Asian Journal of Biology

16(1): 41-48, 2022; Article no.AJOB.92963
ISSN: 2456-7124

Biological Study on Aerial Parts of *Hyoscyamus boveanus* (Dunal) Asch. & Schweinf

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJOB/2022/v16i1295

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/92963

Original Research Article

Received 10 August 2022
Accepted 15 October 2022
Published 19 October 2022

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. This work, aim to study the biological effect of *Hyoscyamus boveanus* alkaloid fraction as Antimuscarenic, mydriatic and anti microbial agent. Basal alkaloid fraction was isolated from collected wild plant by using authentic atropine (sigma USA). Fifteen rabbits weighing 1700 – 2000 gram used in this study, divided into three groups (5 each); control (solvent) group, standard (atropine) group and the *Hyoscyamus boveanus* Dunal basic alkaloid fraction group. Two cm rabbit intestine muscle was isolated to study anti spasmodic activity of the basic alkaloid fraction of *Hyoscyamus boveanus* Dunal. 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. In conclusion, *Hyoscyamus boveanus* Dunal basic alkaloid fraction, showed mydriatic, Antimuscarenic and anti microbial activity against gram negative and gram-positive bacteria.

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Keywords: Antimuscarenic; mydriatic; anti microbial activity; Hyoscyamus boveanus Dunal; biological activity.

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 [1]. 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 [2].

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 [3]. 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 [4] [5] 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 [4].

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 [6]. The genus Hyoscyamus is a rich source of tropane alkaloids which show pharmacological activities such as antispasmodic, mydriatic, anticholinergic and antiemetic effect [7]. This work, aim to study the biological effect of *Hyoscyamus boveanus* alkaloid fraction as Antimuscarenic, mydriatic and anti microbial agent

2. MATERIAL AND METHODS

2.1 Plant Material

The plant material used in this work of *Hyoscyamus boveanus* Dunal. Family Solanaceae was collected from Saint Catherine, Egypt. The identification was verified by prof Dr. Wafaa Amer 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 4OC 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 27oC 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* Dunal basic alkaloid fraction group. Atropine (1% solution) was prepared in hydroxypropyl methycellulose 0.5 % vehicle. *Hyoscyamus boveanus* Dunal 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 [8]. 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 37o C. The anti spasmodic activity of the basic alkaloid fraction of *Hyoscyamus boveanus* Dunal, Was studied using the isolated rabbit intestine method [9,10]. 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* Dunal. 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* Dunal. 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

Staphylococcus aureus (RCMB010016), sarcina lutea (RCMB 010032) and bacillus subtilis (RCMB 010068) used as gram-positive cocci. Pseudomonas aeruginosa (RCMB 010048) and Escherichia coli (RCMB 010052) used as gram-negative bacilli and candida albicans (RCMB 015039) used as fungus. 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 [11].

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 [12,13]. 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), The control has no significant difference in the eye pupil diameter before and after solvent application. While, application of atropine solution 1% and basic alkaloid fractions induced a significant increments in the eye pupil diameter after 5,15,35 and 60 min interval when compared with control group. 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* Dunal.1% solution. Thus, from the previous, it was clear that the *Hyoscyamus boveanus* Dunal basic alkaloid fractions produced dilation of the eye pupil, showing equal potency with that of atropine used as standard.

These results agree with [14] who reported that atropine, apoatropin and scopolamine as a major alkaloidal components in *Hyoscyamus boveanus* Dunal. 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. As well as, these drugs are listed on the World Health Organization's Model List of, Essential Medicines [15].

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

Groups	Diameter of the rabbit eye pupil (mm)				
	Before application	After 5 min	After 15 min	After 35 min	After 60 min
solvent (control group)	6.14±0.41	6.08±0.29	6.20±0.47	6.19±0.32	6.13±0.31
Atropine (standard group)	6.12±0.38	8.2* ± 0.43	8.37* ± 0.45	8.64* ± 0.78	8.23* ± 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

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.

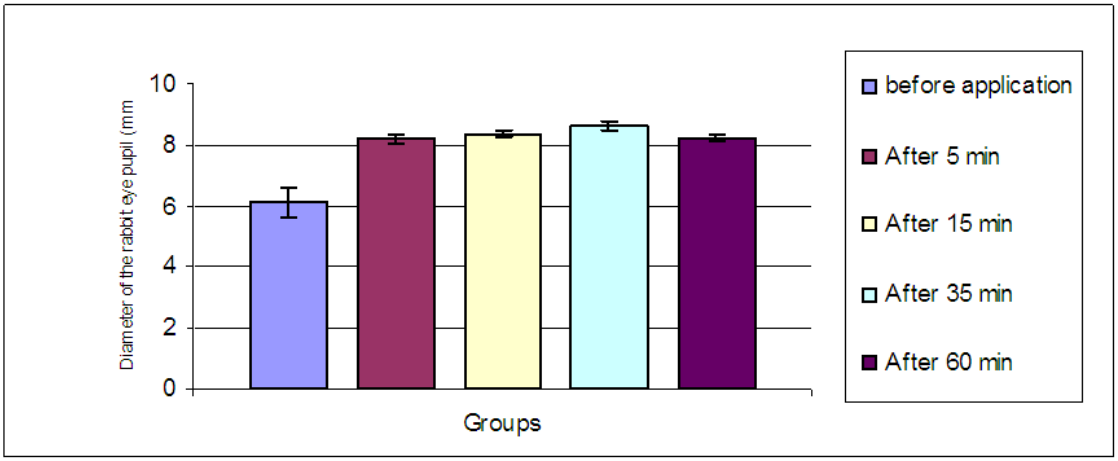

Fig. 1. Diameter of the rabbit eye pupil (mm) before and after application of three drops of atropine, in the right eye pupil

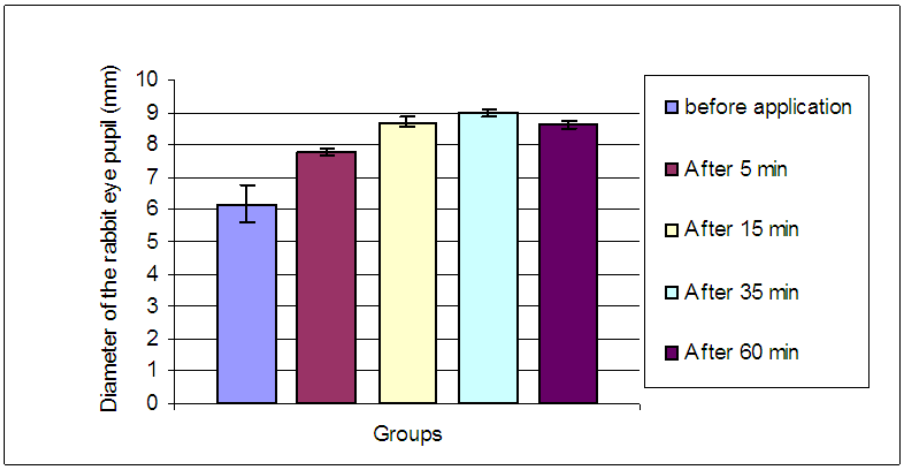

Fig. 2. Diameter of the rabbit eye pupil (mm) before and after application of three drops of basic alkaloid fraction (1%), in the right eye pupil

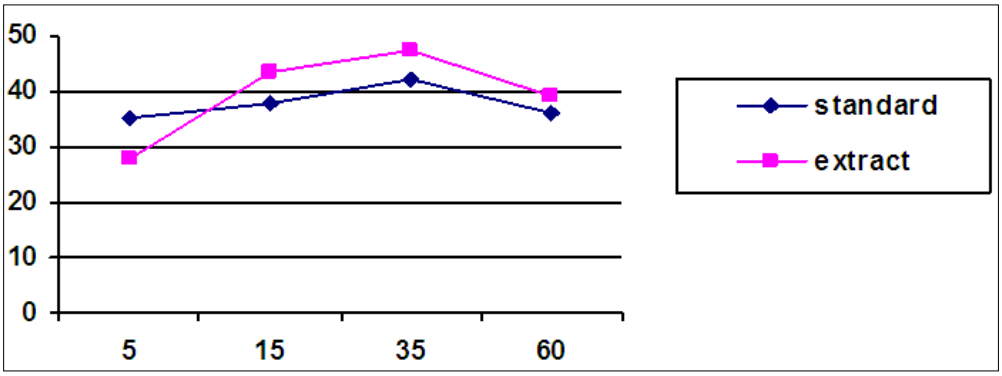

Fig. 3. Increment percentage in the rabbit eye pupil diameter (mm) after application of three drops of either the standard (1%) or the basic alkaloid fraction (1%) solution in the right rabbit eye pupil

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* Dunal 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* Dunal 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

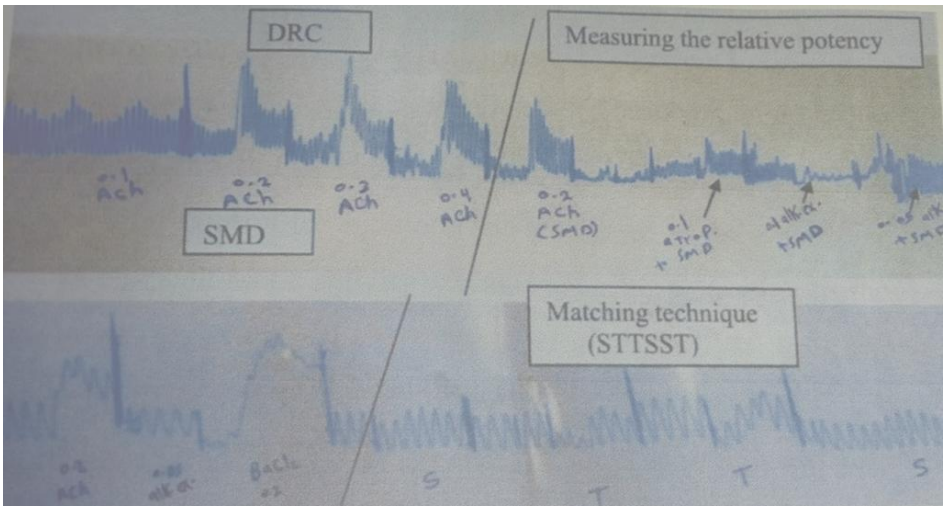

Fig. 4. Screening and assay of the antimuscarinic activity of the basic alkaloid fraction of *Hyoscyamus boveanus* Dunal

3.3 Anti Microbial Activity

None of the tested samples showed activity against candida albicans.

Only Crude alcoholic extract (sample No 1) 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 [16,17] 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.

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Table 3. Inhibition of microbial growth by different samples from *Hyoscyamus boveanus* Dunal.

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

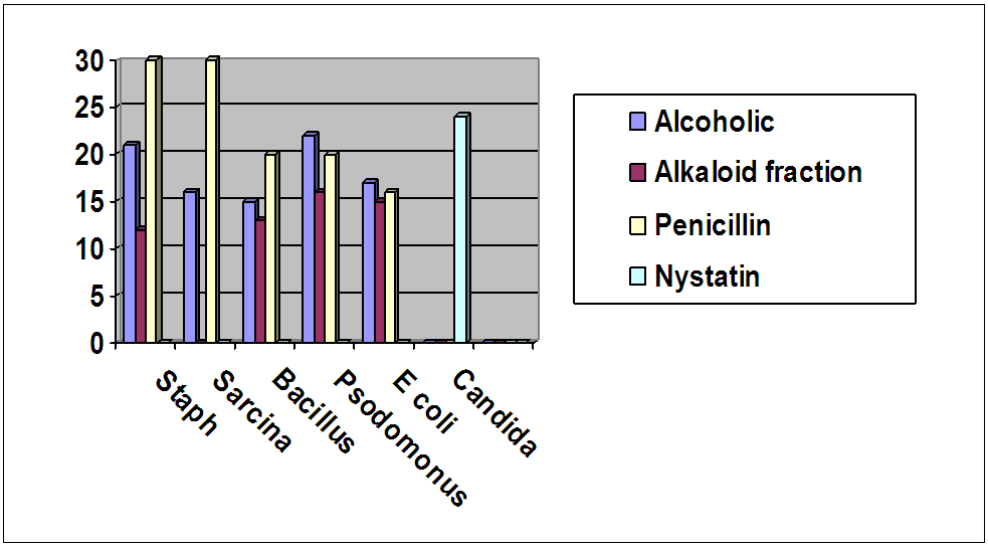

Fig. 5. Antimicrobial activity by different samples from *Hyoscyamus boveanus* Dunal. Family Solanaceae against tested microorganisms

Each bar expressed an average of three measurements

4. CONCLUSION

The basal alkaloid fraction of *Hyoscyamus boveanus* Dunal. 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. So, this work suggest the usage of basal alkaloid fraction of *Hyoscyamus boveanus* as anti muscarenic, anti mydriatic as well as, anti microbial agent.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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