Cytotoxic and insect -repellent activities of surface flavonoids from *Datura stramonium* L. Grown in Egypt

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Abstract: Three flavonoidal aglycones viz; F1 (Chrysin), F2 (Kampferol) and novel F3(3,7- dimethylether quercetin), were isolated from acetone wash of fresh leaves of *Datura stramomium* L. Isolated compounds were identified on the basis of their physico-chemical properties, Co-TLC, ^{1}H-NMR, ^{13}C-NMR and CIMS specrtum. Significant cytotoxic activity against three tumor cell lines (liver, cervix & breast), was recorded for the isolated compounds. In addition, remarkable repellent action of surface flavonoids against House Flies (*Musca domestica*) was recorded.

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1. Introduction:

Leaf surface flavonoids (exudates) are usually found in plants, which grow in arid or semi-arid habitats, and often in association with aromatic terpenoids on the leaf surface (Williaims *et al*.,1997). The leaf surfaces of many plants are covered by various non glandular and glandular trichomes. Trichomes with their lipophilic exudates (flavonoid aglycones, waxes, terpenes and lipids) may protect leaves against extensive light, UV-B radiation, and desiccation (Ehleringer, 1982; Karabourniotis *et al*., 1993; Cockell and Knowland, 1999; Tattini *et al*., 2000; Juma *et al*., 2001), or they may form the first line of defense against insects and herbivores by entrapping, deterring, or poisoning (Harborne, 1991; Wagner, 1991; Hare, 2002). For Solanaceae, in particular, trichome exudates are responsible for the mortality of neonate larvae; the removal of exudates using an ethanol solution increase larval survival (Gurr and McGrath, 2002).

Datura stramonium L., known by the common names Jimson weed or Datura is a plant in the Solanaceae (nightshade) family, which is believed to have originated in the Americas, but is now found around the world (*Datura stramonium* information from NPGS/GRIN).

Datura stramonium L. is a foul-smelling, erect annual, freely-branching herb that forms a bush up to 2 to 5 feet (60–150 cm) tall. The leaves are approximately 3 to 8 inches (8–20 cm) long, smooth, toothed, soft, irregularly undulate. The upper surface of the leaves is a darker green, and the bottom is a light green. The leaves have a bitter and nauseating taste, which is imparted to extracts of the herb, and remains even after the leaves have been dried.(Stace, 1997).

For centuries, *Datura* has been used as an herbal medicine to relieve asthma symptoms and as an analgesic during surgery or bone setting. It is also a powerful hallucinogen and deliriant, which is used spiritually for the intense visions it produces. However, the tropane alkaloids which are responsible for both the medicinal and hallucinogenic properties are fatally toxic in only slightly higher amounts than the medicinal dosage, and careless use often results in hospitalizations and deaths.

Many studies showed that certain genera of Solanaceae plants, particularly *Datura stramonium* L., produce a range of biologically active alkaloids, including tropane alkaloids (Sato *et al*., 2001). In recent decades Solanaceae have only rarely been studied for the occurrence of externally accumulated surface flavonoid aglycones, although in many species glandular trichomes and sometimes even their resinous exudates are obvious. Results on some Solanaceae genera have been published previously (Wollenweber, 1990; Wollenweber and Dörr, 1995 and Wollenweber *et al*., 2005).

Earlier, glycoside patterns have been reported for five Datura spp. (Pate and Averett, 1986) but concerning free aglycone exudates only one report has been published (Wollenweber *et al*., 2005).

The aim of this study was therefore designed to isolate the surface flavonoids from leaves exudates of *Datura stramonium* L. and evaluate cytotoxic potentials of these flavonoids against selected tumor cell lines. Another specific goal was to determine the insect repellent activity of the exudate against house flies**.**

2. Material and methods

2.1. Plant material

Fresh leaves of *Datura stramonium* L. was collected from plants cultivated in the Experimental Station of Medicinal and Aromatic Plants, Faculty of Pharmacy, Cairo University, Giza, Egypt. The authentication of the plant was kindly confirmed by

Prof. Dr. Mohamed El-Gebally, Prof. of Plant Taxonomy, NRC, Dokki, Giza. Voucher specimens are kept in the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University.

2.2. Chemicals

2.2.1 Reference samples:

Reference phenolic samples: Quercetin, kampferol and chrysin (E. Merck, Darmstadt, Germany).

2.2.2 Material for chromatography:

Silica gel G (60 mesh) for TLC, silica gel (70-230 mesh) for CC, precoated TLC plates (silica gel 60 GF_{254}) from E. Merck (Darmstadt, Germany), sephadex LH-20 from Pharmacia (Uppsala, Sweden).

Solvent systems:

Chloroform - methanol (in different ratios v/v).

Spray reagents; aluminium chloride reagent for flavonoids (Markham, ,1982).

2.3. Material for biological evaluation:

2.3.1. Plant extracts:

The biological evaluation was performed on the three isolated compounds.

2.3.2. Tumor cell lines:

Tumor cell lines (cervix, HELA), (liver, HEPG2) and (breast, MCF7) from National Cancer Institute of Egypt were used for cytotoxic screening.

2.3.3. Insects for testing repellent effect:

House Flies (*Musca domestica*) were collected from flies trap in the Garden of Misr International University.

2.4. Apparatus:

2.4.1. NMR Jeol GLM, Jeol TMS Route instrument (^{1}H-NMR, 300 MHZ, ^{13}C, 75 MHZ, Japan).

2.4.2. Mass spectrometer: chemical ionization, Finnigan, CA, USA

2.5. Phytochemical study:

2.5.1. Extraction, isolation and Identification:

One Kg of fresh leaves was dipped briefly (10 second) in acetone. The concentrated acetone wash was chromatographed on Sephadex LH-20 column (l.5x30cm) using chloroform/methanol 98:2 as eluent. Similar fractions were pooled and the solvent was then evaporated under reduced pressure and some fractions yielded on concentration compounds F1 & F2 (15mg and 17mg; respectively). Another combined fraction was further purified on Sephadex LH-20 column (1 x 20 cm) using methanol as eluent. Subfractions were collected together and the solvent was stripped off under reduced pressure to yield, on concentration, compound F3 (13 mg). The structures of these isolated compounds were established on the basis of physicochemical data, Co TLC, 1H-NMR, 13CNMR and CIMS (Table 1).

2.6. Biological study:

2.6.1. Cytotoxic activity:

Potential cytotoxicity of the tested samples (Compounds F1, F2 and F3) was tested at the National Cancer Institute of Egypt adopting the method of Skehan (Skehan & Strong, 1990). Cells were plated in a 96-wells plate (10^{4}cells/well) for 24hrs before treatment with the tested sample to allow attachment of the cells to the wall of the plate. Different concentrations of each of the tested samples under study (0, 1, 2.5, 5 and 10 µg/ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose and were incubated for 48hrs at 37°C in an atmosphere of 5% CO_2. After 48hrs, cells were fixed, washed and stained with Sulphorodamine B stain. Excess stain was washed with acetic acid and attached stain was recovered with Tris-EDTA buffer. Colour intensity was measured in an ELISA reader. The survival curves of each of the tumor cell lines (cervices, liver and breast) were plotted and IC50 was calculated for each of the tested samples (Table 2)

2.6.2. Repellent activity against house flies:

Flies repellency value of surface flavonoids was tested adopting the method of Carloina (Carloina *et al*.,2006). Comparative study between two leaves of *Datura stramonium* one unwashed fresh leaf (Fig. 9A) & other is acetone-washed leaf (Fig. 9B). The leaves were allowed to dry for 5 minutes before being placed in a 10 cm petri dish . Ten House Flies (*Musca domestica*) were released in the center of each Petri dish, and both flies & their residual distribution were recorded after 3hours later. Repellency values were calculated by dividing the number of flies that move away from each leaf by the total number of Flies. Each experiment was replicated 4 times and results were recorded in table (3) and shown in figure (2).

3. Results and Discussion

3.1. Investigation of flavonoidal exudate content:

Compound F1 is white residue, exhibited a molecular ion peak at m/z 255. **^{1}HNMR spectrum of compound F1(Table1 & Figure 1)** showed the signals characteristic for chrysin (Harborne *et al*., 1975 and Mabry *et al*. 1996). **Ring B** is free from any substitution and this was confirmed by presence of 2 multiplets at δ 7.8 and δ 7.24 ppm; one integrated for two protons 2' and 6' while the other integrated for three protons 3',4' and 5'. On the other hand, **ring A** is substituted only in positions 5 and 7 which was verified by the presence of two *meta* coupled doublets of protons 8 and 6 at δ 6.3 and δ 6.1, respectively.

Compound F2 is pale yellow solid, exhibited a molecular ion peak at m/z 286. The **^{1}HNMR** spectrum of F2 **(Table1 & Figure 1)** showed the signals characteristic for kaempferol (Harborne *et al*., 1975 and Mabry *et al*., 1996). Spectrum showed the presence of two meta-coupled aromatic protons at δ 6.3 and 6. 4 corresponds to H-6 and H-8 protons

appeared separately as doublets having coupling constants 2.3 and 2.1 Hz; respectively.

In addition, the **^{1}HNMR** spectrum of F2 also showed the presence of two doublet of doublets at δ7.3 and 7.8 with coupling constants 8.1/2.2 Hz and 8.3/2.1 Hz respectively corresponds to the 4 aromatic protons of ring B; characteristic for the 3,5,7,4'-tetrasubstituted flavones

Compound F3 is yellowish white solid, exhibited a molecular ion peak at m/z 330. **^{1}HNMR spectrum of compound F3(Table1 & Figure 1)** showed the signals characteristic for quercetin (Harborne *et al*., 1975 and Mabry *et al*., 1996). **Ring B** is substituted in 3' and 4' positions and this was confirmed by presence of a multiplet at 7.5 ppm, integrated for two protons, 2' and 6' and an *ortho* coupled douplet ascribed to proton 5'. Singlet signal at 3.9 ppm integrated for 6 protons attributed to dimethoxy groups. **^{13}CNMR sectrum** had signals for carbons essentially identical to those of quercetin 3,7-dimethyl ether. Spectrum recorded two methoxy signals at 60 and 58 ppm attributed for C-3 & C-5 & this was confirmed by molecular ion peak in of F3 (330m/z) ascribed to dimethoxy derivative of quercetin contrasting from tri-methoxy derivative reported previously (Wollenweber *et al*., 2005).

Aglycones were isolated from hydrolyzed extracts of *D. stramonium* L. (Lakshmi and Krishnamoorthy, 1991), but free aglycones exudates detected in the leaf wash of this species, has been found only once before (Wollenweber *et al*., 2005).

3.2. Cytotoxic activity:

Considering cytotoxic activity (Table 2) a high potency of compound F1 was noticed only against liver cell line. On the other hand highly oxygenated compounds F2 & F3 recorded higher activities against both cervix and breast tumor cell line than compound F1. Highest activity of oxygenated isolated compound (F2 & F3) may be attributed to nature of these types of cancer as both cervix & breast cancers are hormone-dependant cancer

3.2. Repellent activity against house flies:

The average repellency percentage of houseflies for the unwashed fresh leaves recorded 82% of total number of tested flies after 3hrs (Table 3 & Fig. 9B). This compared to the average repellency percentage of acetone- washed leaves recorded (15%) (Table 3 & Fig. 9A). The results showed unwashed leaves provided significantly better repellency compared to the acetone- washed leaves. The results appraise the importance of surface flavonoids exudes on the surface of *Datura stramonium* L. leaves and their role as defensive barrier against different insects and herbivores.

Table 1. 1H-NMR, 13C-NMR & CIMS data of compounds F1, F2 & F3 isolated from the leaves exudate of *Datura stramonium* L. grown in Egypt.

Carbon number	**F1**	**F2**	**F3**	
	δ_H	**δ_C**	**δ_H**	**δ_C**
2	-	-	-	153.1
3	**6.8,** *s*	-	-	138.5
4	-	-	-	176.6
5	-	-	-	159.1
6	**6.1,** *d (1.9 Hz)*	**6.3,** *d (2.3 Hz)*	**6.2,** *d (2.1 Hz)*	100.1
7				165.4
8	**6.3,** *d (1.9 Hz)*	**6.4,** *d (2.1 Hz)*	**6.6,** *d (2.1 Hz)*	95.3
9	-	-	-	154.1
10	-	-	-	103.4
1'	-	-	-	119.8
2'	**7.8,** *m*	**7.3,** *dd (8.1 , 2.2 Hz)*	**7.5,** *d (2.2 Hz)*	111.4
3'	**7.24,** *m*	**7.8,** *dd (8.3 , 2.1 Hz)*	-	142.6
4'	**7.24,** *m*	-	-	148.7
5'	**7.24,** *m*	**7.8,** *dd (8.3 , 2.1 Hz)*	**6.8,** *d (8.1 Hz)*	117.9
6'	**7.8,** *m*	**7.3,** *dd (8.1 , 2.2 Hz)*	**7.5,** *dd (8.1 , 2.2 Hz)*	119.8
3 OH	-	**12.5,** *s*	-	-
5 OH	**12.3,** *s*	**12.5,** *s*	**12.5,** *s*	-
7 OH	**12.3,** *s*	**12.5,** *s*	-	-
3' OH	-	-	**12.5,** *s*	-
4' OH	-	**12.5,** *s*	**12.5,** *s*	-
3 OMe	-	-	**3.9,** *s*	60
7 OMe	-	-	**3.9,** *s*	58
CIMS m/z	**255** (M^+, 98%), **226**(27%),	**286** (M^+, 100%), **258**(44%).	**330** (M^+, 100%)	

Table 2. Cytotoxic activity of compounds F1,F2 & F3 isolated from the leaves exudate of *Datura stramonium* L. grown in Egypt.

Cell Line	IC_{50} (υg/ml)		
	Compound F1	Compound F2	Compound F3
Cervix (MCF7)	1.51	0.83	0.79
Breast (HELA)	0.96	0.66	0.61
Liver (HEPG2)	0.67	0.87	0.91

Table 3. Repellent activity of leaves exudate of *Datura stramonium* L. grown in Egypt against House Flies (*Musca domestica*)

% repellency	Fresh leaf without wash	Fresh leaf after washing
First determination	90 %	20 %
Second determination	80 %	10 %
Third determination	90 %	10 %
Fourth determination	70 %	20 %

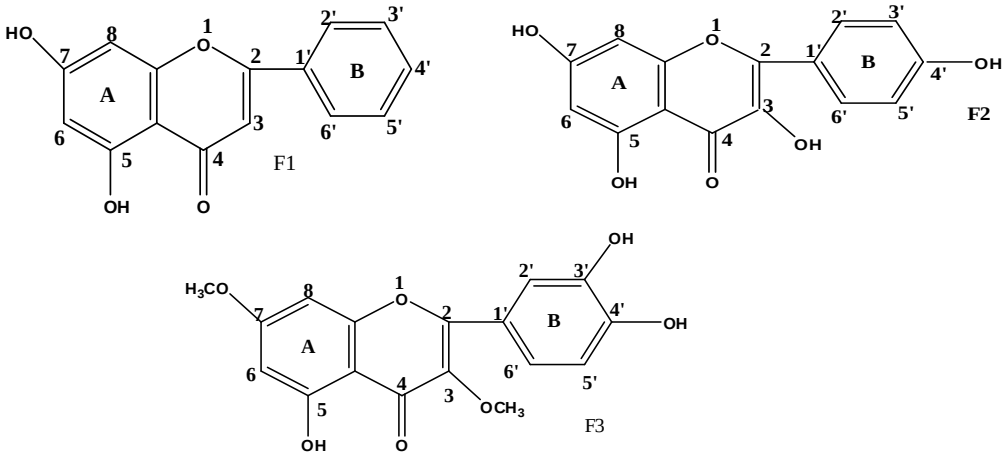

Figure 1. Isolated compounds from acetone wash of *Datura stramonium* L. leaves.

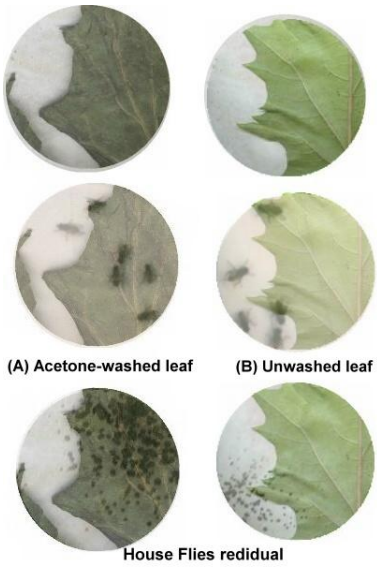

Figure 2. Repellent activity of leaves exudate of *Datura stramonium* L. grown in Egypt. against House Flies (*Musca domestica*)

4. Conclusions

Two known aglycones chrysin & kampferol in addition to dimethoxy quercetin derivative were isolated from the wash of *Datura stramonium* L. leaves for the first time. The structures of isolated compounds were identified on the basis of spectroscopic and chemical studies as well as by comparing their physical and spectral properties reported in the literature. Free aglycones could be detected in the leaf wash of Datura species, indicating that these aglycones most probably occur in glycosidic form as tissue constituents.

All isolated compounds exhibited significant cytotoxic activities against liver, cervix and breast cell lines. Consequently, simple wash of *Datura stramonium* leaves instead of destructive extraction revealed a new hope for designing novel molecules for treatment & prevention of liver and hormone dependent cancer**.**

Consumers now have an array of "natural" insect repellents from which to choose. These are made from benign-sounding plant extracts and exudates. Many natural insect repellents, deemed "minimum-risk pesticides" by the Environmental Protection Agency, are exempt from safety testing because their active and inert ingredients have been deemed safe for the intended use. Significant insect repellent effect has ecological importance where the observed effects of leaf exudates of *Datura stramonium* L. on the House Flies (*Musca domestica*) offer an eco – friendly new natural formula act as insect repellent and fight herbivores.

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