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ANABOLIC AND ANDROGENIC EFFECTS OF CERTAIN *ATRIPLEX* SPECIES GROWN IN EGYPT

BY

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ABSTRACT

20-Hydroxyecdysone (20-HE) was detected by TLC in the ethyl acetate fraction of two Atriplex species grown in Egypt: A. lindleyi subsp. inflata and A. leucoclada. EIA quantification of 20-HE proved its presence in a concentration of 9.15 and 7.3 µg/g dried aerial parts in each of the two species, respectively. Significant anabolic and androgenic activities were demonstrated for 20-HE comparing to testosterone; the study also revealed that the total alcohol extract and the ethyl acetate fraction of A. lindleyi subsp. inflata exhibited greater activities than their analogues in A. leucoclada. Column chromatographic fractionation of the ethyl acetate fraction of the most active species A. lindleyi subsp. inflata resulted in the isolation of 20-HE (I) in addition to three flavonol glycosides: Isorhamnetin 3-O- galactoside (II), Isoquercitin (III) and Rutin (IV) identified via spectral analysis. DNA were amplified using seven decamer primers as a contribution to the characterization and discrimination between the two Atriplex species indicating only one polymorphic band between the two species and displaying a similarity coefficient of 88.88%.

INTRODUCTION

20-hydroxyecdysone (20-HE) has been known by its effectiveness as anabolic and is marketed as an ingredient in nutritional supplements for various sports, particularly body building (Bathori *et al*., 2008). It is a phytoecdysteroid produced by various plants including *Atriplex* species (Dinan, 1995, Dinan *et al*., 1998 and Keckeis *et al*., 2000). TLC screening revealed the presence of 20-HE in *A. lindleyi* subsp. *inflata* and *A. leucoclada* grown in Egypt. Previous reports indicated also its isolation from *A. lindleyi* subsp. *inflata* (Ateya *et al*., 2005). This study aimed to evaluate - for the first time - the anabolic and androgenic potentialities of *Atriplex* species under investigation in correlation to their content of 20-HE. Moreover, RAPD (Random Amplified Polymorphic DNA) technique was performed for genotyping characterization and discrimination between the two plant species.

EXPERIMENTAL

I-Plant Material

Samples of *Atriplex lindleyi* (Moq.) subsp. *inflata* (F. Muell) P.G. Wilson were collected from El-Salhya road, Egypt (May-2007) while samples of *Atriplex leucoclada* (Boiss) were collected from km 69 Cairo-Alexandria desert road, Egypt (July-2007), both in flowering and fruiting stage. Identification of *A. lindleyi* was carried out by Prof. Mohamed Tantawy, Professor of Taxonomy, Faculty of Science, Ain Shams University and verified by Prof. Lotfy Boulos, Professor of Taxonomy, Faculty of Science, Alexandria University; while *A. leucoclada* was identified by Dr. Mohamed El-Gibaly, ex-curator of National Research Center Herbarium, Dokki, Giza. A voucher specimen has been deposited in the Herbarium, Faculty of Pharmacy, Misr International University.

Plant extract

Aerial parts were collected, air-dried and reduced to fine powder. A sample (5kg) was exhaustively extracted, by maceration in 70% ethanol. The residue left after evaporation of the solvent was successively fractionated with *n*-hexane, chloroform, ethyl acetate and *n*-butanol (6 x 300ml, each).

II- Material and Methods for Enzyme Immune Assay (EIA) of 20-hydroxy ecdysone

Preparation of sample

A sample of the air dried aerial parts (1g) of each species of *Atriplex* under investigation was separately extracted twice with 10 ml methanol (HPLC grade; 5 minutes, each). The methanolic extracts were centrifuged and evaporated. EIA sample buffer (1ml) was added on each residue and 1/1000 dilution was then prepared using the sample buffer. The prepared samples were stored at -20^{o}C (Porcheron, *et al.* 1989 and Blais, *et al.* 2010).

Apparatus

Microtitration plates (96 wells, flat bottom), microplate washer (S8/12), plate agitator, multipipettes, 8-channeled micropipettes with propylene tips and a spectrophotometer (Multiskan Plus MK II) were used in the determination.

Buffer solutions

Washing buffer: 0.01M phosphate buffer prepared by adding 10ml of 1M phosphate buffer stock solution to 500µl of tween 20 and distilled water to complete 1 liter; **sample buffer**: 185mg EDTA (10^{-3}M) and 0.5g bovine serum albumin were added to a mixture of 50ml 1M phosphate buffer and 11.7g NaCl, the volume was completed to 500ml with distilled water and stored at 4^{o}C; **coloration buffer** (0.1M phosphate-citrate buffer): 14.7g of sodium citrate and 7.8g sodium phosphate were dissolved in 1L distilled water and the pH was adjusted to 5 by sufficient amount of citric acid.

Reagents

The enzymatic activity was measured using ortho-phenylene-diamine (Sigma Co. Ltd) as **substrate**; an **enzymatic tracer** 2-succinyl-20-hydroxyecdysone coupled to peroxidase was homemade; **coloration reagent** was prepared by adding 12.5 ml phosphate-citrate buffer to 1 tablet of orthophenylene diamine (OPD, Sigma P3804:100), followed by 3 µl of 30% H_2O_2.

Determination of 20-hydroxyecdysone (20-HE)

Measurement of 20-hydroxyecdysone depends on binding the enzyme tracer (peroxidase + 20-HE) to 20-HE rabbit antibody (100% coloration $\mathbf{B_0}$), then the sample or standard 20-HE replace the tracer on its binding site (**B**). Difference in absorbance of the yellow color produced was measured at 450 nm and expressed as **(B / B_0) %.** The percentages in samples were compared to a calibration curve obtained with standard 20-HE concentrations (15.6 – 2000 ηmol). Results were the average of duplicate determinations (Porcheron, *et al.* 1989 and Blais, *et al.* 2010).

III- Material and Methods for biological study

Plant extracts

The alcohol extracts of the aerial parts of each of *Atriplex* species under investigation, their ethyl acetate fractions and 20-hydroxyecdysone (20-HE) were, separately, tested.

Experimental animals

Albino mice of 25-30g body weight (for toxicity study) and adult male albino rats of Sprauge-Dawely strain weighing 130-150g (for anabolic and androgenic investigation) were obtained from the animal house colony, national research Centre and kept under the same hygienic conditions and on standard laboratory diet. Water was supplied *ad lib*.

Drugs and chemicals:

Testosterone (Primotestone Depot®, 250 mg/ml, Chemical Industries Development, S.A.A., Giza, Egypt).

Toxicity study:

Determination of median lethal dose (LD_{50})

The LD_{50} of the alcoholic extracts of each plant under investigation were, separately, estimated following karber's procedure (Karber, 1931).

Anabolic and androgenic activities

Castration in adult male albino rats was performed through median incision in the scrotum under anaesthesia by inhalation (Schalm, 1961). Animals were left for two weeks before treatment then divided into seven groups, each of 6 rats. The first group served as control receiving 1ml saline, the second group received a dose of testosterone hormone (0.1 mg/kg, b.wt., *i.p.*) for 10 days and served as positive control. Four groups were separately received a dose (50 mg/kg, b.wt., *i.p.*, 10 days) of each of the tested samples (alcoholic extracts and ethyl acetate fractions of each of the two plants under investigation). The seventh group received a dose of 20-hydroxyecdysone (10 mg/kg, b.wt., *i.p.*, 10 days). Finally, a group of six uncastrated rats received 1 ml saline and served as negative control.

The ventral prostate gland, seminal vesicles, epididimis, vas difference and levator ani muscle were removed and cleared of extraneous tissue then weighed. All organs weighed were normalized to body weight and compared.

The anabolic activity was estimated by increase the ratio of weights of levator ani muscle to prostate gland; while the androgenic activity was evaluated by measuring the changes in weights of prostate gland, seminal vesicles, epididimis and vas difference (Slama and Lafont, 1995).

Statistical analysis

The data obtained from the pharmacological study were statistically analyzed using the Student's t-test (Snedecor and Cochran, 1971). All values are expressed as mean ± S.E. of a number of experiments (n).

IV- Material and Methods for Phytochemical Study

General

Melting points were uncorrected and measured on a digital melting point apparatus (Büchi melting point B-540). ^{1}H- NMR (300 MHz) and ^{13}C- NMR (75 MHz) spectra were recorded on Varian mercury apparatus at 25^{o}C using TMS as an internal standard and chemical shifts were given in δ values. Jasco V-630 and Shimadzu-265 spectrophotometer was used for recording the U.V. absorption spectra. Silica gel H (E. Merck) was employed for VLC technique and Sephadex LH-20 (Pharmacia) was used for column chromatography; TLC separations were performed on pre-coated silica gel 60 F_{254} plates (0.25mm thickness, E. Merck) and chromatograms developed with ethyl acetate: formic acid: acetic acid: water "100: 11: 11: 20" (S_1) and *n*-butanol: glacial acetic acid: water "3: 1: 1" (S_2) as solvent systems; PC were conducted on sheets of Whatmann No.1 filter paper, developed with *n*-butanol: acetic acid: water "4:1:5, upper phase" (S_3). Visualization was accomplished in day and U.V. (254 / 365 nm) lights before and after exposure to ammonia vapors and by spraying with aluminium chloride (for flavonoids; Mabry *et al.*, 1970), *p*-anisaldehyde/ sulphuric acid, followed by heating at 110ºC (for 20-hydroxyecdysone; Stahl, 1969) and aniline phthalate, followed by heating at 110ºC (for sugars; Stahl, 1969).

Column chromatographic fractionation of the ethyl acetate fraction of *A. lindleyi* subsp. *inflata*

An aliquot of the ethyl acetate soluble extractive (5g) was chromatographed on a VLC column (10 cm L x 4 cm D) on silica gel H (50 g), elution being gradient with $CHCl_3$/Ethyl acetate and Ethyl acetate/Methanol mixtures. The fractions were monitored (TLC, spots located in day and U.V. lights, before and after exposure to ammonia vapors and spraying with $AlCl_3$) then similar fractions pooled. Fractions were further, separately, subjected to rechromatography on Sephadex LH-20 columns, elution being with 100% methanol to afford **compounds I-IV**, with respective R_f values 0.54, 0.80, 0.63 and 0.29 (S_1), which were further identified through spectral analysis.

Reference material and chemicals: Reference samples of 20-hydroxyecdysone, flavonoids and sugars were obtained from E. Merck, Darmstadt, Germany. Shift reagents for U.V. spectral analysis of flavonoids were prepared from analytical grade chemicals according to Mabry *et al.*, (1970).

V- Material and Methods for Molecular Investigation

Plant material

A sample of fresh leaves from both species under investigation was separately stored at -70^{o}C, freeze-dried and ground under liquid nitrogen to a fine powder prior to DNA isolation.

Apparatus

DNA thermocycler (Hybaid PCR Express) used for amplification of DNA, agarose gel electrophoresis tool (Biorad Wide Mini Sub Cell) for separation of RAPD fragments according to size and UV Polaroid camera for visualization of RAPD fragments.

Buffer solutions and gel

Extraction buffer: 0.7 M NaCl, M Tris (pH 7.5), 0.01 M EDTA, 1% (w/v) N-cetyl-N, N, N- trimethylammonium bromide (CTAB), 1% (v/v) β-mercaptoethanol (added immediately before use); **washing buffer 1**: 76M Na-acetate; **washing buffer 2**: 76% ethanol, 10mM NH_4O-acetate; **TE-buffer** : 10mM tris (pH 8.0), 1 mM EDTA; **10% reaction buffer:** 100mM tris HCl (pH 8.3), 500mM KCl, 0.01% (w/v) gelatin, chloroform/propyl alcohol 24:1 (v/v), isopropanol, dNTP mix (Pharmacia, Sweden), Taq DNA polymerase (Perkin-Elmer/Cetus, USA, Advanced Biotechnologies, UK). **Agarose gel:** 1.4% agarose gel (Sigma), with running buffer TE buffer, was used for electrophoresis.

Primers and Molecular size marker

Seven primers (Operon Technologies Inc., Almeda, California, USA) were used for RAPD analysis with the following sequence: TGCTCTGCCC (OPB-06), TGCGCCCTTC (OPB-05), GTTTCGCTCC (OPB-01), AGGGGTCTTG (OPA-05), AATCGGGCTG (OPA-04), AGTCAGCCAC (OPA-03) and CAGGCCCTTC (OPA-01). **Molecular size marker:** A 100 bp ladder (Promega Corporation, Madison, USA) was used as standard marker.

DNA extraction and quantification

A sample (50mg) of the frozen leaves was powdered in liquid nitrogen, extracted with 0.8ml CTAB and precipitated with isopropanol. The precipitate was washed with washing buffers and dissolved in deionized water (Doyle and Doyle, 1987).

Amplification of RAPD markers: The polymerase chain reactions were carried out with 100ηg of genomic DNA template following a thermal cyclic program. Amplified products were analyzed by electrophoresis on 1.4% agarose gel and finally stained with ethidium bromide. A molecular size marker was used as standard marker.

Analysis of RAPD data: RAPD bands were treated as presence or absence, without considering their percentage. Similarity coefficient is calculated by genetic similarity equation: $GS = 2N_{1x2}/(N_1+N_2)$, where N_{1x2} is the number of shared fragments between the two plants under investigation; N_1 and N_2 are the number of scored fragments of *A. lindleyi* subsp. *inflata* and *A. leucoclada*, respectively (Jaccard, 1908).

RESULTS AND DISCUSSION

The percentage yields of the alcohol extractive and its fractions: *n*-hexane, chloroform, ethyl acetate and *n*-butanol of the aerial parts of *A. lindleyi* subsp. *inflata* were 10, 0.47, 0.12, 0.14 and 0.56 % w/w, respectively while those of the aerial parts of *A. leucoclada* were 17.78, 0.26, 0.24, 0.11 and 0.28 % w/w, respectively.

TLC screening (S_1) against reference sample, revealed the presence of 20-hydroxyecdysone in total alcohol extract and ethyl acetate fraction of each *Atriplex* species.

EIA quantification revealed that the amount of 20-HE was 9.15 and 7.30 µg/g dried aerial parts in each of *A. lindleyi* subsp. *inflata* and *A. leucoclada*, respectively. From previous reports (Dinan *et al*., 1998), we can conclude that the amount of 20-HE in the aerial parts of *A. lindleyi* subsp. *inflata* was found to be higher in concentration than the seeds of the same species grown in Australia.

LD_{50} of alcoholic extracts of each of the two *Atriplex* species under investigation was up to 5g/ kg, b.wt., consequently they could be considered safe according to Buck *et al*., (1976).

The results recorded in tables (1 and 2) revealed that all tested extracts and fractions of both *Atriplex* species under investigation exhibited significant anabolic and androgenic activities (at the given doses) in castrated rats, comparable to that of testosterone hormone.

The anabolic activity was proved by the increase in the ratio of weights of levator ani muscle to prostate gland: the ethyl acetate fraction of *A. lindleyi* subsp. *inflata* demonstrated a higher activity than the alcohol extract of the same species. In the contrary, the alcohol extract of *A. leucoclada* showed a higher anabolic activity than its ethyl acetate fraction.

In addition, the androgenic activity was verified by the increase in weights recorded in genital organs *viz*. prostate gland, seminal vesicles, epididimis and vas difference: in *A. lindleyi* subsp. *inflata*, the activity of the alcohol extract was greater than its ethyl acetate fraction, while in *A. leucoclada*, the ethyl acetate fraction was more effective than the alcohol extract.

The result of anabolic effect of the isolated 20-hydroxyecdysone is in agreement with previous literature (Lafont and Dinan, 2003). Furthermore, it was also previously proved that it possess androgenic-like anabolic action by its ability to increase the weights of prostate gland, seminal vesicle, levator ani muscle of the castrated rats and accelerate the growth of mice and protein content in the liver (Slama and Lafont, 1995).

Taking in consideration that 20-hydroxyecdysone was detected mainly in the ethyl acetate fractions of both *Atriplex* species, we assume that these activities are attributed, at least in part, to its content in the tested fractions.

The total alcohol extract and the ethyl acetate fraction of *A. lindleyi* subsp. *inflata* showed greater anabolic and androgenic activities than their analogues in *A. leucoclada*. The higher amount of 20-HE in the aerial parts of *A. lindleyi* subsp. *inflata* may give explanation to these biological findings.

Column chromatographic fractionation of the ethyl acetate of *A. lindleyi* subsp. *inflata* (the most active) resulted in the isolation of a steroidal compound **(I)** and three flavonol derivatives (**II-IV**), 100, 6, 40 and 10mg, respectively.

Compound I, was obtained as yellowish white amorphous powder; its melting point was 241-243^{o}C, it gave positive Liebermann's and Salkowski's tests which suggested the presence of steroid or triterpenoid skeleton.

The **U.V. spectrum** of compound **(I)** run in methanol showed only one absorption frequency at wavelength 235 nm due to the 7-ene-6-one conjugation. the **EIMS** showed a molecular ion at m/z 480 which is in a good accordance with the molecular formula $C_{27}H_{44}O_7$. **^{1}HNMR data (CD3OD, 300 MHz)** of this compound showed a set of five singlet methyl signals at δ 0.89 (3H, *s*, H-18), 0.97 (3H, *s*, H-19), 1.19 (3H, *s*, H-21 and 3H, *s*, H-26) "overlapped" and 1.20 (3H, *s*, H-27); an olefinic proton H-7 at δ (5.81, 1H, *d*, J= 2.2 Hz) showed long range zig-zag (W) coupling "characteristic for ecdysteroid" with H-9 δ 3.15 (*m*), which was coupled to two methylene protons of position 11 (δ 1.62 and 1.81 ppm) and in turn coupled with the two protons at position 12 (δ 1.91 and 2.13 ppm).

By comparing the physicochemical and spectral data with those reported (Girault and Lafont, 1988; Sarker *et al*., 1998 and Ateya *et al*., 2005) and by direct comparison with authentic sample, **compound I** was identified as **20-hydroxyecdysone** (fig. 1A).

Compounds II-IV, all were obtained as yellow powders; they showed deep purple color under U.V., changing to yellow on exposure to ammonia vapors or spraying with $AlCl_3$ reagent. Positive Molisch's test suggested their glycosidic nature. Their melting points were 235-237, 225-227 and 210-213^{o}C, respectively. Their U.V., ^{1}HNMR and ^{13}CNMR spectral data are displayed in tables 3, 4 and 5, respectively and their structures represented in figure 1B.

The **U.V. spectra** in methanol of the isolated compounds (table 3) were significant to flavonol derivatives, having substituted 3-hydroxyl group. The bathochromic shift in band I (relative to methanol) in presence of sodium methoxide without decrease in intensity and that in band II in presence of sodium acetate indicated free 4' and 7- hydroxyl groups, respectively in all isolated compounds. Absence of *ortho* dihydroxyl pattern in ring B in **compounds II** was deduced from the absence of bathochromic shift (band I) upon addition of boric acid and from the absence of hypsochromic shift (band I) upon addition of HCl. However, this *ortho* dihydroxyl pattern was detected in **compounds III and IV**.

In **^{1}HNMR data** (table 4), two doublets (J~ 2.0Hz) in the range of 6.12-6.80 ppm were assigned for the protons at C-6 and C-8 and proved the presence of 5,7-dihydroxy substitution pattern in all isolated compounds. Signals of the protons at 2', 5' and 6' positions in all isolated compounds showed 3' and 4' substitutions appearing as two doublets at 6.87 and 6.92 ppm corresponding to H-5' and H 2', respectively

and a doublet of doublet at 7.57-7.71 ppm (J= 2.5, 8.2Hz) corresponding to H-6'. A signal at 3.90 ppm indicated the presence of a methoxy group in **compound II** only. Galactose and glucose were identified as the sugar moieties in **compound II** and **III**, respectively through their characteristic doublets of anomeric protons, while in **coumpound IV**, both glucose and rhamnose could be identified where a doublet at 0.8-0.9 (J~ 6.0Hz) confirmed the presence of methyl protons of rhamnose.

^{13}CNMR data (table 5) verified those of U.V. and ^{1}HNMR in all of the isolated compounds and revealed the presence of a carbonyl carbon signal at δ 179-180 ppm for 4-keto flavonoids. Signals of methine carbons, ascribed for C-6 and C-8 indicated no substitution in these positions. Oxygenated carbon signals in the range of 145.9-149.9 ppm confirmed the presence of 3'-4' dihydroxy pattern in **compounds II-IV**. Presence of one anomeric carbon signal in **compounds II** and **III** revealed the presence of one sugar in each (δ 103.86 and 104.42 ppm, respectively). In **compound IV**, the presence of two sugars were confirmed by two anomeric carbon signals (104.76 and 102.94 ppm). The remaining sugar carbons signals appeared at 56-78 ppm except C6''' at 17.89 ppm corresponding to L-rhamnose.

Acid hydrolysis of **compounds II-IV** (Mabry *et al*., 1970) yielded isorhamnetin in the hydrolysate of **compound II** and quercetin in those of **compounds III** and **IV** (yellow under U.V., R_f 0.68 and 0.57, respectively [TLC, S_2]). Galactose (in **compound II**), glucose (in **compound III**), glucose and rhamnose (in **compound IV**) were identified as sugar moieties (brown with aniline phthalate, R_f 0.30, 0.34 and 0.57, respectively [PC, S_3])

By comparing the abovementioned data with those reported (Mabry, *et al.*, 1970 ; Harborne *et al.*, 1975 and Agrawel, 1989), and by direct comparison with authentic samples, **compounds II**, **III** and **IV**, were identified as, **Isorhamnetin 3-*O*-galactoside**, **Isoquercitin** (quercetin 3-*O*- ß-D-glucopyranoside) and **Rutin** (quercetin 3-*O*- α-L-rhamnosyl ß-D-glucoside), respectively.

TLC comparison (S_1) of the ethyl acetate fractions of the two *Atriplex* species under investigation revealed the presence of compound II and the absence of compounds III and IV in *A. leucoclada*.

To the best of our knowledge, this is the first report on the isolation of compounds II and IV from genus *Atriplex* while compound III was previously isolated from *A. lindleyi* subsp. *inflata* (Ateya *et al*., 2005).

Moreover, the DNA of the two *Atriplex* species under investigation when amplified using seven decamers (table 6 and figure 2); a total of 43 and 44 different RAPD fragments were recorded for *A. lindleyi* subsp. *inflata* and *A. leucoclada*, respectively. A number of fragments amplified using six primers "OPB-06, OPB-05, OPA-05, OPA-04, OPA-03, OPA-01" was found to be identical and the primer "OPB-01" was the only effective primer in generating the polymorphic band. Fragments of both *Atriplex* species showed 9 bands by OPA-01 primer ranging from 1188 to 355 bp, 7 bands by OPA-03 primer (1747 – 164 bp), 6 bands by each of OPB-06, OPB-05 and OPA-04 primers (1028 – 523, 1132 – 523 and 1028 – 323 bp, respectively), and 5 bands by OPA-05 primer (1924 – 323 bp). *A. lindleyi* subsp. *inflata* showed 4 bands

by OPB-01 primer (933 – 199 bp) whereas *A. leucoclada* showed 5 bands by the same primer (1308 – 199 bp). The only different polymorphic band between the two *Atriplex* species displayed similarity coefficient of 88.88% (table 7). The analysis of RAPD data, under the experimental conditions, can thus be used to distinguish each of the two plants from other species.

Table (1): Anabolic effect of the alcoholic extracts, ethyl acetate fractions of the aerial parts of each of *Atriplex* species and the isolated 20-hydroxyecdysone on castrated adult male albino rats

Parameters / Groups	Weigt of prostate gland (g)	Weight of levator ani muscle (g)	Ratio[!]
Uncastrated rats (negative control)	0.23±0.01	0.21±0.01	--
Castrated rats receiving saline	0.19±0.001*	0.14±0.001*	-1.75
Castrated rats receiving alcoholic extract of *A. lindleyi* subsp. *inflata*	0.46±0.02	0.47±0.02	1.13
Castrated rats receiving alcoholic extract of *A. leucoclada*	0.43±0.01	0.39±0.03	0.9
Castrated rats receiving ethyl acetate fraction of *A. lindleyi* subsp. *inflata*	0.38±0.01	0.41±0.03	1.3
Castrated rats receiving ethyl acetate fraction of *A. leucoclada*	0.37±0.01	0.32±0.01	0.8
Castrated rats receiving 20-hydroxyecdysone	0.41±0.02	0.43±0.02	1.2
Castrated rats receiving testosterone (positive control)	0.52±0.03	0.63±0.01	1.5

Results are expressed as mean ± S.E., n=6. * Significantly different from control, P<0.01.

[!] Ratio calculated as change in weight of levator ani muscle /change in weight of prostate gland

Table (2): Androgenic effect of the alcoholic extracts, ethyl acetate fractions of the aerial parts of each of *Atriplex* species and the isolated 20-hydroxyecdysone on castrated adult male albino rats

Parameters / Groups	Weigt of prostate gland (g)	% change	Potency	Weigt of epididimis (g)	% change	Potency	Weigt of seminal vesicle (g)	% change	Potency	Weigt of vas difference (g)	% change	Potency	Body weight (g)
Uncastrated rats (negative control)	0.23 ±0.01	--	--	0.26 ±0.01	--	--	0.31 ±0.01	--	--	0.08 ±0.002	--	--	163.7 ±4.2
Castrated rats receiving saline	0.19* ±0.001	--	--	0.11* ±0.01	--	--	0.16* ±0.01	--	--	0.06* ±0.001	--	--	151.1 ±3.4
Castrated rats receiving alcoholic extract of *A. lindleyi* subsp. *inflata*	0.46 ±0.02	142	0.82	0.29 ±0.02	163.6	0.72	0.38 ±0.01	137.5	0.59	0.002 ±0.013	966.6		173.8 ±3.6
Castrated rats receiving alcoholic extract of *A. leucoclada*	0.43 ±0.01	63.2	0.36	0.21 ±0.01	90.9	0.4	0.23 ±0.02	43.75	0.20	0.09 ±0.002	966.6		165.7 ±4.1
Castrated rats receiving ethyl acetate fraction of *A. lindleyi* subsp. *inflata*	0.38 ±0.01	100	0.58	0.22 ±0.01	100	0.44	0.26 ±0.002	62.5	0.27	0.11 ±0.001	83.3	0.38	153.4 ±4.2
Castrated rats receiving ethyl acetate fraction of *A. leucoclada*	0.37 ±0.01	94.7	0.55	0.24 ±0.01	118.2	0.52	0.19 ±0.002	18.75	0.08	0.12 ±0.001	100	0.46	161.2 ±5.8
Castrated rats receiving 20-hydroxyecdysone	0.41 ±0.02	115.8	0.66	0.26 ±0.01	136.4	0.60	0.26 ±0.01	62.5	0.27	0.12 ±0.001	100	0.46	161.2 ±5.8
Castrated rats receiving testosterone (positive control)	0.52 ±0.03	173.7	1	0.36 ±0.03	227.3	1	0.53 ±0.012	231.3	1	0.19 ±0.01	216.6	1	177.8 ±3.9

Results are expressed as mean ± S.E., n=6. * Significantly different from control, P<0.01.

Table (3): U.V. spectral data and shifts of compounds (II-IV)

U.V. shift reagent	λ max (nm)		
	II	**III**	**IV**
MeOH	255, 268*sh*, 303*sh*, 357	258, 268*sh*, 299*sh*, 359	258, 266*sh*, 299*sh*, 361
NaOMe	272, 326, 415	274, 329*sh*, 410	272, 328*sh*, 410
$AlCl_3$	270, 299*sh*, 366*sh*, 406	275, 304*sh*, 435	273, 304*sh*, 433
$AlCl_3$/HCl	268, 299*sh*, 359, 403	271, 304, 367*sh*, 404	272, 294*sh*, 364*sh*, 402
NaOAc	274, 316, 389	271, 326, 393	271, 325, 393
NaOAc/H_3BO_3	257, 268*sh*, 307*sh*, 363	264, 298*sh*, 389	263, 294*sh*, 387

Table (4): ^{1}H-NMR spectral data, 300 MHz (CD_3OD) of compounds (II-IV)

Position No	δ_H (*ppm*), J (*Hz*)		
	II	**III**	**IV**
H-6	6.12 (1H, *d*, J= 2.1)	6.19 (1H, *d*, J= 2.0)	6.20 (1H, *d*, J= 2.1)
H-8	6.80 (1H, *d*, J= 2.3)	6.37 (1H, *d*, J= 1.8)	6.40 (1H, *d*, J= 2.1)
H-2'	7.57 (1H, *d*, J= 2.4)	7.71(1H, *d*, J= 2.8)	7.69 (2H), overlapped
H-6'	7.35 (1H, *dd*, J= 2.5, 8.1)	7.56 (1H, *dd*, J= 2.8, 8.2)	
H-5'	6.92 (1H, *d*, J= 8.5)	6.87 (1H, *d*, J= 8.6)	6.88 (1H, *d*, J= 9.0)
OCH_3-3'	3.90 (3H, *s*)	--	--
Anomeric H	5.25 (1H, *d*, J= 7.1)	5.20 (1H, *d*, J= 7.1)	5.08(1H, *d*, J= 7.4), 4.51 (1H, *d*, J= 2)
Sugar H's	3.47 (*m*)	3.65 (*m*)	3.37 (*m*)

Table (5): ^{13}C-NMR spectral data, 300 MHz (CD_3OD) of compounds (II-IV)

Position No		δ_c (*ppm*)		
		II	**III**	**IV**
2		158.31	158.39	158.49
3		135.19	135.63	135.68
4		179.25	179.41	179.35
5		159.10	162.91	163.98
6		100.06	99.92	100.02
7		166.33	165.96	162.92
8		94.81	94.76	94.90
9		159.15	159.04	158.49
10		105.41	105.64	105.63
1'		123.33	123.19	123.09
2'		116.39	115.99	116.10
3'		145.89	145.82	145.98
4'		149.76	149.79	150.49
5'		117.31	117.60	117.42
6'		123.11	123.04	123.51
Sugar carbons	**1''**	103.86	104.42	104.76
	2''	71.75	75.69	75.70
	3''	75.68	78.29	78.14
	4''	64.32	71.21	71.49
	5''	78.06	78.07	78.07
	6''	56.48	62.54	69.19
	1'''	--	--	102.94
	2'''	--	--	71.49
	3'''	--	--	72.39
	4'''	--	--	74.01
	5'''	--	--	69.46
	6'''	--	--	17.89

Table (6): Molecular size in base pairs of amplified DNA fragments produced by seven decamer primers in *Atriplex* species under investigation.

Molecular size (bp)	OPB-06		OPB-05		OPB-01		OPA-05		OPA-04		OPA-03		OPA-01	
	A.i	*A.l*	*A.i*	*A.l*	*A.i*	*A.l*	*A.i*	*A.l*	*A.i*	*A.l*	*A.i*	*A.l*	*A.i*	*A.l*
1924	-	-	-	-	-	-	+	+	-	-	-	-	-	-
1747	-	-	-	-	-	-	-	-	-	-	+	+	-	-
1373	-	-	-	-	-	-	-	-	-	-	+	+	-	-
1308	-	-	-	-	-	+	+	+	-	-	-	-	-	-
1188	-	-	-	-	-	-	-	-	-	-	-	-	+	+
1132	-	-	+	+	-	-	-	-	-	-	-	-	-	-
1028	+	+	-	-	-	-	+	+	+	+	-	-	+	+
979	-	-	-	-	-	-	-	-	-	-	+	+	-	-
933	-	-	+	+	+	+	-	-	-	-	-	-	+	+
889	+	+	-	-	-	-	-	-	-	-	-	-	-	-
847	-	-	-	-	-	-	-	-	-	-	-	-	+	+
807	-	-	-	-	-	-	-	-	+	+	-	-	-	-
769	+	+	+	+	-	-	-	-	-	-	-	-	-	-
733	-	-	-	-	-	-	-	-	-	-	-	-	+	+
666	+	+	+	+	-	-	-	-	-	-	-	-	-	-
634	-	-	-	-	-	-	-	-	-	-	+	+	-	-
604	+	+	-	-	-	-	-	-	+	+	-	-	-	-
576	-	-	-	-	+	+	-	-	-	-	-	-	+	+
523	+	+	+	+	-	-	+	+	+	+	+	+	-	-
475	-	-	-	-	-	-	-	-	-	-	-	-	+	+
452	-	-	-	-	+	+	-	-	-	-	-	-	-	-
431	-	-	-	-	-	-	-	-	+	+	-	-	+	+
355	-	-	-	-	-	-	-	-	-	-	-	-	+	+
323	-	-	+	+	-	-	+	+	+	+	-	-	-	-
308	-	-	-	-	-	-	-	-	-	-	+	+	-	-
199	-	-	-	-	+	+	-	-	-	-	-	-	-	-
164	-	-	-	-	-	-	-	-	-	-	+	+	-	-
Total	6	6	6	6	4	5	5	5	6	6	7	7	9	9

(+) and (-): presence and absence of bands, respectively.

A.i: *Atriplex lindleyi* subsp. *inflata* A.l.: *Atriplex leucoclada*

Table (7): Total number of RAPD fragments, distribution of monomorphic and polymorphic bands and similarity coefficients by seven decamer arbitrary primers in *Atriplex* species under investigation.

Primers	Number of fragments	Monomorphic fragments	Polymorphic fragments	Percentage Polymorphism	Similarity coefficient*
OPB-06	6	6	0	0	100
OPB-05	6	6	0	0	100
OPB-01	5	4	1	20	88.88
OPA-05	5	5	0	0	100
OPA-04	6	6	0	0	100
OPA-03	7	7	0	0	100
OPA-01	9	9	0	0	100
Total	44	43	1	Mean: 2.86	Mean: 98.85

*: calculated by genetic similarity equation

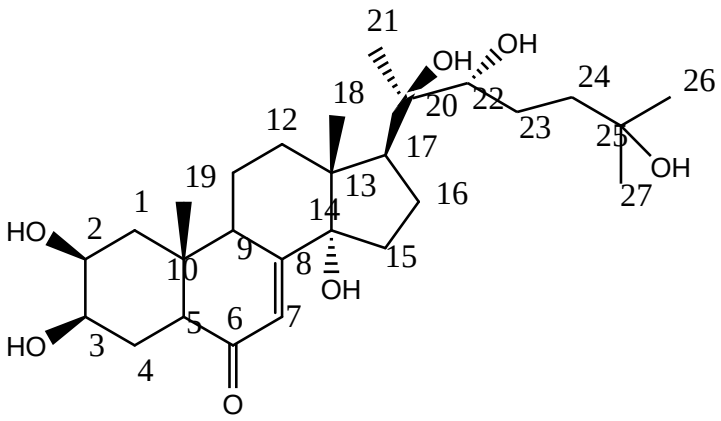
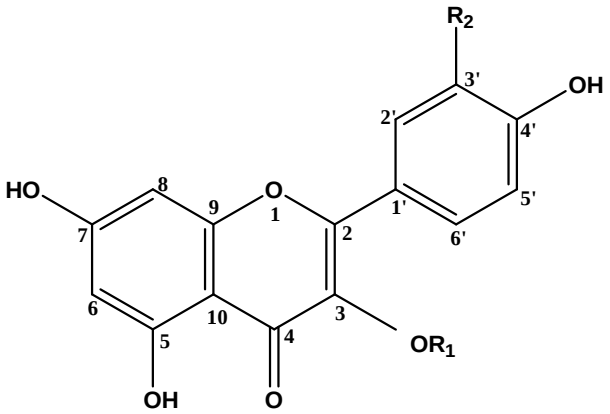
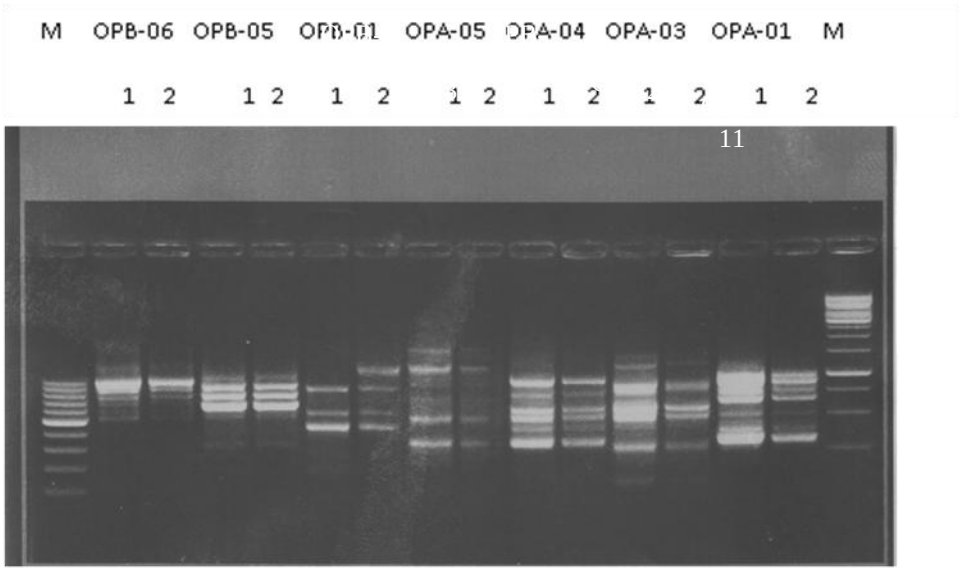


Fig. (1A): Structure of compound I



II R_1= galactose, R_2= OCH_3
III R_1= glucose, R_2= OH
IV R_1= rhamnoglucose, R_2= OH

Fig. (1B): Structures of compounds II-IV



M: standard DNA molecular size marker

1: *Atriplex lindleyi* subsp. *inflata* 2.: *Atriplex leucoclada*

Fig. (2): The RAPD electrohporetic profile of *Atriplex* species under investigation generated by the seven decamer primers

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ARABIC SUMMARY

التأثير الابتنائى والتأثير الذكورى لجنس *أتريبلكس* الذى ينمو فى مصر

للسادة الدكاترة

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**قسم الفارماكولوجى – المركز القومى للبحوث – الدقى – جيزة

كشفت كروماتوجرافيا الطبقة الرقيقة عن وجود مركب 20–هيدروكس اكديسون فى مستخلص خلات الاثيل لنباتين من جنس *أتريبلكس* اللذان ينموان فى مصر وهما: *أتريبلكس ليندلى* سلالة *انفلاتا* و *أتريبلكس ليوكوكلادا*. وتم التقدير الكمى لهذا المركب بطريقة EIA فى كل من النباتين وأسفرت النتيجة عن مقدار 9,15 و 7,3 ميكروجرام /جرام للمركب فى النباتين محل الدراسة على التوالى.

أثبتت الدراسة الحيوية أن لمركب 20–هيدروكس اكديسون تأثيراً ابتنائياً وتأثيراً ذكورياً بالمقارنة لهرمون تستستيرون، كما تبين أن المستخلص الكحولى ومستخلص خلات الاثيل لنبات *أتريبلكس ليندلى* سلالة *انفلاتا* لهما تأثير ابتنائى وتأثير ذكورى أعلى من مثيليهما فى نبات *أتريبلكس ليوكوكلادا*.

و قد تم تجزئة مستخلص خلات الاثيل لنبات *أتريبلكس ليندلى* سلالة *انفلاتا* (وهو الأعلى فى التأثير الحيوى) باستخدام كروماتوجرافيا العمود وأسفرت الدراسة عن فصل 20–هيدروكس اكديسون (I)، ايزورامنتين–3–جلاكتوسيد (II)، ايزوكيرستين (III) وروتين (IV) وتم التعرف على المواد المفصولة بواسطة الوسائل الطيفية المختلفة.

كما أسفرت دراسة البصمة الوراثية للحمض النووى عن وجود دلائل جينية يمكن بها تمييز النباتين محل الدراسة واظهار الفرق بينهما حيث تعين معامل تشابه بنسبة 88,88%.