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LIPID FRACTION CONSTITUENTS AND EVALUATION OF ANTI-ANAPHYLACTIC ACTIVITY OF *PRUNUS MAHALEB* L. KERNELS

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Abstract

The lipid fraction constituents as well as evaluation of anti-anaphylactic activity of *Prunus mahaleb* L. Kernels were studied. *Prunus mahaleb* L. kernels were obtained from the local market in Cairo, Egypt. Investigation of the fatty acids revealed that oleic and linoleic acids are the major constituents. 12 compounds were identified from the hydrocarbon fraction. The sterol fraction comprises of cholesterol, stigmasterol, β-sitosterol and campesterol. The pharmacotoxicity studies were carried out on total and defatted ethanolic extracts as well as the oil fraction. The oil fraction proved to be extremely safe and free from any acute lethal toxicity in intraperitoneal (i.p.) and oral doses up to 100 ml/kg. *Invivo* assessment of prophylactic efficacy was afforded by 7 days course of daily medication schedule of sensitized adult male guinea pigs against ovalbumin bronchospasm. The prophylactic anti-inflammatory activity of the total ethanolic extract was higher than that of the defatted ethanolic extract. In addition, the lipid fraction of *Prunus mahaleb* L. kernels evoked complete anti-inflammatory efficacy among the survival animals receiving low and medium doses.

Key words: *Prunus mahaleb* L., Rosaceae, lipids, fatty acids, toxicity, antianaphylactic activity.

Introduction

The genus *Prunus* belongs to the family Rosaceae and comprises more than 400 species including many desirable ornamentals as well as the stone fruit-plums, apricots, almonds, peaches, and cherry laurels (Hortus Third, 1976). *Prunus mahaleb* L. commonly known in Europe as santa lucia cherry and in the Arabia as mahleb, has been used in folk medicine as a tonic for sensory organs and the heart, in the treatment of asthma, and relief of pains arising from liver, kidney and gastro intestinal troubles. Also in the Arabia, the kernels are used as sedative and vasodilator as well as for scenting and preservation purposes (Al-Said and Hifnawy, 1986). In Egypt, the plant is not cultivated, but the fruits are imported to be used in the baking and candy industries. The inner most kernel is ground and mixed with white flour as a flavoring material (Marcos and El-Dakhakhany, 1962).

Experimental

General

GLC analysis was carried out using Pye Unicum gas chromatograph series 304 GC.
Column: Coiled glass column (2.8 m X 4 mm) packed with Diatomite C (100-120 mesh) and coated with 1% OV-17.

Plant material

Prunus mahaleb L. kernels (voucher number s.n.118/4MP) were obtained from the local market in

Cairo, Egypt and were identified by Prof. Kamal H. El-Batanouny, Botany Dept., Cairo University, Egypt. They were freed from foreign materials and carefully rubbed between soft cloth to remove dust and then macerated.

Extraction and isolation

1 kg of the ground kernels were extracted with pet. ether (40-60°C) using the percolation method until exhaustion. The pet. ether extract was evaporated at 40 °C *in vacuo* giving a light yellow transparent oil with yield about 36%. 5 g oil was saponified by refluxing with 50 ml N/2 alcoholic KOH for 6 hours. The unsaponifiable matter was extracted with ether (3 X 50 ml). The combined ethereal extract was washed with distilled water, dehydrated over anhydrous sodium sulphate and evaporated in *vacuo* to give a semisolid residue which was subjected to GLC analysis under the following conditions:

Temperature programming:
Column: 70 - 270 °C, 10 °C/min
Injector: 300 °C
Detector: 300 °C, FID.

Flow rate of gases:
N_2 : 30 ml/min
H_2 : 33 ml/min
Air: 330 ml/min.
The obtained results were tabulated in Table 1

Table 1: Retention times and relative percentages of the hydrocarbons fraction components

n-alkane	Retention time	Relative percentage
C_{10}- unsat.	0.60	1.75
C_{10}	1.35	0.17
C_{18}- unsat.	4.00	0.47
C_{18}	4.95	4.86
C_{19}	6.25	4.56
C_{20}	6.65	9.10
C_{23}	7.20	25.00
C_{24}	7.60	9.97
C_{25}	8.30	25.93
C_{27}	9.30	4.77
C_{28}	9.75	7.15
C_{32}-unsat.	10.10	6.27
Cholesterol	11.50	14.83
Stigmasterol	12.20	17.47
B- sitosterol	13.20	48.94
Campesterol	13.45	18.76

The hydro-alcoholic soap solution after saponification (mother liquor) was rendered acidic (pH 2) with 5 % sulphuric acid, extracted with ether (3 X 50ml). The combined ethereal extract was washed with distilled water till free from acidity and dehydrated over anhydrous sodium sulphate. After filteration, the ether was evaporated in *vacuo* at 40 °C till dryness to give the fatty acids.

1 gm of the total fatty acids was dissolved in 50 ml dry methanol containing 4-5 % dry HCl and refluxed on a boiling water bath for two hours. The reacted mixture was diluted with distilled water and extracted with successive portions of ether (3 X 50 ml). The combined ether extract was washed with distilled water till free from acidity and then over anhydrous sodium sulphate, filtered and evaporated in *vacuo* at 40 °C, to give the methyl esters of the total fatty acids.
GLC analysis of the fatty acids methyl ester was carried out under the following conditions:

The obtained results were tabulated in Table 2

Pharmacological studies
Test material

Three extracts of crude powdered *Prunus mahaleb* L. kernels were prepared and subjected to acute lethal

toxicity in adult normal albino mice of both sexes and subsequently employed in pharmacological screening procedures for beneficial anti-anaphylactic activity. These comprised:

- 80% ethanolic extract containing (13% w/w).
- Defatted ethanolic extract (8.225% w/w).
- Petroleum ether extract incorporating lipid fraction (36% w/w).

Table 2: Retention times and relative percentages of the fatty acids fraction

Fatty acid	No. of carbons	Retention time	Relative percentage
Hexanoic	6:0	1.86	0.06
Heptanoic	7:0	2.77	0.03
Octanoic	8:0	3.86	0.27
Nonanoic	9:0	5.09	0.08
Decanoic	10:0	6.45	0.08
Undecanoic	11:0	7.88	0.04
Dodecanoic	12:0	8.66	0.18
Tridecanoic	13:0	10.58	0.18
Myristic	14:0	11.73	0.07
Myristoleic	14:1	12.39	0.18
Pentadecanoic	15:0	12.76	0.99
Palmitic	16:0	14.12	8.20
Heptadecanoic	17:0	15.13	0.41
Stearic	18:0	15.51	0.46
Oleic	18:1	17.08	53.12
Linoleic	18:2	17.89	35.04
Arachidic	20:0	20.44	0.60

Test animals

Adult normal albino mice of uniform strain with equal sex distribution ranging in body weight from 18-22 gm were used for carrying out the acute toxicity study.
Dunkin Hartley adult male albino guinea pigs ranging in body weight from 250-300 gm were used for carrying out the anti-anaphylactic activity testing.
All animal procedures were performed after approval from the Ethics Committee of the National Research Centre and in accordance with the recommendations for the proper care and use of laboratory animals (NIH publication No. 85-23, revised 1985).

Toxicological studies

Acute i. p. lethal toxicity tests in adult normal albino mice of both sexes over 24 hours observation period were carried out.
The LD_{50} was determined using mice according to the procedure described by (Balazs, 1970). The LD_{16} and LD_{84} were also determined. The symptoms of acute toxicity and post-mortem findings were recorded.

Anti-anaphylactic activity testing

This study aims at comparative assessment of prophylactic efficacies of short term daily repeated medication schedule of sensitized adult male guinea pigs with three graded oral dose levels of total and defatted ethanolic extracts as well as the oil fraction against anaphylactic bronchospasm induced by injection of ovalbumin in conformity with procedure of (Engineer et al. 1976). Three equal sized medicated groups of 15 animals each, subdivided into three specified graded dose levels yielding 45 total medicated animals. Ten guinea pigs serving as parallel non-medicated controls divided into two groups; non-sensitized non-medicated group and sensitized non- medicated group were also used. Each animal was initially injected with pure ovalbumin colloidal solution (10%) in a dose of 30 mg subcutaneously coupled with 70 mg by i. p. route of systemic administration. Subsequent repetition of dual ovalbumin dosing after the lapse of three weeks from initial sensitization is expected to evoke hypersensitivity reaction reflected by severe bronchospasm and consequent development of asphyxial convulsive fits in control guinea pigs. Recommended graded oral dose levels of the test *Prunus mahaleb* L. extracts adapted in short term daily repeated prophylactic medication schedules were

selected on basis of their comparability to 2.5%, 5% and 10% fractions of extrapolated guinea pigs equivalent of acute median lethal dose "A-LD_{50}" in mice. These are stated here after:

G. P. equivalent	**total ext.**	**defated ext.**	**oil fraction**
2.5% fraction	1	0.7	1.5
5% fraction	2	1.4	3
10% fraction	4	2.8	6

Doses in g/kg b. wt. and ml/kg

Medication courses were started on the 15th day post ovalbumin injection and continued till the 21st day. Two hours after the last dose, challenge doses of 1% ovalbumin solution were given by subplantar injection.
The skin thickness was measured using ODITEST (OD120 RK/K GAUGE) immediately after the subplantar injection.

Statistical analysis

Statistical evaluation of acute toxicity was done using (Litchfield Jr. and Wilcoxon, 1949).

Results and discussion

Lipid fraction investigation

The chemical investigation of the oil fraction resulted in the isolation and identification of 12 hydrocarbons (n-c_{10} – n-$c_{32\ unsat.}$), sterols which were identified as sitosterol (major constituent), campesterol and relatively small amounts of stigmasterol and cholesterol and 27 fatty acids, 17 of them were identified. Oleic acid ($C_{18:1}$) and linoleic acid ($C_{18:2}$) were the major constituents. Neither GC or GC/MS could confirm the presence of eleostearic acid as reported earlier (Saffet, 1949)

Acute toxicity studies

Post-mortem findings detectable by naked eye inspection of the internal organs and viscera of the fatally intoxicated mice evidence of death by asphyxia, secondary to respiratory failure, verified by marked congestion and bluish discoloration of the liver, spleen and kidneys as well as dilatation and stagnation of blood on the right of the heart coupled with multiple scattered foci of petechial haemorrhages in the lungs and discrete areas of pulmonary collapse. Marked distention of the stomach and colon was also noted in some of the dead animals at autopsy. Petroleum ether extract of *Prunus mahaleb* L. kernels containing lipid proved to be extremely safe, innocuous and free from any lethal toxicity in i. p. doses reaching up to two folds the lipid content in i. p. LD_{84} of the total ethanolic extract and reaching up to eight folds higher peak for oral dosage. It is also noteworthy that the larger i. p. doses of the petroleum ether extract elicited short phases of initial hyper-excitability, hyper-reflexia, and jumping behaviour before the onset of dose-dependent hypnosedation subsiding within 2 hours post dosing. In contrast, oral overdosage with the petroleum ether extract of *Prunus mahaleb* L. kernels evoked reversible dose proportional grades of hypnosedation not preceded by any psychomotor manifestations. For the total and the defatted ethanolic extracts, it was found that both showed low toxicity (LD_{50} = 7 and 4.5 g/kg, respectively) and that the defatted ethanolic extract despite its relatively lower content (8.225%), emerged as a more potent lethal toxic agent than the total ethanolic extract.

Anti-anaphylactic activity

In vivo assessment studies revealed insignificant fluctuations among the mean initial body weight records in the various extracts treated subgroups from the mean respective control values. In contrast, the discrepancies between the mean terminal body weight records in guinea pigs in the treated subgroups mostly exhibiting variable magnitudes of reductions below the corresponding mean control value proved to be statistically significant in 7 out of 9 medicated subgroups. The death rate among the ovalbumin-sensitized non medicated control amounted to 60% while mortality was peculiarly lacking among non- sensitized non medicated control. No fatalities were encountered among the three subgroups receiving daily repeated oral medication with total ethanolic extract of the crude powdered kernels confirming complete prophylactic efficacy for this plant extract against crude anaphylactic lethal toxic reaction.

The outcome of oral medication with defatted ethanolic extract was reflected by freedom from any deaths among subgroups receiving medium and high doses. However, low dose medication afforded protection in 4 out of 5 guinea pigs yielding low grade residual fatality of the order of 20%. Likewise, the same order of partial

prophylactic efficacy coinciding with 80% was not among the two medicated subgroups receiving low and medium doses of the oil fraction. In striking contrast, high dose medication of the three surviving guinea pigs failed to elicit any protection against lethal anaphylactic reactions yielding 100% incidence frequency of mortality. The anti-inflammatory activities of the three specified extracts were as follow:

- Among the three subgroups receiving the total ethanolic extract, virtual abolition of oedema swelling was achieved in subgroups receiving medium and high doses while the low dose medication eluicited considerable reduction by 92.9% in the intensity of oedema swelling.
- Among the three subgroups receiving the defatted ethanolic extract, weaker prophylactic anti-inflammatory activity, relative to the total ethanolic extract, was achieved.
- Among the three subgroups receiving the oil fraction, complete protective anti-inflammatory efficacy was achieved among the subgroups receiving the low and medium doses. The failure to achieve any evidence of oedema swelling among the three surviving guinea pigs receiving high dose medication could be ascribed to the occurrence of rapid death allowing no time for odeama development.

It should be added that the sedation, hypnosis and CNS depression noticed on the animals during experiments could be attributed to the coumarins present in these kernels (Marcos and El-Dakhakhany 1962; El-Dakhakhany and Fayez 1963). Also, the noticed death among the subgroups receiving high dose medication of the oil fraction could be due to benzaldehyde accumulation with repeated daily medication.

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