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Research Article

**PHYTOCHEMICAL AND ANTIMICROBIAL SCREENING OF
HYOSCYAMUS MUTICUS, A PLANT FOUND IN THE
NORTHERN BORDER REGION OF SAUDI ARABIA****Mohd. Imran*¹ and Nudrat Fatima²**¹Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Northern Border University, Rafha - 91911, P.O. BOX 840, Kingdom of Saudi Arabia.²Department of Phytochemistry and Natural Product, Faculty of Pharmacy, Northern Border University, Rafha - 91911, P.O. BOX 840, Kingdom of Saudi Arabia.

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Abstract:

Hyoscyamus, commonly known as henbane, comprise of a small genus of flowering plants in the Solanaceae family. *Hyoscyamus muticus* L. or (Sakran al Misri) Egyptian henbane is a small perennial plant belonging to this family, which is famous for its tropane alkaloid content. This plant of the Northern Border Region of Saudi Arabia origin was studied for its pharmacognostic features, phyto-constituents and antimicrobial activity. The study was conducted on leaf, flower and stem extract. The extracts were fractionated with chloroform, hexane and water, which were also investigated for antimicrobial studies along with the ethanolic extract. The extracts of the leaf, flower and stem contained alkaloids, carbohydrates and sterols. Antimicrobial activity was investigated on some selected pathogenic organisms like *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi*, and *Candida albicans* using the agar well diffusion method. The flower and stem extracts showed insignificant antimicrobial activity, while the leaf extract exhibited significant antimicrobial activity against all strains in all fractions. The ethanol extract showed the prominent activity which was followed by the chloroform extract and the hexane extract, respectively. The further studies may provide fruitful insight about the main constituents of the ethanolic extract responsible for the antimicrobial activity.

Kew words: *Hyoscyamus muticus*, Northern Border Region, Saudi Arabia, Antimicrobial activity.**Corresponding Author:****Dr. Mohd. Imran,**

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INTRODUCTION:

Medicinal plants have been used since thousands of years ago and are still in use by a great percentage of the world population for being good. A number of scientists are trying to analyze the traditional medicines for their beneficiary effects and the results are promising [1,2]. Phytotherapy can be suggested only if biologically active constituents are present in plants [3,4]. The most important application range of these plant extracts is dealing with the growth inhibition or retardation of the serious and resistant pathogens [5-7]. The Kingdom of Saudi Arabia is one of the oldest inhabited regions of the world of mankind. It has a vast variety of plants from the Northern region to the Southern region. The Northern region is rich in plants suitable for Mediterranean climates. *Hyoscyamus*, commonly known as the henbanes, comprise of a small genus of flowering plants in the nightshade family. It comprises 11 species, all of which are toxic. It, along with other genera in the same family, is a source of the drug hyoscyamine or daturine [8]. *Hyoscyamus muticus* L. (Sakran al Misri or Egyptian henbane) belonging to the family solanaceae, is a small perennial plant of 0.3-0.6 m in height with a thick and highly branched stem. The leaves are alternate and succulent with broad lamina and pale green

color, narcotic odor and salty bitter taste. Flowers are in the form of racemose inflorescence, hermaphrodite and zygomorphic. Calyx is tube shaped with funnel shaped corolla. The fruits are small in size, brown in color containing many seeds [9,10]. *Hyoscyamus muticus* L. is famous for its tropane alkaloid contents. The main alkaloids present in *H. muticus* are scopolamine, nor-scopolamine, hygrine, nor-hygrine, tropine, pseudotropine, homotropine and hyoscyamine. It is also reported to contain other phyto-constituents like steroids, isofucosterol and muticin in leaf and root extract [11]. The effects of these alkaloids include stimulation of the central nervous system and simultaneous depression of the peripheral nerves typical for a para-symphathomimetic. The medicinal uses include spasmolytic, anti-asthmatic, anti-cholinergic, narcotic and anesthetic properties [12,13]. The ascaricidal activity of this plant is also reported [11]. Although, *H. muticus* is reported to possess a wide variety of biological activities, we believe that there is no report mentioning the antimicrobial screening of *H. muticus* found in the Northern Border Region of the Saudi Arabia. Accordingly, it was thought to perform the phytochemical and antimicrobial screening of *H. muticus* found in the Northern Border Region of the Saudi Arabia.



Fig 1: *Hyoscyamus muticus* plant samples collected from the Northern Border Region of Saudi Arabia

MATERIALS AND METHODS:**Collection of the plant material**

The plant was collected from different regions of the Northern Border Province after approval of the method by the Local Committee of Bioethics Decision No. (1/38/P) dated 14/12/2016. The plant was identified by Dr. Nudrat Fatima, (Department of Phyto-chemistry and Natural Product, Faculty of Pharmacy, Northern Border University) and a sample was submitted in the Herbarium (No. HM/007/2016).

Pharmacognostic studies

For pharmacognostic studies powdered material was crushed well in a mortar and pestle, then a little quantity was put on a slide, soaked with glycerin, mixed well and covered with cover slit. The slide was observed under microscope.

Preparation of the extract

The plant was cleaned, different aerial parts (leaves, stem and flower) were separated, grinded and weighed. About 50 g powder of each part was soaked in 100 ml ethanol, chloroform and hexane which were left for 6 days at room temperature in an airtight jar. The jars were shaken daily for complete extraction. After six days the samples were filtered and the solvent was evaporated on a rotary evaporator under reduced pressure and vacuum. Three samples were obtained the leaf extract, the flower extract and the stem extract weighing about 23 g, 16 g and 19 g, respectively [14].

Phytochemical studies

Phytochemical studies were carried out on leaf, stem and flower extracts by reported method. The tests were performed to find out the presence of alkaloids (Mayer's test, Wagner's test and Dragendorff's test), carbohydrates / glycosides (Molisch test, Fehling test), tannins (Ferric chloride test), saponins (Froth test), flavonoids (Sodium hydroxide test), triterpenoids (Acetic anhydride test) and

anthraquinones (Borntragers test). All chemicals used were of analytical grade [15].

Antimicrobial activity

Antimicrobial activity was investigated using the agar well diffusion method [16,17]. All plant extracts were mixed with DMSO in a ratio of 2:5 and applied in triplicate on the agar plates. The mean zone of inhibition in mm \pm standard deviation beyond well diameter (6 mm) produced on a range of clinically pathogenic microorganism. The activity of testing samples was studied against certain Gram positive, Gram negative bacterial and fungal strains.

Antibiotic susceptibility testing

The antibiotic susceptibility tests were performed using the Kirby-Bauer method on Mueller-Hinton agar in accordance with the Clinical and Laboratory Standards Institute guidelines (NCCLS 2002) and using standard antibiotic discs. The antibiotic discs were placed 30 mm apart and 10 mm away from the edge of the plate. Plates were inverted and incubated aerobically at 35 \pm 2°C for 16 to 18 hours. The zone of inhibition and resistance was measured, recorded, and interpreted according to the recommendation of the CLSI (NCCLS 2002) [18,19].

RESULTS:**Pharmacognostic studies**

Following major diagnostic elements were seen clearly:

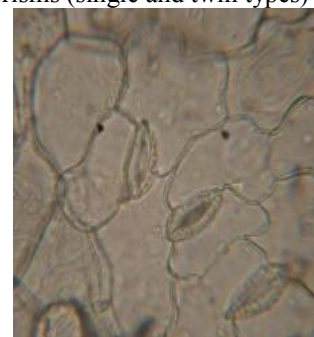
1. Anisocytic stomata
2. Epidermal cells (sinuous walled)
3. Striated cuticle
4. Wood fibres
5. Vessels (lignified, spiral, pitted, reticulate)
6. Pericyclic fibres
7. Non glandular hair (uniseriate, multicellular conical)
8. Glandular hair (branched and non-branched with unicellular globular head)
9. Calcium oxalate prisms (single and twin types)



Glandular branched hair



Twin Prisms



Anisocytic stomata

Fig 2: Major diagnostic elements of *H. muticus* under microscope

Phytochemical screening

The main phyto-constituents detected are presented in Table 1.

Table 1: Phyto-constituents present in different parts of *H. muticus*

Phyto-Constituents	Leaf fraction	Flower fraction	Stem fraction
Alkaloids	Present	Present	Present
Carbohydrates	Present	Present	Present
Tannins	Absent	Absent	Absent
Saponins	Absent	Absent	Absent
Flavonoids	Present	Present	Absent
Triterpenes / sterols	Present	Present	Present
Anthraquinones	Absent	Absent	Absent

Antimicrobial activity

The antimicrobial activity data is presented in Table 2.

Table 2: Antimicrobial activity of the leaf extract against selected microorganisms

Tested microorganisms	Ethanol fraction	Chloroform fraction	Hexane fraction	Ofloxacin	Gentamicin
<i>Candida albicans</i>	18.33±0.288	18.0±0.5	14.10±0.36	25.33±0.29	20.50±0.5
<i>Staphylococcus aureus</i>	18.23±0.25	17.33±0.15	16.17±0.29	25.17±0.29	20.50±0.5
<i>Enterococcus faecalis</i>	18.73±0.25	17.60±0.17	17.07±0.11	29.93±0.11	20.17±0.29
<i>Pseudomonas aeruginosa</i>	18.76±0.25	17.43±0.40	14.60±0.10	29.83±0.29	20.50±0.5
<i>Escherichia coli</i>	19.50±0.5	18.60±0.53	17.00±0.50	29.67±0.58	24.83±0.29
<i>Salmonella typhi</i>	21.33±0.76	20.83±0.29	17.50±0.50	37.10±0.25	20.17±0.29

Size of zone of inhibition is measured in mm. Data is represented as mm ± SD

DISCUSSION:

The results of the pharmacognostic study confirmed the identification of the plant, which were matching with the results of some other researchers of different region. This shows that *Hyoscyamus muticus* plant grown wildy in the Northern Border Region of Saudi Arabia possess same key elements as those grown in other regions, for example, Egypt and India. This suggests that similar phyto-constituents must also be found in the plant grown in this region when compared to other habitat, although quantity may vary [20].

Exploring the phytochemicals in a plant is itself a vast field and a plant is searched for phytochemicals again and again and some researchers find new compounds in the repeated sampling. A little work is reported regarding phytochemistry of *Hyoscyamus muticus*. The initial phytochemical screening of leaf, flower and stem fractions of *Hyoscyamus muticus* ethanol extracts revealed that all fractions contain alkaloids, carbohydrates, and triterpenes / sterols while saponins, anthraquinones and tannins were not found in any fraction. Flavonoids were found in leaf and flower extracts. The alkaloids found in all aerial parts play a significant role in curing various

ailments, including certain pathogenic organisms which justifies its use as a traditional medicine [9].

The antimicrobial investigation revealed that leaves possess significant antibacterial and antifungal activity. These results are concordant with research conducted on *H. muticus* growing in Sudanese habitat by some researchers [21], while mild activity was found in the stem and flower portion which was tested for the first time. The activity was conducted in ethanol, chloroform and hexane fractions of leaf, flower and stem parts. The ethanol extract showed the prominent activity which was followed by the chloroform extract and the hexane extract, respectively.

CONCLUSION:

Hyoscyamus muticus L or (Sakran al Misri) growing in the Northern Border Region of Saudi Arabia possesses same pharmacognostic features as growing elsewhere. The plant possesses a variety of phyto-constituents. Flower and stem extracts showed insignificant antimicrobial activity while the leaf extract exhibited significant antimicrobial activity against all the strains in all the fractions. This may be due to the rich alkaloidal contents in leaves. The

ethanol extract showed the prominent activity which was followed by the chloroform extract and the hexane extract, respectively.

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