Selim SA, Adam ME, Hassan SM, Albalawi AR. Chemical composition, antimicrobial and antibiofilm activity of the essential oil and methanol extract of the Mediterranean cypress (Cupressus sempervirens L.). BMC Complementary and Alternative Medicine 14(179), 2014, 1-8.

**[20]** Van den Berghe DA, Viletinck AJ. Screening Methods for Antibacterial and Antiviral Agents from Higher Plants. In: Hostett-man K, ed. Methods in Plant Biochemistry, Assays for Bioactivity. London, San Diego, New York, Boston, Sydney, Tokyo and Toronto: Academic Press, 6, 1991, 47-69.

**[21]** Firdaus J, Rubina L, Vinod K, Mohd J. Evaluation of antimicrobial activity of plant extracts on antibioticsusceptible and resistant *Staphylococcus aureus*strains. Journal of Chemical and Pharmaceutical Research 3(4), 2011, 777-789

**[22]** Coccia A, Carraturo A, Mosca L, Masci A, Bellini A, Campagnaro M, Lendaro E. Effect of methanolic extract of sour cherry (Prunus cerasus L.). International Journal of Food Science and Technology, 47, 2012, 1620-1629.

**[23]** Dhiman A, Nanda A, Ahmad A, Narasimhan B. In vitro antimicrobial activity of methanolic leaf extract of (Psidium guajiava L.). Journal of Pharmacy and Bioallied Science, 3(2), 2011, 226-229.

**[24]** Umer S, Tekewe A, Kebede N. Antidiarrhoeal and antimicrobial activity of Calpurnia aurea leaf extract. BMC Complementary and Alternative Medicine, 13(21), 2013, 1-5.

**[25]** Haobin H, Xudong Z, Huaishing H, Yan L. Chemical Compositions and Antimicrobial Activities of Essential oils Extracted from Acanthopanax brachypus. Archives of Pharmacal Research, 32 (5), 2009, 699-710.

**[26]** Valgas C, Machado de Souza S, Smânia EFA, Smânia AJ. Screening methods to determine antibacterial activity of natural products. Brazilian Journal of Microbiology, 38, 2007, 369-380

**[27]** Wilkins TD, Thiel T. Modified broth-disk method for testing the antibiotic susceptibility of anaerobic bacteria. American Society for Microbiology, 3, 1973, 350-356.

**[28]** Kim JS, Kim YH. The inhibitory effect of natural bioactives on the growth of pathogenic bacteria. Nutrition Research and Practice, 1, 2007, 273-278.

**[29]** Shan B, Cai Y, Brooks JD, Corke H. The in vitro antibacterial activity of dietary spice and medicinal herb extracts. International Journal of Food Microbiology, 117, 2007, 112-119.

**[30]** Chan EWC, Lim YY, Omar M. Antioxidant and antibacterial activity of leaves of Etlingera species (Zingiberaceae) in Peninsular Malaysia. Food Chemistry, 107, 2007, 1586–1593.

**[31]** Cristani M, D'Arrigo M, Mandalari G, Castelli F, Sarpietro MG, Micieli D, Venuti V, Bisignano G, Saija A, Trombetta D. Interaction of four monoterpenes contained in essential oils with model membranes: implications for their antibacterial activity. Journal of Agricultural and Food Chemistry, 55, 2007, 6300-6308.

**[32]** Duke JA. Handbook of Medicinal Herbs, CRC Press, Boca Raton FL, 1985, pp. 240-243.

**[33]** Ma CY, Williams ID, Che CT. Withanolides from *hyoscyamus niger* seeds. Journal of Natural Products, 62, 1999, 1445-1447.

**[34]** Ma CY, Liu WK and Che CT. Lignanamides and nonalkaloidal components of *Hyoscyamus niger* seeds. Journal of Natural Products, 65, 2002, 206-209.

**[35]** Steinegger E, Sonanini D. Solanaceous flavones. II. Flavones of *Hyoscyamus niger*. Pharmazie, 15, 1960, 643-644.

**[36]** Saeedi M, Morteza-Semnani K, Mahdavi M, Rahimi F. Antimicrobial studies on extracts of four species of Stachys. Indian Journal of Pharmaceutical Sciences , 70, 2008, 403-406

**[37]** Begum S, Sahai M, Suessmuth R, Asai T, Hara N, Fujimoto Y. Hyosgerine, a new optically active coumarinolignan from the seeds of Hyocyamus niger. Chemical and Pharmaceutical Bulletin, 5, 2006, 538-541.

**[38]** Supria KB, Szoke E, Toth K, Laszlo I, Kursinszki L. Investigation of tropane alkaloids in genetically transformed Atropa belladonna L. cultures. Chromatographia 60, 1998, 555-559

**[39]** Cuneyt C, Kudret K, Birsen S. Physical and Physiological Dormancy in Black Henbane (*Hyoscyamus niger* L.) seeds. Plant Biology 47, 2004, 391 - 395.

**[40]** Robbers JE, Speedie MK, Tyler VE. Pharmacognosy and pharmacobiotechnology. Williams and Wilkins, Baltimore, 1996, PP: 80-140.

**[41]** Zehra M, Banerjee S, Naqvi AA, Kumar S. Variation in Growth and Tropane Alkaloid Production Capability of the Hairy Roots of *Hyoscyamus albus*, *H. muticus* and their Somatic Hybrid. Plant Science 136, 1998, 93 -99.