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BIOLOGICAL STUDIES OF INDIGENOUS MEDICINAL PLANTS-I: PHYSIOCHEMICAL AND ANTIMICROBIAL SCREENING OF NON-ALKALOIDAL CONSTITUENTS OF SOME SOLANACEOUS SEEDS

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ABSTRACT

The seeds of *Atropa belladonna*, *Datura fastuosa*, *D. stramonium* and *Hyoscyamus niger* were investigated for the isolation and anti-microbial activity of non-alkaloidal constituents. The compounds were separated by column chromatography and spectral studies i.e. IR and UV were performed to ascertain their chemical mature. The anti-microbial activity of crude extracts and purified compounds was tested against Gram positive and Gram negative bacteria and a fungus. All the micro-organisms tested were markedly inhibited, however, the crude extracts as well as the purified compounds isolated from the seeds of *A. belladonna* and *D. fastuosa* showed little inhibitory activity.

Introduction

The present investigation is a part of research work being carried out on the physicochemical, biopharmaceutical and biological activities of indigenous medicinal herbs (Alam *et al*, 1986; Ahmed *et al*, 1988). The plants belonging to family solanaceae are rich in tropane alkaloids, which are present in most parts, particularly in leaves and flowering tops. Little work on the non-alkaloidal constituents of these plants has been reported. Giebel (1970) detected monophenols in the root extract of *Hyoscyamus niger*. The seeds were reported to contain 14.85-22.13% of oil belonging to the oleic and linoleic groups (Aslanov, 1978). Myoung and Minsuk (1978) analysed the sterol composition in solanaccous seed oil and identified cholesterol, cholest-7-enol, compesterol and fucosterol as constituents. In this communication an attempt has been made to isolate and purify non- alkaloidal components from the seeds of *Atropa belladonna*, *Datura fastuora D.*, *stramonium* and *Hyoscyamus niger* and to examine their antimicrobial activities.

Materials and Methods

Unless otherwise stated all the chemicals used were of analytical grade. The seeds of d[i]fferent plants used in this study were obtained from Pakistan Forest Institute, Peshawar.

The pure cultures of *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 8739 and *Staphylococcus aureus* ATCC 6536, were obtained from Schazoo

Laboratories, Lahore, while those of *Aspergillus niger* and *Pseudomonas aeruginosa* were obtained from our local culture collection at the Department of Pharmacy, University of the Punjab. Lahore. All the bacterial strains were maintained on nutrient agar medium at 37°C. *A. niger* was maintained on Sabouraud's dextrose agar medium at 25°C.

Isolation and purification of non-alkaloidal constituents

(A) Removal of Alkaloids:

The seeds (300 g) of *H. niger* were crushed to coarse powder and then soaked in 5% v/v H2SO4 (one liter) for 3 days. The suspended material was stirred occasionally. The suspended solid was collected on Buchner hazel, washed with water (4x350 ml), then washed with 5% w/v Na_2CO_3 solution (2650 ml) and finally washed with water (5x350 ml). The alkaloidal free residue was dried at 20-30°C.

The alkaloidal free residues from other seeds were obtained by using the method described above.

(B) Separation of non-alkaloidal components:

The dried non-alkaloidal residues obtained were separately extracted with methanol in soxhlet extractor. The methanolic extracts were evaporated under reduced pressure.

The crude extracts obtained from the seeds of D. stramonium and *H. niger* were applied on thin layer kieselgel/cellulose (1:1) plates and developed in pure chloroform. Whereas for the crude extracts of A. belladonna and *D. fasuosa*, pure benzene and benzene: methanol (4:1, v/v) were used as solvent systems respectively. The plates were dried and spots were detected with iodine vapours. The R_f values of various compounds were determined.

Column chromatography

The alkaloidal free crude residues were separated on silica gel column and eluted with benzene. The separation of fractions was monitored by TLC. The fractions having the same R_f values were combined. The UV spectra of the purified compounds were obtained in ethanol on Pye-Unicam SP 8-400 spectrophotometer and the IR spectra were obtained on Hitachi 270-30 spectrophotometer.

Anti-microbial activity

The suspensions of microorganisms were prepared by suspending a loopful of pure culture in 10 ml of sterile distilled water. One ml of bacterial suspensions were separately mixed withy 14 ml of sterile molten NA. medium in different sterile petri dishes (already labelled with bacterial name/ compound under study) and 1D ml of fungus suspension was mixed with 14 ml of sterile Sabouraud's dextrose agar medium. The media of petri dishes were divided into four equal parts after solidification and uniform holes were made in the center of each part with a cork borer Not. Two drops of crude extracts and purified compounds were placed into the respective holes and incubated at 37°C for 24 hours for bacteria and at 27°C for 72 hours for fungus. The zones of inhibition were measured by vernier calipers.

Results and Discussion

It is apparent from the data given in Table 1 that the maximum yields of non-alkaloidal constituents in methanol extract were obtained from the seeds of *A. belladonna* and *H. niger* and minimum from *D. fastuosa*.

Table 1

Yields of non-alkaloidal constituents in methanol extract

Species	wt. of seed powder (g)	wt. of alkaloidal free powder (g)	wt. of crude residue (g)	yield of crude residue (%)
Atropa belladonna	300	252	27	10.7
Datura fastuosa	300	271	18	6.3
D. stramonium	300	268	22	8.2
Hyoscyamus niger	300	260	28	10.8

The components of non-alkaloidal crude extracts were separated on TLC plates. The R_f values and colour reaction with iodine vapours along with other physical characteristics are given in Table 2.

When residues of methanol extracts were subjected to column chromatography, two major compounds from *A. belladonna* (AB-1, AB- 2) and *H. niger* (RN-1, HN-2) and one each from *D. fastuosa* (DF-1) and *D. stramonium* (DS-1) were isolated. The purification of these compounds was further confirmed by TLC.

Table 2

Physicochemical characteristics and R_f values of compounds isolated from Solanaceous seeds.

Characteristics	Compounds					
	AB-1	AB-2	DF-1	DS-1	HN-1	HN-2
Colour	Pale yellow	Pale yellow	Light yellow	Light brown	Yellowish	Yellowish
Physical state	Oily viscous liquid	Oily viscous liquid	Oily liquid	Oily liquid	Oily liquid	Oily liquid
Refractive index (25°C)	1.42	0.83	1.72	1.58	1.12	0.96
Fluorescence under UV light	Nil	Nil	Nil	Nil	Nil	Nil
Colour reaction with iodine varpours	Dark yellow	Brownish yellow	Yellow	Dark brown	Yellow	Yellow
R_f value (solvent system)						
(a) Chloroform	-	-	-	0.77	0.81	0.40
(b) Benzene	0.67	0.36	-	-	-	-
(c) Benzene: Methanol (4:1)	-	-	0.57	-	-	-

The general appearance of the IR spectrum of compound AB-1 suggests that it is an aliphatic or alicydic ester. The strong band at 1720 cm^{-1} could be attributed to a saturated aliphatic ester. The characteristic UV spectrum exhibits absorption maximum at 236 nm (log ε 4.61). The IR band of AB-2 at 1730 cm^{-1} suggests the presence of an aldehyde group. There is an absorption at 1580 cm^{-1} due to C=C stretching. The characteristic UV absorption maxima appear at 306 (log e 3.72) and 327 nm (log ε 2.93).

The compound DS-1 appears to be an aliphatic ester because it showed absorption at 2940 (CH2) and 1740 cm^{-1} (C = 0). The compound RN-1 also exhibits similar features and showed absorption at 1730 cm^{-1} due to C=O and two peaks at 2940 and 1470 cm^{-1} for CH2 groups. The characteristic UV spectra of both compounds exhibit absorption maxima at 304 (log ε 4.52) and 291 um (log ε 3.68), respectively.

The compound DF-1 showed absorption in IR spectrum at 1745 cm^{-1} which indicates the presence of an ester C=0 group. There are absorption bands at 1590 and 1610 cm^{-1} which suggest the presence of aromatic ring. The characteristic UV absorption maximum appears at 296 nm (log ε 3.82).

The C = O band in the IR spectrum of compound HN-2 exhibited absorption at 1717 cm^{-1} (broad) together with the CH stretching band at 2940 cm^{-1} and weak C=C band at 1590 cm^{-1} which indicates the presence of an ester group. The UV absorption peak appears at 300 am (log ε 3.28).

The Bit and UV spectral characteristics of the isolated compounds give some indication of their chemical nature, however, complete identification of these compounds remains to be achieved.

The anti-microbial screening of the non-alkaloidal extracts of *D. stranonium* and *H. niger* and the purified major components, DS-1, HN-2, revealed a marked antibacterial and antifungal activity (Table 3). The effect proved to be potent against *A. niger, B. subtilis E. col*i, *P. aeruginosa* and *S. mucus*. The non-alkaloidal extract of *A. belladonna* and its purified component, AB-2, showed inhibitory activity against *B. subtilis E. coli* and *S. aureus*. However, component AB-1 was inactive against either microorganism. The non-alkaloidal extract of *D. fastuosa* and its purified major component, DF-1, were active only against *B. subtilis* (Table 3).

Table 3

Zones of inhibition (mm) observed for crude extracts and purified compounds

Micro-organisms	*A. belladonna*			*D. fastuosa*		*D. stramonium*		*H. niger*		
	N.A.E.*	AB-1	AB-2	N.A.E.*	DF-1	N.A.E.*	DS-1	N.A.E.*	HN-1	HN-2
Aspergillus niger	-		-	35.2	-	28.5	21.1	30.0	30.4	28.5
Bacillus subtilis	9.0	-	8.6	13.0	25.1	14.1	17.6	24.0	31.9	26.0
Escherichia coli	7.3	-	7.1		-	10.3	22.0	23.8	30.8	28.8
Pseudomonas aeruginosa	-	-	-		-	6.1	10.2	25.0	31.8	27.0
Staphylococcus aureus	9.0	-	7.4		-	21.7	23.3	27.0	34.0	32.0

*N.A.E. -- Non-alkaloidal crude extract.

Hyoscyamus oil has been employed as an ingredient of skin ointment used for the treatment of scars and tissue proliferations (Pfirrmann and Wicki, 1981). The antibacterial and antifungal activities of the non-alkaloidal methanol extracts of D. ammonium and *H. niger* and to some extent that of *A.* belladonna may account for their use in traditional medicine.

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