COMPARATIVE STUDY OF ALKALOIDS COMPOUNDS FROM SOME TAXA IN CUCURBITACEAE FAMILY IN NAJAF CITY

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Abstract
Alkaloid compounds were studies in this research and it was found that Citrullus colocynthis is unique in the presence of quinoline, nicotinamide, 4-methoxy quinoline and 4,7 benzoquinoline alkaloid compounds, while Citrullus lanatus and Cucumis melo share the presence of pyrrolezanthine, b-(1- pyrazolyl) alanine and pyro imidazole alkaloid compounds, but in different proportions, as for Cucumis melo. var. flexuosus and Cucumis sativus, they share the presence of quinine, nicotine, strychnine and ephedrine and also in different proportions, as for Cucurbita maxima, Cucurbita pepo and Lagenaria siceraria, they share the presence of heliotrine, isoquinoline and restrosine in different proportions.
Keyword: Cucurbitaceae, alkaloid compound, secondary metabolism.

Introduction

The cucurbitaceae is a plant family consisting of about 965 species in around 95 genera (Christenhuszand Byng, 2016). The plant in this family is grown ever were the tropics and in temperate areas. Cucurbithave economic importance, the fruits of many species are used as human foods, numerous species of Cucurbit plants have some important chemical compounds with important medicinal potential (Luchian and Tedosiu 2019). Cucurbit are annual or perennial herbs, most species are climbing or prostrate, fast- growing vines with long-stalked palmate leaves, tendril is a simple branched spirally coiled (Rezk et-al, 2022).

Secondary metabolites are compounds used in many aspects, both pharmaceutical and industrial, they are believed to be produced for defensive purposes, (AL-Aridhee et-al, 2019). There are three major classes of plant secondary metabolites, namely alkaloids, phenols, and terpenoids. Alkaloids originate from aromatic amino acids or from aspartate, glutamate, or glycine in the plant cell, (Luna-Guevara, et- al, 2018). Medicinal plants including cucurbitaceae receive attention to research centers because of their special importance in safety of communities, the curative properties of medicinal plants are mainly due to the presence of various complex chemical substances of different composition which occur as secondary metabolites (Salama, 2012). Most vegetables from the cucurbitaceae family have a rich chemical composition, which improves food health and stability because of their antioxidant power (Busuioc et-al, 2020). During the regular metabolic activities of plants, certain chemical compounds known as phytochemicals are formed, plants produce these chemicals for their defense mechanism (Harborne, 1973). (Mukherjee et-al, 2022) showed there are two types of metabolites in food plants of the cucurbitaceae family, which are represented by primary metabolites, which include proteins, vitamins, minerals, carbohydrates, dietary fiber, and saturated and unsaturated oils, and secondary metabolites, which include terpenes, phenols, and alkaloids.

Materials and methods:

Samples were collected from the leaves of the species and varieties of plants of the cucurbit family under study, represented by field visits and tours to some agricultural areas in Najaf Governorate, startingin September 2023, then the leaves were cleaned of any dust and impurities stuck to them, and they weredried under room temperature conditions on filter papers, taking care to turn them daily and ensuring thatthey did not rot or become infected with viral or fungal diseases, then they were collected in dry paper bags and stored under room temperature conditions, free of moisture, until use, then the leaves of the plants under study were ground individually using an electric grinder to obtain a smooth mixture and stored in plastic containers until use (Hussain, 2023).

Quantitative estimation of alkaloid compounds in the leaves of the species under study by thehigh-performance liquid phase separation method.

Extraction

Plant samples 1.0 gram of leaves homogenized, grinding to fine powder, dissolved in 3% H2SO4 for2 h at room temperature. Filtration on 2.5 um filter paper, the supernatants were made alkaline with 25% NH4OH (PH 9.5) and applied to Extrelut (Merck) columns. The alkaloids were eluted by CH2Cl2 (6 ml/1 g Extrelut) and the extracts were evaporated to dryness by using stream of nitrogen, thus obtained residueswere resolved in 1 ml CH3OH for the further analysis by HPLC according the optimum separation of authentic standard, then the concentration were determined by comparison between area of standard withthat of sample under the same separation condition (Pellati et-al, 2008).

Calculation:

concentration of sample ug/ml = area of sample\area of standard x concentration of standard x dilution factor (Behbahani et al., 2011).

Equipment's:

The separation occurred on liquid chromatography Shimadzu 10AV-LC equipped with binary deliverypump model LC-10A

shimadzu, the eluted peaks were monitored by UV -Vis 10 A-SPD spectrophotometer (Pellati et-al, 2008).

Results

A table (1) showing the retention time and area under the curve for standard alkaloid compounds of thespecies *Citrullus colocynthis* diagnosed with the HPLC device:

Seq.	Subjects	Retention time minute	Area µ volt	Con. 100 µg\ml	Percentage
1	Quinoline	3.077	375306	21.614	21.6%
2	Nicotinamide	3.988	436094	25.1148	25.1%
3	4-methoxy quinoline	4.905	472229	27.1959	27.1%
4	4,7 benzoquinoline	6.722	452772	26.0753	26%

A table (2) showing retention time and area under the curve for alkaloid compounds of the species *Citrullus colocynthis* diagnosed with the HPLC device:

Seq.	Subjects	Retention time minute	Area µ volt	Con. 100 µg\ml	percentage
1	Quinoline	3.053	203783	1085.95	30.5%
2	Nicotinamide	3.975	169510	777.40	21.8%
3	4-methoxy quinoline	4.902	123797	545.48	15.3%
4	4,7 benzoquinoline	6.727	259700	1147.1	32.2%

A table (3) showing the retention time and area under the curve for standard alkaloid compounds of thespecies *Citrullus lanatus* and *Cucumis melo* diagnosed with the HPLC device:

Seq.	Subjects	Retention time minute	Area µ volt	Con. 100 µg\ml	percentage
1	Pyrrolezanthine	2.775	440582	29.3559	29.3%
2	b-(1-pyrazolyl) alanine	3.873	395226	26.3339	26.3%
3	Pyro imidazole	4.95	415267	27.6691	27.6%

A table (4) showing the retention time and area under the curve for alkaloid compounds of the species *Citrullus lanatus* diagnosed with the HPLC device:

Seq.	Subjects	Retention time minute	Area µ volt	Con. 100 µg\ml	percentage
1	Pyrrolezanthine	2.773	122680	835.34	16.3%
2	b-(1-pyrazolyl) alanine	3.883	264729	2009.45	39.3%
3	Pyro imidazole	4.957	313661	2265.97	44.3%

A table (5) showing the retention time and area under the curve for alkaloid compounds of the species *Cucumis melo* diagnosed with the HPLC device:

Seq.	Subjects	Retention time minute	Area µ volt	Con. 100 µg\ml	percentage
1	Pyrrolezanthine	2.773	146448	997.1	21.3%
2	b-(1-pyrazolyl) alanine	3.883	168881	1281.9	27.4%
3	Pyro imidazole	4.943	330949	2390.8	51.1%

A table (6) showing the retention time and area under the curve for standard alkaloid compounds of thespecies *Cucumis melo. Var. flexuosus* and *Cucumis sativus* diagnosed with the HPLC device:

Seq.	Subjects	Retention time minute	Area µ volt	Con. 100 µg\ml	Percentage
1	Quinine	2.16	3600064	25.4883	25.4%
2	strychnine	3.78	351737	24.8989	24.8%
3	Nicotine	4.69	382521	27.078	27%
4	ephedrine	5.965	318340	22.5348	22.5%

A table (7) showing the retention time and area under the curve for alkaloid compounds of the species *Cucumis melo. Var. flexuosus* diagnosed with the HPLC device:

Seq.	Subjects	Retention time minute	Area µ volt	Con. 100 µg\ml	Percentage
1	Quinine	2.6	229647	1913.3	24.3%
2	strychnine	3.775	130442	1112.5	14.1%
3	Nicotine	4.683	210410	1650.1	20.9%
4	ephedrine	5.945	338420	3189.2	40.5%

A table (8) showing the retention time and area under the curve for alkaloid compounds of the species *Cucumis sativus* diagnosed with the HPLC device:

Seq.	Subjects	Retention time minute	Area µ volt	Con. 100 µg\ml	Percentage
1	Quinine	2.583	121769	1014.5	20.6%
2	strychnine	3.767	128562	1096.5	22.2%
3	Nicotine	4.672	179767	1409.8	28.6%
4	ephedrine	5.937	148742	1401.7	28.8%

A table (9) showing the retention time and area under the curve for standard alkaloid compounds of thespecies

Cucurbita maxima. Cucurbita pepo and Lagenaria siceraria diagnosed with the HPLC device:

Seq.	Subjects	Retention time minute	Area µ volt	Con. 100 µg\ml	percentage
1	Quinine	2.483	461109	32.2039	32.2%
2	Heliotrine	3.08	561817	39.2373	39.2%
3	Restrosine	4.253	408916	28.5587	28.5%

A table (10) showing the retention time and area under the curve for alkaloid compounds of the species *Cucurbita maxima* diagnosed with the HPLC device:

Seq.	Subjects	Retention time minute	Area µ volt	Con. 100 µg\ml	percentage
1	Quinine	2.487	170732	925.65	22.4%
2	Heliotrine	3.095	271916	1209.98	29.3%
3	Restrosine	4.222	325893	1992.42	48.2%

A table (11) showing the retention time and area under the curve for alkaloid compounds of the species *Cucurbita pepo* diagnosed with the HPLC device:

Seq.	Subjects	Retention time minute	Area µ volt	Con. 100 µg\ml	percentage
1	Quinine	2.435	174055	1887.3	22.1%
2	Heliotrine	3.043	369749	3290.6	38.6%
3	Restrosine	4.192	272517	3332.1	39.1%

A table (12) showing the retention time and area under the curve for alkaloid compounds of the species *Lagenaria siceraria* diagnosed with the HPLC device:

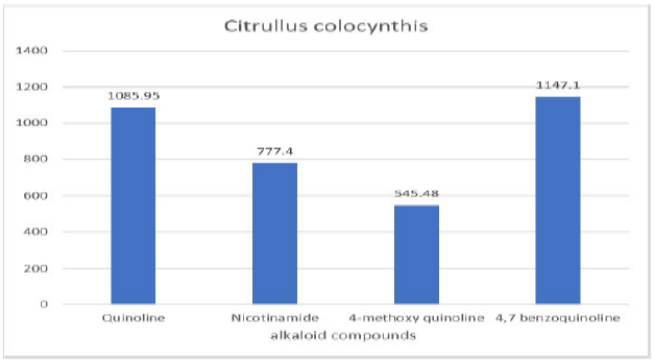
Seq.	Subjects	Retention time minute	Area µ volt	Con. 100 µg\ml	percentage
1	Quinine	2.372	226287	490.7	27%
2	Heliotrine	3.005	324257	577.1	31.8%
3	Restrosine	4.137	304572	744.8	41%

Result and Dissection:

Through our study of alkaloid compounds in the leaves of the species under study, we found that some species share and alkaloid compounds, while some species did not record the presence of some of the compounds mentioned above.

Alkaloids compounds isolated and characterized in *Citrullus colocynthis:*

The leaves of the species *Citrullus colocynthis* contained four alkaloid compounds, and differences were recorded in the concentration of these compounds and the time of their detention, the compound 4,7 benzoquinoline recorded the highest concentration percentage, as it amounted to this (1147.1) µg\gm equivalent to (32.2) % while the lowest concentration percentage was for the compound 4-methoxy quinoline, which amounted to this (545.48) µg\gm the equivalent of (15.3) %, the concentration of the remaining compounds ranges between the two range, as shown in the following figure:



The figure (1) shows the concentration of alkaloid compounds in the species *Citrullus colocynthis*

Alkaloids compounds isolated and characterized in *Citrullus lanatus:*

The leaves of the species *Citrullus lanatus* contained five alkaloids compounds, and differences were recorded in the concentration of these compounds and the time of their detention, the highest concentrationpercentage was for the compound pyro imidazole, which amounted to this (2265.97) µg\gm the equivalent of (44.3) % while the compound pyrrolezanthine recorded the lowest concentration percentage, as it amounted to this (835.34) µg\gm equivalent to (16.3) the b-(1-pyrazolyl) alanine falls within these the two ranges, were the concentration was recorded of (2009.45) the equivalent of (39.3), as shown in thefollowing figure (2):

Alkaloids compounds isolated and characterized in *Cucumis melo:*

The leaves of the species Cucumis melo contained three alkaloid compounds, and differences were recorded in the concentration of these compounds and the time of their detention. The highest concentration percentage was for the compound Pyroimidazole, which amounted to this (2390.8) µg\gmthe equivalent of (51.1) % while the compound Pyrrolezanthine recorded the lowest concentration percentage, as it amounted to this (997.1) µg\gm Equivalent to (21.3) %, the b-(1-pyrazolyl) alanine fallswithin these the two ranges, were the concentration was recorded of (1281.9) µg\gm which is equivalentto (27.4) %, as shown in the following figure:

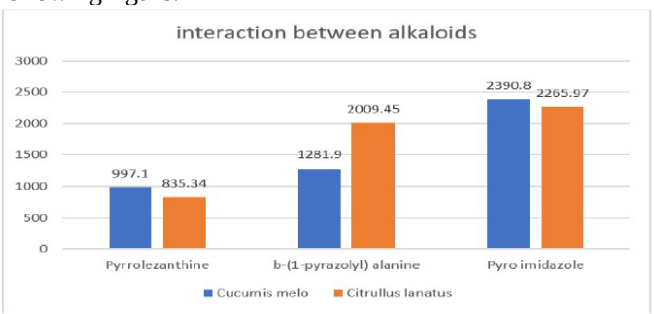


Figure (2) shows the interaction between the concentrations of alkaloids compounds for species *Citrulluslanatus and Cucumis melo.*

Alkaloids compounds isolated and characterized in *Cucumis melo. var. flexuosus*

The leaves of the species *Cucumis melo Var. flexuosus* contained four alkaloid compounds, and differences were recorded in the concentration of these compounds and the time of their detention. The highest concentration percentage was for the compound ephedrine, which amounted to this (3189.2) µg\gm the equivalent of (40.5) % while the compound strychnine recorded the lowest concentration percentage, as it amounted to this (1112.5) µg\gm equivalent to (14.1) % while the concentration of the nicotine compounds recorded (1650.1) µg\gm which is equivalent to (20.9) % and the quinine compounds recorded (1913.3) µg\gm which is equivalent to (24.3) %, as shown in the following figure:

Alkaloids compounds isolated and characterized in *Cucumis sativus:*

The leaves of the species *Cucumis sativus* contained four alkaloid compounds, and differences were recorded in the concentration of these compounds and the time of their detention. The highest concentration percentage was for the compound ephedrine, which amounted to this (1401.7) µg\gm the equivalent of (28.8) % while the compound quinine recorded the lowest concentration percentage, as it amounted to this (1014.5) µg\gm equivalent to (20.6) % while the concentration of the strychnine compounds recorded (1096.5) µg\gm which is equivalent to (22.2) % and the nicotine compounds recorded (1409.8) µg\gm which is equivalent to (28.6) %, as shown in the following figure:

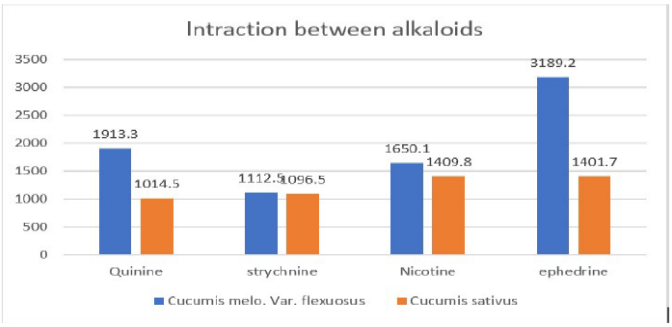


Figure (3) shows the interaction between the concentrations of alkaloids compounds for species *Cucumis melo Var. flexuosus* and *Cucumis sativus.*

Alkaloids compounds isolated and characterized in *Cucurbita maxima*:

The leaves of the species *Cucurbita maxima* contained three alkaloid compounds, and differences were recorded in the concentration of these compounds and the time of their detention. The highest concentration percentage was for the compound restrosine, which amounted to this (1992.42) µg\gm the equivalent of (48.2) % while the compound quinine recorded the lowest concentration percentage, as it amounted to this (925.65) µg\gm equivalent to (22.4) % while the concentration of the heliothrine compounds recorded (1209.98) µg\gm which is equivalent to (29.3) %, as shown in the following figure (8):

Alkaloids compounds isolated and characterized in *Cucurbita pepo*:

The leaves of the species *Cucurbita pepo* contained three alkaloid compounds, and differences were recorded in the concentration of these compounds and the time of their detention. The highest concentration percentage was for the compound restrosine, which amounted to this (3332.1) µg\gm the equivalent of (39.1) % while the compound quinine recorded the lowest concentration percentage, as it amounted to this (18873) µg\gm equivalent to (22.1) %, the concentration of the heliotrine compounds recorded (3290.6) µg\gm which is equivalent to (38.6) %, as shown in the following figure (9):

Alkaloids compounds isolated and characterized in *Lagenaria siceraria:*

The leaves of the species *Lagenaria siceraria* contained three alkaloid compounds, and differences were recorded in the concentration of these compounds and the time of their detention. The highest concentration percentage was for the compound restrosine, which amounted to this (744.8) µg\gm the equivalent of (41) % while the compound quinine recorded the lowest concentration percentage, as it amounted to this (490.7) µg\gm equivalent to (27) % while the concentration of the heliotrine compounds recorded (577.1) µg\gm which is equivalent to (38.8) %, as shown in the following figure:

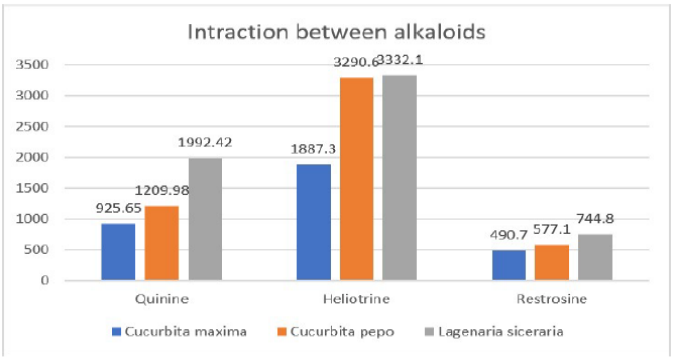


Figure (4) shows the interaction between the concentrations of alkaloids compounds for species *Cucurbita maxima, Cucurbita pepo* and *Lagenaria siceraria.*

Discuss the chemical results

Nowadays, phytochemicals identification in complex plant matrices is a difficult task due to the complexity of their structures and the limited standards commercially available. High performance liquidchromatography (HPLC) is one of the most commonly used separation techniques used to determine thesekinds of bioactive compounds in plant matrix. Lately, the capability of HPLC to separate polyphenols has been well known, being the most commonly used separation technique for determining these compounds. currently, mass spectrometry (MS) is the detection system mainly used due to its high sensitivity and its great potential for identifying compounds Moreover, the use of MS/MS aid enabling the detection of hundreds of compounds within a single extract supplying very useful structural information. (Abu-Reidah,2013).

The studied species of plants of the cucurbit family showed wide variations in terms of their content of secondary metabolite compounds, which increased interest due to their medical and economic importance, in addition to the major taxonomic role

they played in separating the species of the cucurbit family. The results of the chemical study, which reached the isolation and identification of 13 alkaloid compounds in all the studied species belonging to the family, using the high-performance liquid phase separation (HPLC) technique.

for alkaloid compounds *Citrullus colocynthis* was unique in the presence of four alkaloid compounds which includes quinoline' nicotinamide' 4-methoxy quinoline and 4,7 benzoquinoline while the study presented by (Salama, 2012) indicated the presence of tow alkaloid compounds. The following compounds were found in *Citrullus lanatus and Cucumis melo'* represented by pyrrolezanthine' b-(1-pyrazolyl) alanineand Pyro imidazole in, compounds were also found Quinine' strychnine' Nicotine and ephedrine in both*Cucumis melo. var. flexuosus Cucumis sativus and the compounds* heliotrine isoquinoline restrosine in *Cucurbita maxima' Cucurbita pepo and Lagenaria siceraria*.

Conclusions

The chemical study showed the extent of the variations shown by the studied varieties in terms of their content of secondary metabolic compounds in quantity and quality. Thus, these compounds were considered to have comparative taxonomic characteristics separating the varieties and their efficiency in showing the extent of genetic closeness and divergence between the studied varieties. Citrullus colocynthis is unique in the presence of quinoline, nicotinamide, 4-methoxy quinoline and 4,7 benzoquinoline alkaloidcompounds, while Cucumis melo was recorded the highest percentage of pyrrolezanthine and pyro imidazole, Citrullus lanatus was recorded the highest percentage of b-(1-pyrazolyl) alanine, Cucumis melo. var. flexuosus was recorded the highest percentage of quinine, nicotine, strychnine, Lagenaria siceraria, of heliotrine, isoquinoline and Cucurbita maxima was recorded the highest percentage restrosine.

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