Comparative cytotoxic and antimicrobial activities of the alkaloid content of Egyptian *Pancratium maritimum* L. fruits and flowers

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Abstract: The aim of this study is comparing the effect of the alkaloid mixtures of fruits and flowers of the Egyptian *Pancratium maritimum* L. on colon carcinoma cells and selected strains of fungi and bacteria. The chemical composition of the alkaloids of both organs has been analyzed using GC/MS technique. Seventeen and thirteen compounds were detected, from which 14 and 11 were identified for fruits and flowers respectively. Four alkaloids were detected in high concentrations: Tazettine, Lycorine, N-demethyl galantamine and galantamine (in fruits: 21.97%, 17.09%, 14.93 and 14.20% while in flowers: 25%, 8.48%, 10.03% and 14.20% respectively). The alkaloids exhibited promising cytotoxic activity (IC50=5.3 and 13.8µg/mL, respectively) when compared to the standard Doxorubicin (IC 50 = 0.469µg/mL). The antimicrobial activity of total ethanol and alkaloid mixtures of both organs showed significant activity for all the extracts but more prominent for the alkaloid mixtures, against all the selected strains of fungi, Gram(+ve) and Gram(-ve) bacteria except *Enterococcus faecalis*. MICs of the alkaloid mixtures of both organs were also estimated. It could be concluded that the alkaloid mixtures of fruits and flowers of the Egyptian *Pancratium maritimum* L. exhibit promising cytotoxic activity against colon carcinoma cells and effective antimicrobial natural source.
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Key Words: *Pancratium maritimum* L.; Amaryllidaceae; alkaloids; colon carcinoma; antimicrobial.

1.Introduction

Natural products provide safe alternatives to synthesized medicines. Only 15% of the known sources in the world have been screened for their therapeutic purpose. Plants of family Amaryllidaceae showed a great attraction due to presence of specific bioactive metabolites. It consists of about 60 genera and around 800 species, widely distributed in the world (**Kornienko and Evidente, 2008**). Genus *Pancratium*, belonging to Amaryllidaceae, includes about 21 species. The wild Egyptian *Pancratium maritimum* L. (sea daffodil) is a bulbous perennial plant growing along the sandy coasts of the Mediterranean Sea (**Kaya *et al.*, 2010**). Bulbous plants have proven to contain unique biologically active compounds which could provide a lead in novel pharmaceutical products. Investigation of the Egyptian *P. maritimum* L. resulted in the isolation of several types of alkaloids as lycorine, tazettine, pancracine, lycorenine, galanthamine, homolycorine, haemanthidine, sickenbergine, demethylhomolycorine, hippadine, trispheridirine, haemanthamine, pseudolycorine and 11-hydroxyvittatine (**Kornienko and Evidente, 2008**). The majority of compounds present in Amaryllidaceae are bioactive alkaloids with different structures and are uniquely associated with its members. This fact encourages the authors to test the alkaloid mixtures for their synergistic activity.

The present study aims testing the synergistic effect of the alkaloid mixture of each of the two organs (fruits and flowers) of the Egyptian *Pancratium maritimum* L. on the cytotoxic and antimicrobial activities and analyzes their components of alkaloids using GC/MS analysis technique.

2. Material and Methods

Plant material

The fresh fruits and flowers of *Pancratium maritimum* L. were collected in August, 2010, from Mediterranean cost, Matrouh, Egypt. The plant was kindly identified by Dr. Mohamed Gibali, Senior Botanist and a voucher specimen, no. BUPD24**,** is deposited in Pharmacognosy Department, Faculty of Pharmacy, Beni-Suef University.

Extraction and isolation of alkaloids mixture

Fresh plant organs (fruits and flowers, 200g each) were cut into small pieces and extracted separately with 95% ethanol. The extracts were concentrated *in vacuo*, acidified with dil. HCl acid to pH 1-2 and defatted with petroleum ether (40-60). The acidic aqueous phase was then alkalinized with NH_4OH (25%) to pH 10-11 and the alkaloids were extracted three times with petroleum ether (40-60). The petroleum ether extracts were combined, dried over anhydrous Na_2SO_4 and evaporated. The residues obtained were dissolved in methanol and subjected to GC/MS analysis.

(a)

(b)

Fig. (1): *Pancratium maritimum* L. Fruits (a) and Flowers (b)

Gas chromatography coupled with mass spectrometry (GC/MS) analysis of alkaloid mixture

Analyses with GC/MS were performed using a Hewlett Packard 6890/MSD 5973A instrument operating in EI mode at 70 eV. HP_5 MS column (30 m/0.25 mm/0.25 µm) was used. The flow rate of carrier gas (He) was 1 ml/min. Injector temperature was 280°C. The temperature program was 80°C – 280°C at 10°C/min and 10 min hold at 280°C. Identification of the alkaloids was confirmed by comparing the mass spectral data with the standard compounds from database NIST 05 (a Hewlett Packard Mass Spectral Library, Hewlett Packard, Palo Alto, CA, USA) or comparing with the reported data. Results are presented in Table (1).

Cytotoxic activity using viability test

The mammalian colon cell lines, HCT (obtained from American Type Culture Collection (ATCC), were propagated in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% heat – inactivated fetal bovine serum, HEPES buffer, 1% L-glutamine and 50µg/ml gentamycin (from BioWhittaker® Lonza, Belgium). All cells were maintained at 37°C in a humidified atmosphere with 5% CO_2 and subcultured two times a week. The cytotoxic assay was carried out according to **Mosmann, 1983 and Vijayan *et al.*, 2004.** All experiments were carried out in triplicate and viable cells yield was determined by a colorimetric method (absorbance at 490 nm) using Dimethyl sulfoxide (DMSO) as control and Doxorubicin as standard.

Antimicrobial activity

The antimicrobial assay was determined using agar well diffusion method as described by **Holder and Boyce, 1994**. The tested strains were subcultured on nutrient agar medium for bacteria and Saboroud glucose agar for fungi (Oxoid laboratories, UK). Ampicillin was used as standard for gram positive bacteria, gentamicin for gram negative and amphotericin B for fungi. The plates were done in triplicates. Bacterial cultures were incubated at 37°C for 24hrs while fungal cultures were incubated at (25 - 30°C) for 3 – 7 days. Antimicrobial activity was determined by measurement of inhibition zone (**Agwa *et al.*, 2000**) and results were compiled in table (2).

The minimum inhibitory concentration (MIC) of the samples was estimated in triplicate for each tested microorganism (**Doughari, 2006**) and results were presented in table (3).

3. Results and Discussion

Alkaloids of fruits and flowers of *Pancratium maritimum* L

Analysis of the underivatized alkaloid mixture by GC/MS techniques was reported to retain the characteristic mass fragmentation pattern and showed better results than silylation before analyses **(Berkov *et al.*, 2004).** The main alkaloid in both organs (fruits and flowers) of *Pancratium maritimum* L. was Tazettine (21.97% and 25% respectively). Results from Table (1) showed the presence of higher number of alkaloids in fruits than flowers (17 and 13 peaks respectively). For fruits, 14 alkaloids were identified among 17, where as in flowers 11 alkaloids were identified among 14. The most predominant alkaloids in both organs were Tazettine, Lycorine, N-demethyl galantamine and galantamine (21.97%, 17.09%, 14.93 and 14.20% respectively) while in flowers (25%, 8.48%, 10.03% and 14.20% respectively). Trisphenidine was not present in fruits, whereas Crinine, N-formyl galanthamine, Buphanisine, Buphanidrine, Buphanamine, Anhydro-lycorine, Crinamine and Crinane – 3 – one where absent from flowers. This is the first report on the alkaloid content of the fruits of Egyptian *Pancratium maritimum*. The alkaloid content of flowers of *Pancratium maritimum* L. was previously studied (**Abou-Donia *et al.*, 1992; Youssef and Frahm, 1998;.Youssef, 1999; Youssef, 2003**). It is the first report on GC/MS analysis of the alkaloid mixture of flowers. Structures of the major identified alkaloids are presented in Figure (3).

Cytotoxic activity against HCT

The alkaloid mixtures of both organs of *Pancratium maritimum* L. showed a promising inhibition activity against colon carcinoma cells, whereas the fruits were more potent (IC 50= 5.3 µg/mL) than flowers (IC 50 = 13.8µg/mL) when

compared to the standard Doxorubicin (IC 50 = 0.469µg/mL).

Amaryllidaceae alkaloids are well known for their cytotoxic activity (**Pettit *et al.*, 1995**; **Weniger *et al.*, 1999**; **Mc Nulty *et al.*, 2007**). The latter could be attributed to the presence of Lycorine which is the most frequent alkaloid found in Amaryllidaceae plants (**Sener *et al.*, 1993**). It is a pyrrolo[*de*]phenathridine ring type of alkaloid. It has been proven to have cytotoxic activity **(Cambell *et al.*, 1998; Van Goietsenoven *et al.*, 2010**) by reducing the cell survival by inducing apoptosis of tumor cells (**Zupko *et al.*, 2009).** It is present in fruits in a double amount (17.09%) when compared to flowers (8.48%). Tazettine, which predominates in the alkaloid mixture of both organs (21.97% and 25% in fruits and flowers respectively), exhibited cytotoxicity (**Weniger *et al.*, 1995).** The effect could also be due to presence of the crinine type of alkaloids (like Crinan – 3 – ol, Crinamine and Crinane – 3 – one) which are identified in flowers (1.2%, 5.64% and 0.0% respectively) in fruits (0.05%, 4.82% and 0.19% respectively). It was reported that Crinamine induces apoptosis, a programmed cell death, which eliminates the damaged cells and plays a physiological role during embryonic development (**McNulty *et al.*, 2007**). It was also reported that Trispheridine, which is present in flowers and absent from fruits, was able to induce apoptosis (**Zupko *et al.*, 2009**). These previous results could explain the cause of activity of both organs as cytotoxic with increase in activity of the fruits.

Table 1: Alkaloids mixture analyzed by GC/MS of fruits and flowers organs of Egyptian *Pancratium maritimum* L.

Peak No.	Alkaloid Compound	[M+] and characteristic ions m/z (% rel. int.)	Fruits		Flowers		Reference (of MS data)
			Rt	(%)	Rt	(%)	
1.	Trispheridine	223 (100), 222 (36), 164 (13), 138 (18)	-----	-----	17.61	4.37	NIST05
2.	Fr1[b]	228 (15), 227 (100), 226 (70), 212 (13), 199 (36)	16.43	0.41	-----	------	NIST05
3.	N- Formyl galanthamine	243 (24), 225 (17), 224 (100), 167 (6), 166 (9)	16.68	0.72	-----	------	Berkov *et al.*, 2004
4.	Fr-2[b]	257 (33), 239 (16), 238 (100), 223 (41), 222 (15)	16.97	7.89	-----	------	NIST05
5.	Fr-3[b]	240 (6), 239 (41), 238 (100), 180 (10), 139 (5)	17.73	2.68	-----	------	NIST05
6.	Galantamine[a]	287 (80), 286 (100), 244 (22), 216 (28), 174 (22)	18.99	14.20	18.63	10.03	NIST05
7.	N- Demethyl galantamine[a]	274 (15), 273 (95), 272 (100), 230(32), 202 (22)	19.69	14.93	19.01	23.71	Berkov *et al.*, 2004
8.	Buphanisine	285 (55), 284 (89), 271 (100), 266 (21), 254 (20)	19.16	0.85	-----	------	Berkov *et al.*, 2004
9.	Crinan- 3-ol	272 (28), 271 (100), 199 (60), 187 (52), 115 (23)	20.03	0.05	19.19	1.20	NIST05
10.	Fl-1[b]	282 (19), 281 (100), 263 (17), 262 (20), 250 (34)	-----	------	19.46	3.39	NIST05
11.	Buphanidrine	315 (26), 300 (53), 232 (15), 231 (100), 70 (37)	20.25	1.45	-----	------	Cahlikova *et al.*, 2011
12.	Fl-2[b]	249 (56), 248 (100), 191 (9), 190 (20), 95 (9)	-----	------	20.30	4.65	NIST05;
13.	Galanthane	281 (10), 251 (40), 250 (100), 192 (11), 191 (11)	20.47	3.67	-----	-----	NIST05
14.	Tazettine[a]	331 (29), 298 (22), 248 (15), 247 (100), 201 (15)	22.78	21.97	20.63	25.00	NIST05
15.	Pancracine	287 (100), 286 (25), 223 (23), 199 (23), 185 (31)	23.43	1.78	20.96	2.58	NIST05
16.	Fl-3[b]	259 (18), 258 (100), 211 (15), 186 (16), 181 (16)	-----	------	21.15	1.88	NIST05
17.	Lycorine[a]	287 (29), 286 (32), 268 (22), 227 (24), 226 (100)	24.25	17.09	21.56	8.48	NIST05
18.	Buphanamine	287 (100), 188 (66), 176 (95), 175 (62), 174 (91),	21.72	0.97	-----	------	Cahlikova *et al.*, 2011
19.	Anhydro-lycorine	250 (9), 249 (57), 248 (100), 190 (21), 95 (9)	22.00	5.58	-----	------	Berkov *et al.*, 2011
20.	Crinamine	302 (19), 301 (100), 225 (13), 211 (14), 115 (8)	25.02	4.82	22.16	5.64	Cahlikova *et al.*, 2011
21.	Crinane –3– one	345 (24), 239 (19), 238 (100), 210 (39), 152 (18)	32.81	0.19	-----	------	Berkov *et al.*, 2004

Fr-1, 2 and 3: unidentified compounds from fruits Fl-1, 2 and 3: unidentified compounds from flowers
[a] predominant alkaloid in both organs [b] not identified

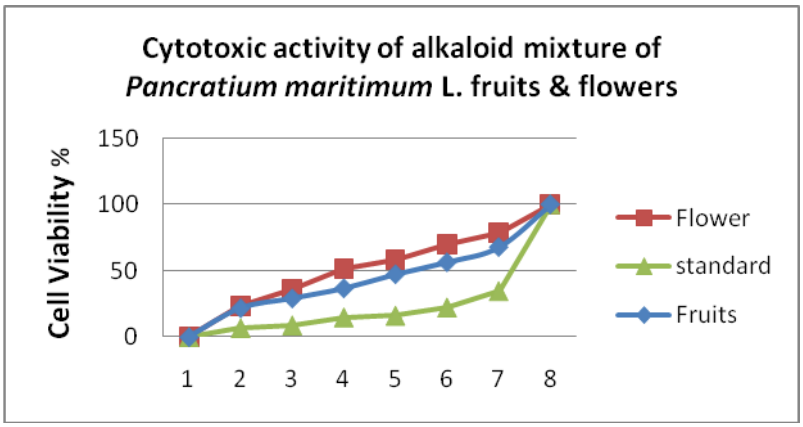


Fig. (2): Evaluation of Cytotoxic activity of alkaloid mixture of fruits and flowers of *Pancratium maritimum* L.

Antimicrobial activity

Table (2) compares the antimicrobial activity of the two organs (fruits and flowers) of *Pancratium maritimum* L. using agar dilution method. Results revealed the significant antimicrobial activities against the selected strains of fungi, Gram (+ve) and Gram (-ve) bacteria of the alkaloid mixtures of both organs when compared to their total ethanol extracts where as the alkaloid mixture of flowers was slightly more active than fruits. Both alkaloid mixtures were inactive against *Enterococcus faecalis* (RCMB 010068). Amaryllidaceae alkaloids (**Ieven *et al.*, 1979**) are known for their antibacterial (**Elgorashi *et al.*, 2003**) and antifungal activities (**Sur-Altiner *et al.*, 1999**). It is reported that Lycorine showed antifungal activity against *Candida albicans* (**Evidente *et al.*, 2004**). Also it inhibits the growth of some yeast (**De Laurentis *et al.*, 2004**) but it is resistant to bacteria.

Table 2: Mean zone of inhibition of total ethanol and alkaloid mixture of fruits and flowers of *Pancratium maritimum* L. against selected microorganisms.

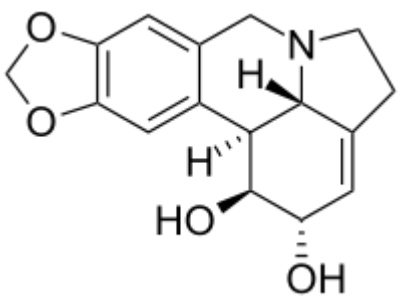
Tested microorganisms	Ethanol extract of fruits	Total alkaloid mixture of fruits	Ethanol extract of flowers	Total alkaloid mixture of flowers	Standard
Fungi					Amphotericin B
Trichophyton mentagrophytes (RCMB 08925)	16.2±0.44	19.3±0.63	15.3±0.25	20.9±0.58	22.3±0.44
Aspergillus fumigatus (RCMB 02569)	17.1±0.58	20.4±0.44	16.7±0.63	22.8±0.44	25.8±0.25
Rhizopus microsporus (RCMB 03628)	19.2±0.63	23.1±0.37	17.8±0.44	25.3±0.25	26.7±0.37
Candida glabrata (RCMB 05274)	15.1±0.25	16.0±0.44	14.6±0.25	18.6±0.63	20.4±0.58
Candida tropicalis (RCMB 05039)	13.9±0.37	14.9±0.25	11.2±0.58	17.2±0.25	21.6±0.25
Gram positive bacteria					Ampicillin
Streptococcus pneumonia (RCMB 010010)	16.9±0.25	18.1±0.37	13.2±0.58	19.3±0.25	26.4±0.37
Enterococcus faecalis (RCMB 010068)	NA	NA	NA	NA	20.3±0.13
Gram negative bacteria					Gentamicin
Klebsiella pneumoniae (RCMB 0010093)	17.3±0.25	19.6±0.44	14.6±0.25	20.6±0.37	22.8±0.22
Salmonella typhimurium (RCMB 010072)	16.9±0.37	17.2±0.37	15.3±0.44	22.8±0.63	28.8±0.24

NA: No activity

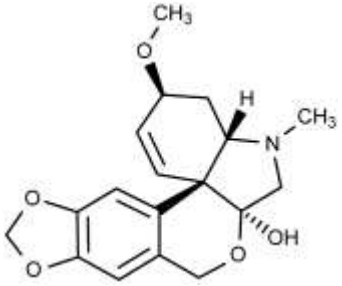
Table 3: Minimum inhibitory concentrations (MIC) of tested alkaloid mixture of fruits and flowers of *Pancratium maritimum* L. against selected microorganisms.

Tested microorganism	**Alkaloid mixture of Fruits**	**Alkaloid mixture of Flowers**	**Standard**
Fungi			Amphotericin B
Trichophyton mentagrophytes (RCMB 08925)	3.9	0.98	0.49
Aspergillus Fumigatus (RCMB 02569)	1.95	0.49	0.03
Rhizopus microsporus (RCMB 03628)	0.24	0.06	0.015
Candida glabrata (RCMB 05274)	31.25	3.9	0.98
Candida tropicalis (RCMB 05039)	62.5	15.63	0.49
Gram positive bacteria			Ampicillin
Streptococcus pneumonia (RCMB 010010)	7.8	3.9	0.03
Enterococcus faecalis (RCMB 010068)	NA	NA	0.98
Gram negative bacteria			Gentamicin
Klebsiella pneumoniae (RCMB 0010093)	1.95	0.98	0.24
Salmonella typhimurium (RCMB 010072)	15.63	0.24	0.007

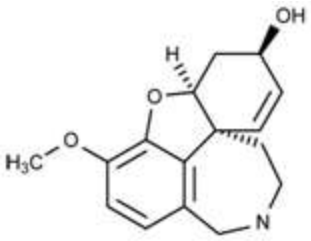
NA: No activity



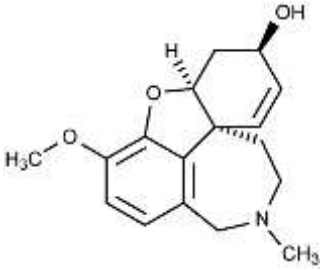
Lycorine



Tazettine



N-demethyl galantamine



Galantamine

Fig. (3): structures of major alkaloids identified in fruits and flowers of *Pancratium maritimum* L.

No conflict of interest.

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