Biological study on aerial parts of *Hyoscyamus boveanus* Dun. Family *Solanaceae*

Abstract

Family *Solanaceae* is one of the largest families in the plant kingdom. A family of 98 genera and over 2700 species; tropical and temperate; herbs, shrubs or small trees. Secondary metabolites of *Solanaceae* plants, sharing tropane skeleton as a common structural feature, sharply divided into two classes: tropine and ecgonine derivatives. The first group, represented by well-known alkaloids: atropine and scopolamine, which are considered to be model anticholinergic drugs, continues to provide inspiration in the search for more selective muscarinic receptor antagonists. In this study, the basal alkaloid fraction was isolated from collected wild plant by using authentic atropine (sigma USA). Fifteen rabbits weighing 1700 – 2000 gram were used in this study, divided into three groups (5 each); control (solvent) group, standard (atropine) group and the *Hyoscyamus boveanus Dun* basic alkaloid fraction group. Two cm rabbit intestine muscle was isolated to study anti spasmodic activity of the basic alkaloid fraction of *Hyoscyamus boveanus Dun*. As well as, anti microbial activity of *Hyoscyamus boveanus* basal alkaloid fraction was studied. The results revealed that *Hyoscyamus boveanus* basal alkaloid fraction showed mydriatic, Antimuscarenic and anti microbial activity against gram negative and gram-positive bacteria.

1 - Introduction

Medicinal plants are the most exclusive source of life saving drugs for the majority of the world population. Bioactive compounds currently extracted from plants used as food additives, pigments, dyes, insecticides, cosmetics and perfumes and fine chemicals ***(Balandrin and Klocke, 1988)***. These compounds belong to a group collectively known as secondary metabolites. Studies on plant secondary metabolites have been increasing over the last fifty years, the molecules known to play a major role in the adaptation of plants to their environments, but also represent an important source of pharmaceuticals ***(Ramachandra Rao and Ravishankar, 2002)***.

Family *Solanaceae* is one of the largest families in the plant kingdom. A family of 98 genera and over 2700 species; tropical and temperate; herbs, shrubs or small trees ***(Olmstead and Bohs, 2006).*** The most common genera included in this family are *Nicotiana* (66 spp.), *Lycium* (80-90 spp.), *Withania* (10 spp.), *Hyoscyamus* (20 spp.), *Physalis* (100 spp.), *Capsicum* (50 spp.), *Solanum* including *Lycopersicon* (1700 spp.), *Datura* (10 spp.), *Cestrum* (150 spp.), *Acnistus* (50 spp.), *Fabiana* (25 spp.). ***(Olmstead and Bohs, 2006; Trease and Evans, 1996).***

(Tackholm, 1974) reported that, there are about 33 species out of eight genera from this family indigenous to Egypt, distributed in different localities. Family *Solanaceae* characterized by the presence of wide range of alkaloids as plant secondary metabolites, which are of great taxonomic interest. The types of alkaloid present in different genera show good correlation with their earlier classification made on purely botanical grounds. Types of alkaloids recorded are tropane, alkaloidal amine, indole, isoquinoline, purine, pyrazole, pyridine, pyrrolidine, steroid alkaloids and glycoalkaloids. Other constituent include steroidal saponins, coumarins, pungent principles (e.g. in *Capsicum*), flavones, and carotenoids ***(Trease and Evans, 1996).***

Hyoscyamus is highly diversified genus in the *Solanaceae* family and comprises twelve to fifteen species; *Hyoscyamus* is one of the important plants in this family. Whoever, all plant parts contain tropane alkaloids (hyoscyamine, scopolamine, and atropine) and are toxic to humans and animals when ingested in large amount. Moreover, these alkaloids have many medicinals importance, i.e. action on autonomic nervous system, Ophtholmic properities, action on the central nervous system, Parkinson's disease, action on respiratory system and anti-inflammatory ***(Khater and Elashtokhy, 2015)***.

2- Material and methods

2.1- Plant material:

The plant material used in this work of Hyoscyamus boveanus Dun. Family Solanaceae was collected from Saint Catherine, Egypt. The identification was verified by one of the professor and head of plant taxonomy, faculty of science, Cairo University. It was left in air for drying without direct sun heat. After drying, the plant was grinded then immersed in 70% ethyl alcohol for 4 days. The extract filtered and concentrated at room temperature. The dried extract was stored at $4^{O}C$ until using.

2.2- Materials for HPLC analysis:

The basal alkaloid fraction was isolated from collected wild plant by using authentic atropine (sigma USA).

2.3- Mydriatic activity of *Hyoscyamus boveanus* basal alkaloid fraction:

Fifteen rabbits weighing 1700 – 2000 gram were used in this study held under standard laboratory conditions in the animal house of faculty of pharmacy Zagazig University at 27^{o}C with 12/12 light dark cycle. They were fed laboratory diet and water ad libitum. .

The rabbits were divided into three groups (5 each); control (solvent) group, standard (atropine) group and the *Hyoscyamus boveanus Dun* basic alkaloid fraction group. Atropine (1% solution) was prepared in hydroxypropyl methycellulose 0.5 % vehicle. *Hyoscyamus boveanus Dun* basic alkaloid fraction (1% solution) was prepared in propylene glycol 2% vehicle. The third group that received propylene glycol was used as control.

The initial eye pupil diameter of the rabbits was determined by using micrometer according to the method of **(Colasanti and Barany, 1979)**. Three drops of either the standard, control or the fraction were applied in the right rabbit's eye. The eye pupil diameter of the rabbit was measured after 5, 15, 35 and 60 min. interval and percentage increment in the eye pupil diameter, were redetermined. Paired student t–test was used to compare the eye pupil diameter before and after test substance application. Unpaired student t–test was used to compare between the mydiratic effects of both standard and basic fraction at ($P < 0.05$).

2.4- Antimuscarenic activity of *Hyoscyamus boveanus* basal alkaloid fraction:

Two cm rabbit intestine muscle was isolated and suspended in organ bath. The preparation was supplied by Tyrode's solutions and oxygen. The temperature of the Tyrode's solutions was kept at 37^{o} C. The anti spasmodic activity of the basic alkaloid fraction of *Hyoscyamus boveanus Dun*, Was studied using the isolated rabbit intestine method ***(Nageib and El – Fayomi, 2002; Tytgat and Guido, 2007).*** A suitable dose response care (DRC) of acetylcholine 0.001% solution (as agonist) was performed and the sub maximal dose was selected.

An equal volume of *Hyoscyamus boveanus Dun*. Basic fraction (0.0003% solution) was injected and the inhibition in the muscle response is examined, compared with the sub maximal dose of acetylcholine (Ach). Barium chloride solution (3%) was injected as direct smooth muscle stimulant. In the same experiment, indirect assay was performed using matching technique.

The test was carried out by comparing the dose of *Hyoscyamus boveanus Dun.* Basic fraction (0.0003 % solution) which causes 50% reduction in the contraction produced by acetylcholine 0.001% solution with that of the standard atropine (0.0003 % solution) which causes the same response (50% reduction).

2.5- Antimicrobial activity of *Hyoscyamus boveanus* basal alkaloidal fraction:

Microorganisms used: *staphylococcus aureus (RCMB010016), sarcina lutea (RCMB 010032) and bacillus subtilis (RCMB 010068)* as gram-positive *cocci*. *Pseudomonas aeruginosa (RCMB 010048) and Escherichia coli (RCMB 010052)* as gram negative *bacilli* and *candida albicans (RCMB 015039) as fungus were used*. bacteria were grown and maintained on neutrant agar medium (Sheet blood agar media composed of casein enzymic hydrolysate 14 g/L, peptic digest of animal tissue 4.5 g/L, yeast extract 4.5 g/L, sodium chloride 5 g/L, agar 12.5 g/L and sheep blood 5 g/L at PH 7.3 ± 0.2, at 25 ^{o} C). While, saburaud 's dextrose agar (composed of peptones (meat and casein) 10 g/L, dextrose monohydrate 40 g/L and agar 15 g/L. at PH 5.6 ± 0.2 , at 25 ^{o} C) medium was used for *candida albicans*. ***(Alkofahi et al, 1997)***

Screening of the potential antimicrobial activity of crude alcoholic extract of the *Hyoscyamus boveanus* (sample 1) and crude basic alkaloid fraction of *Hyoscyamus boveanus* (sample 2) against several microorganisms as gram negative, gram positive bacterial stains and fungal stains in vitro. Penicillin and Nystatin were used as positive control for antibiotic and antifungal drugs, respectively.

In vitro antibiotic activity was assayed using (cup–plate method) according to modified method of ***(Jorgensen and Ferraro., 2009 and CLSI, 2012).*** Petri dishes were prepared with 25 ml agar-solidified media and inoculated with 1ml of diluted culture (made by serial dilution). Excess inoculum was removed and the plates were dried for 30 min at 37 ^{o} C. cups of 10 mm in diameter were made by the means of cork porer in the inoculated agar

and filled with about 20 µl of dimethyl-formamide (DMF) solutions of the different products at 2 mg/ml. A cup was filled with 20 µl DMF, used as negative control and another cup was filled with either Penicillin or Nystatin as positive control. The inhibitions zones around the cups were recorded after 24 hours of incubation at 37o C.

3- Results and discussion:

3.1- Mydriatic activity:

As shown in **Table (1)** and illustrated in **Fig.(1)** and **Fig.(2)**, topical application of atropine solution 1% induced a significant increments in the eye pupil diameter after 5,15,35 and 60 min interval. As shown in **table (2)** and illustrated in **fig.(3)** there was no significant difference between the increments in the eye pupil diameter induced by atropine solution 1% or basic fraction of *Hyoscyamus boveanus Dun*.1% solution. The control has no significant difference in the eye pupil diameter before and after drug application. Thus, from the previous, it was clear that the *Hyoscyamus boveanus Dun* basic alkaloid fractions produced dilation of the eye pupil, showing equal potency with that of atropine used as standard.

These results agree with ***(El Shazly et al, 1997***) who reported that atropine, apoatropin and scopolamine as a majr alkaloidal components in *Hyoscyamus boveanus Dun*. These alkaloids components were reported as a muscarinic antagonist used to treat nausea, vomiting, and motion sickness, whereas atropine is a similar anticholinergic agent, and is used in the treatment of certain poisonings and heart conditions, and to dilate the pupils in ophthalmology. These drugs are listed on the World Health Organization's Model List of, Essential Medicines ***(WHO, 2019)*** .

Groups	**Diameter of the rabbit eye pupil (mm)**				
	Before application	After 5 min	After 15 min	After 35 min	After 60 min
Atropine (standard group)	6.12±0.38	$8.2^{*} \pm 0.43$	$8.37^{*} \pm 0.45$	$8.64^{*} \pm 0.78$	$8.23^{*} \pm 0.48$

Basic alkaloid fraction group	6.17±0.5	7.77*±0.37	8.7*±0.39	8.99*±0.55	8.6*±0.55

Table (1): diameter of the rabbit eye pupil (mm) before and after application of three drops of either the standard (1%) or the basic fraction (1%) solution in the right rabbit eye pupil.

Data is expressed as mean ± SE.
* significantly different from the corresponding initial diameter of the rabbit eye pupil at ((p≤0.05).

Table (2): increment percentage in the rabbit eye pupil diameter (mm) before and after application of three drops of either the standard (1%) or basic alkaloid fraction (1%) solution in the right rabbit eye pupil.

Groups	**Increment percentage in the rabbit eye pupil diameter (mm)**			
	After 5 min	After 15 min	After 35 min	After 60 min
Atropine (standard group)	35.4± 8.6	38.5± 9.04	42.36 ± 11.5	35.9 ± 9.3
Basic alkaloid fraction group	27.8±6.8	43.6±7.6	47.4±7.7	39.2±4.7

Data is expressed as mean ± SE.

Fig. (1): diameter of the rabbit eye pupil (mm) before and after application of three drops of atropine, in the right eye pupil.

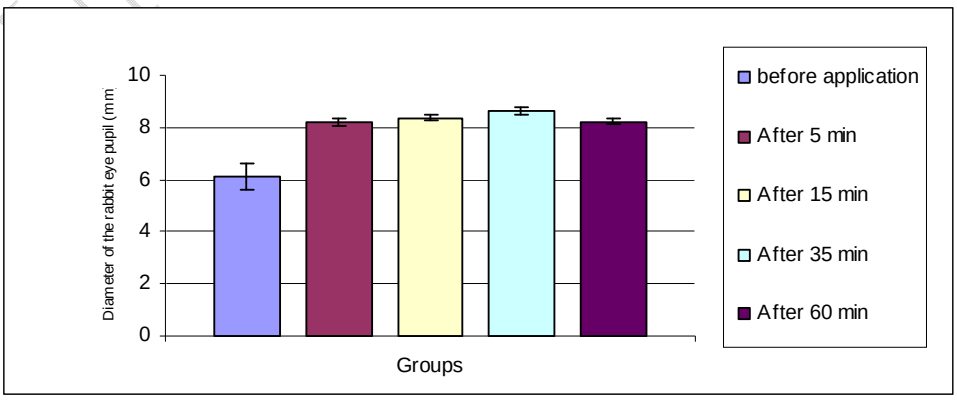


Fig. (2): diameter of the rabbit eye pupil (mm) before and after application of three drops of basic alkaloid fraction (1%), in the right eye pupil.

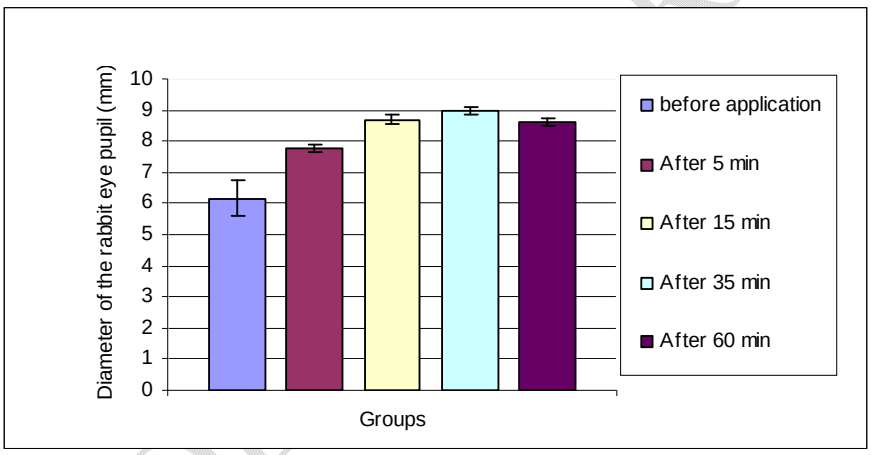
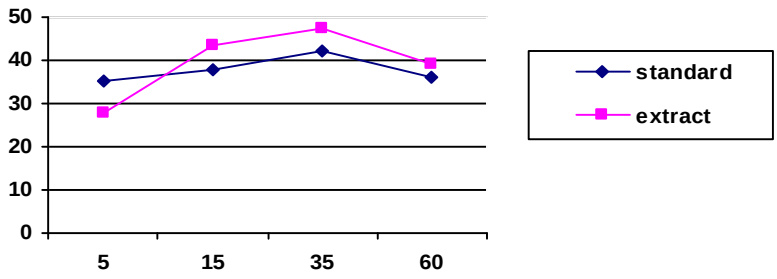


Fig. (3): increment percentage in the rabbit eye pupil diameter (mm) after application of three drops of either the standard (1%) or the basic alkaloid fraction (1%) solution in the right rabbit eye pupil.

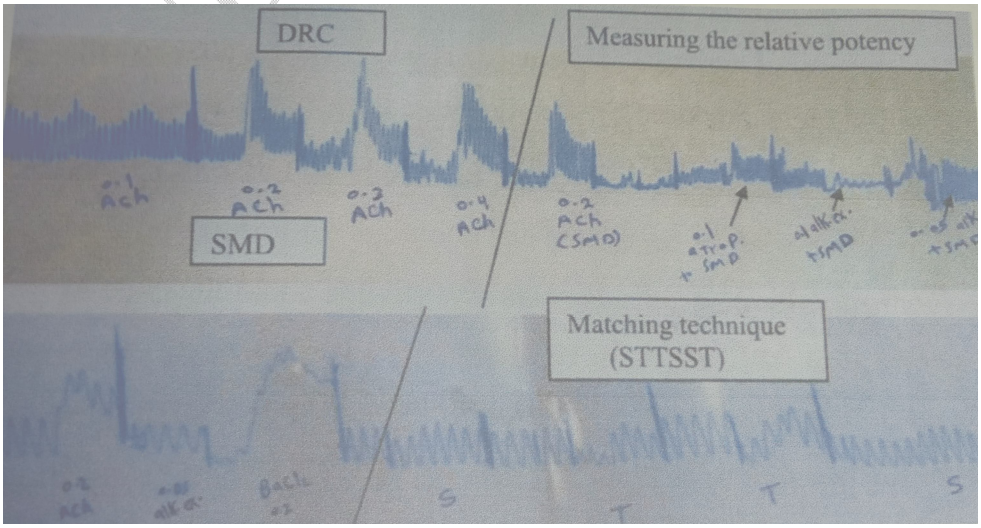


3.2- Anti spasmodic activity (anti muscarenic activity):

As shown in **fig. (4)**, the injection of 0.2 ml acetylcholine 0.001 % solution in the Tyrode 's solution showed an elevation in the muscle response, addition of 0.05 ml *of Hyoscyamus boveanus Dun* basic fraction (0.0003 % solution) resulted in a significant reduction in the elevation in the muscle response caused by 0.2 ml acetylcholine 0.001% solution. Thus, the extract either blocks the muscarinic receptors or directly inhibit the intestine muscle contraction. When barium chloride solution (3%) was added to the Tyrode's solution after the basic fraction addition, an elevation in the response was shown. So, it was suggested that *Hyoscyamus boveanus Dun* basic alkaloid fraction has anti muscarinic activity. As shown in **fig.(4)**, 0.1 ml of the standard atropine (0.0003% solution) caused 50% reduction of the contraction produced by 0.2 ml acetylcholine 0.001% solution (used as agonist) and 0.05 ml of of *Hyoscyamus boveanus* basic alkaloid fraction caused the same response (50% reduction) produced by the standard atropine (0.0003% solution). These results suggested that, *Hyoscyamus boveanus* basic alkaloid fraction was twice as potent as the standard atropine (0.0003% solution) as anti-muscarinic.

Relative potency = atropine potency/alkaloid potency = alkaloid dose/atropine dose =0.05/0.1
Atropine : alkaloidal extract 1:2

Fig.(4): screening and assay of the antimuscarinic activity of the basic alkaloid fraction of *Hyoscyamus boveanus* **Dun.**



3.3- Anti microbial activity:

None of the tested samples showed activity against *candida albicans* Only sample No one could inhibit *sarcina lutea* in the preliminary plate assays. The activity of the other samples against the studied microorganisms was summarized in **Table (3)**. The results showed that the two tested samples were effective against gram positive organisms (*Staphylococcus oureus* and *Bacillus subtilis*), and gram negative organisms (*Pseudomonas aeruginosa* and *Escherichia coli*). The highest activity (measured in terms of zone of inhibition diameters) which was demonstrated by crude alcoholic extract of the plant against *Pseudomonas aeruginosa* (= 22) and *Escherichia coli* (=17) more than that caused by penicillin as illustrated in **Fig.(5)** .

These results agree with that reported by ***(Kadi, et al 2013 ;and Dulger and Dulger, 2015)*** as they documented that alkaloid extracts of *Hyoscyamus albus* showed antibacterial activity against *Pseudomonas stutzeri, Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumonia*. The methanol extracts of the seeds of *Hyoscyamus niger* were investigated for antimicrobial effect against urinary tract pathogens (*Enterococcus faecalis*, *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis* and *Candida albicans*). The extracts showed strong antimicrobial activity against *Enterococcus faecalis, Klebsiella pneumoniae* and *Candida albicans* with inhibition zones of 26.0, 19.0 and 16.0 mm, and moderate activity against the other test microorganisms.

Tested organism	**Zone of inhibition (diameter per mm)**			
	1	**2**	**penicillin**	**Nystatin**

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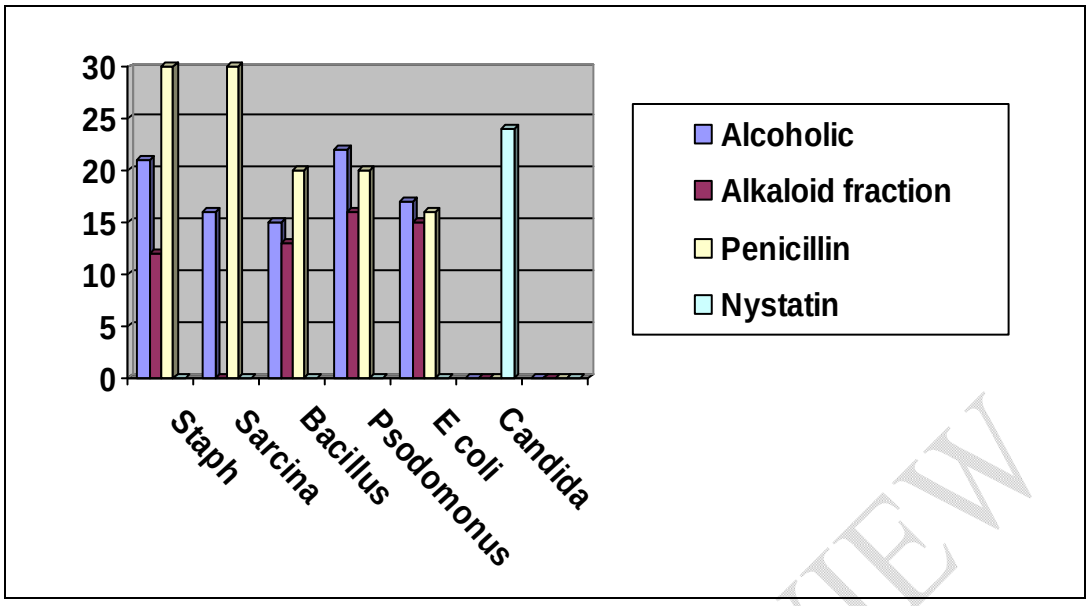
Table (3): Inhibition of microbial growth by different samples from Hyoscyamus boveanus Dun.

Staphylococcus aureus	21	12	30	-
Sarcina lutea	16	-	30	-
Bacillus subtilis	15	13	20	-
Pseudomonas aeruginosa	22	16	20	-
Escherichia coli	17	15	16	-
Candida albicans	-	-	-	24

1- Crude alcoholic extract of the plant.
2- Crude basic alkaloid fraction of the plant.

Fig.(5): Antimicrobial activity by different samples from *Hyoscyamus boveanus* Dun. Family *Solanaceae* against tested microorganisms.



Each bar expressed an average of three measurements.

4- Conclusion:

The basal alkaloid fraction of *Hyoscyamus boveanus* Dun. Showed a clearly anti mydriatic activity and anti spasmodic activity (anti muscarenic activity) as well as the whole alcoholic extract and the basal alkaloid fraction of *Hyoscyamus boveanus* showed a clearly antimicrobial activity against various gram negative and gram positive bacteria.

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