Original Article

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Extraction of Anthocyanins from two Types of Cowpea (White and Red) Vigna Unguiculata L. using GCMS Technique

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Abstract

In this study, the anthocyanin dye was extracted from two cultivars of cowpea (white cowpea and red cowpea) Vigna unguiculata L. using acidified methanol (1% HCl in methanol) and acidified ethanol (1% HCl in ethanol). The extract obtained from the extraction was injected directly into the GCMS device, and GCMS is an advanced technology to determine the composition, molecular weight, scientific name of the anthocyanins and the identified compounds together. In this study, several compounds were identified in the anthocyanin extract, and the anthocyanin type was identified, as it was found that white and red cowpea contain Cyanidin 3-glucoside, while Peonidin 3-glucoside was found in red cowpea only.

Keywords:

Anthocyanins, Vigna unguiculata, GC-MASS, Chemical analysis.

Introduction

Anthocyanins represent a large class of polyphenol pigments within the flavonoid family that are ubiquitous in the human diet. Anthocyanins are characterized by a glycon moiety typically conjugated to an OH hydroxyl group in the C ring, making them glycosides. Their non-glycoside analogues (aglycons) known as anthocyanidins are stable poorly and rarely found in nature [1].

To date, more than 700 anthocyanins have been identified but only six account for about 90% of those found in nature: cyanidin (50%), delfindin (12%),malvidin (12%), peonidin (12%), peonidin (7%), and petonidin (7%) [2]. The anthocyanins in colored cowpea make up about 26.3% of all cowpea, and include cyanidin-3-O-glucoside and peonidin-3-O-glucoside, and they account for 90% of the main active ingredients in colored cowpea [3]. Anthocyanin extracts can improve LDL (low-density lipoprotein) and HDL (High-density lipoprotein) levels in humans [4], and can delay cancer development in carcinogenic rodent models [5].

And since the anthocyanin content is very high in colored cowpea, it is considered one of the superior antioxidants in white and red cowpea, which helps prevent the harmful effects of free radicals [6]. And promote blood circulation, slow tissue damage and aging, reduce cholesterol and blood sugar levels.

Anthocyanins have been shown to inhibit the growth of human cancer cells from leukemia, breast, prostate, uterus, lung, vulva, stomach, colon, and colon cancer [7]. Some studies have shown that anthocyanins are absorbed into the blood [8]. Other studies have indicated that anthocyanins are degraded in the intestine, either by intestinal flora or intestinal enzymes [9].

Materials and working methods

1- Origin of the materials and equipment used

The rotary vacuum evaporator is supplied from UNIKAN company (China), GCMS from Cleaver Scientific company (England), ethyl alcohol, methanol and hydrochloric acid from Bio Basic company (Canada), Cyanidin 3-o-glucoside chloride and Peonidin 3-o - Glucoside chloride, Cyanidin chloride, Peonidin chloride, Delphidin chloride, Delphidin chloride and Malvindin

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chloride from Polypenol Lab compeny (Norway).

2- Extraction of anthocyanins from the two types of cowpea under study (white and red)

The anthocyanin dye was extracted according to the method of Lu and Foo (2001) after Malvidine Chloride was dissolved in a solution of 20 ml of water: ethanol 50:50 with 0.1 of HCl, then 50 g of white cowpea seeds were ground with the addition of 25 Delphinidine amalgam as internal standard. The extraction took place twice, each time with continuous shaking for 6 hours at a temperature of 4°C. Then the extract was placed in a centrifuge at 3200 rpm for 20 minutes, then the supernatants were taken and concentrated in a Rotary Vaccum Evaporator at a temperature of 45 °C to get rid of the solvent, then the extract was filtered using filter paper (Whatman No. 450), then it was hydrolyzed. By mixing the supernatants with a water solution for 60 minutes at 100 degrees Celsius, then the samples were directly cooled to room temperature, and then injected with a GCMS device.

The same steps were repeated in the previous experiment in white cowpea, but using Cyanidin 3-o-glucoside chloride, and a third time using o-glucoside chloride Peonidin 3- and a fourth time using Cyanidin chloride, a fifth using Peonidin chloride, and a sixth using. Delphindin chloride. The same steps were repeated in the previous experiment, but using another solvent (methanol) in white cowpea, so that twelve experiments were obtained for white cowpea. The same steps were repeated in the previous experiment, but using ethanol with red cowpea, then using methanol with red cowpea, and the same steps as the previous experiment.

3- Analysis using Gas Chromatograohy Mass Spectrometry (GC-Mass)

The alcoholic extract of cowpea grains was analyzed using a perkin elmer GC clarus500 GC and a mass-srectrometer according to the following analysis conditions:

- The temperature of the injector is 280 degrees Celsius.
- The temperature of the ionic source is 200 degrees Celsius.
- 99% helium gas was used as a carrier gas at a constant flow rate of one ml per minute.
- The injected fluid was a volume of 8 microliters.
- The temperature of the oven is 110 Celsius, as it is programmed automatically with an increase of 10 Celsius/min. The increase in temperature continues until it reaches 280 Celsius and stabilizes for 8 minutes until the end.
- The total time is 32 minutes from starting the device until it is turned off.
- Elite-1 fused silica capillary column consisting of 100% Dimethyl polysiloxane which works in EV70 (Electron Hunting Detector) mode.
- The pressure inside the device is 100 Kpa, at a rate of one ml.min.

Results and Discussion

1- Diagnosis of anthocyanins with white cowpea and ethanol solvent:

Figure (1) indicates the appearance of several peaks of the compounds that make up the recorded white cowpea when dissolved in ethanol alcohol and placed in the GCMS device.

The third peak with a reading (detention time) of 21.214 can be observed, which indicates the presence of anthocyanins. By noting Table (1), we find that the GCMS device has identified the type of anthocyanin, which is Kuromanine, which is C-3-G Cyanidin-3-o-Glucoside)) Figure (2), with the chemical formula C21H21O11 and molecular weight = 449 molar, and his scientific name: (2S,3R,4S,5S,6R)-2{2-(3,4-dihydroxyphenyl)-5,7-dihydroxychromenylium-3-yl}oxy-6-(hydroxymethyl)oxan-3,4,5-triol.

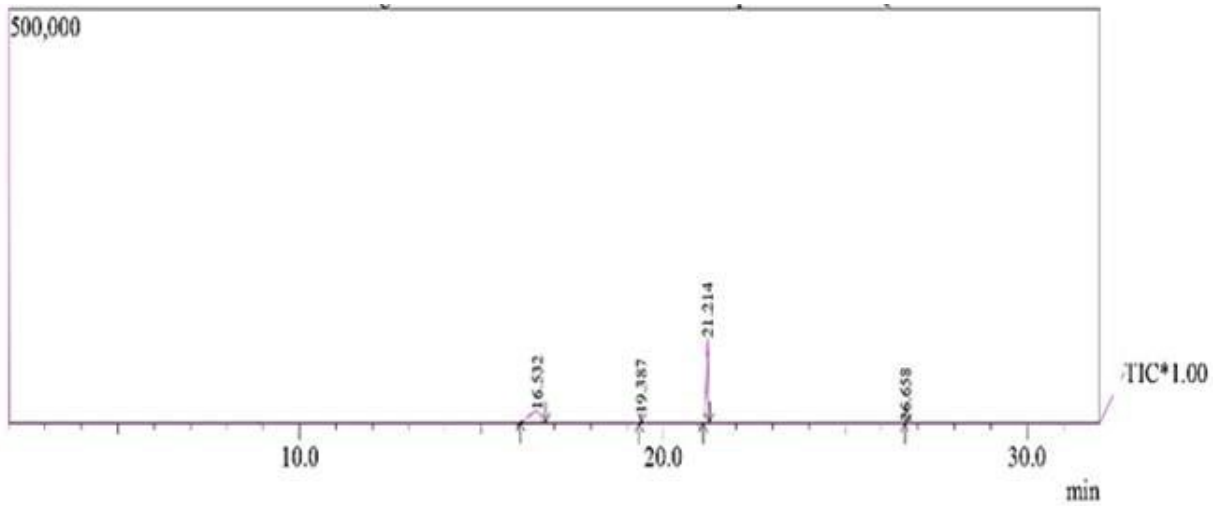

Figure (1): Peaks of anthocyanins and the compounds that make up white cowpea using GCMS and ethanol as a solvent

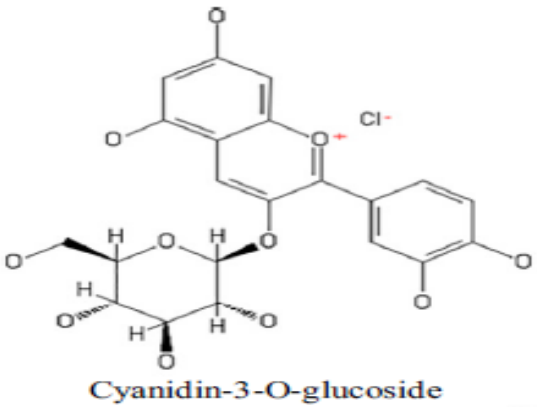

Figure (2): Cyanide-3-glucoside in white cowpea

Table (1): Type of anthocyanins and some of the compounds included with it in white cowpea by GCMS device and using ethanol as a solvent

Peak	Molecular weight	Chemical formula	Compound name	Area%	Area	Detention time
1	208	C8H16O6	Manose	38.30	271331	16.532
2	117	C5H11NO2	Valine	3.29	23329	19.387
3	449	C21H21O11	Kuromanine	58.19	412260	21.214
4	186	C9H14O4	Oxalic acid	0.21	1520	26.658

2- Diagnosis of anthocyanins with white cowpea and methanol solvent:

Figure (3) indicates the appearance of several peaks of the compounds that make up the recorded white cowpea when dissolved in methanol and placed in the GCMS device, and the third peak with the reading (detention time) (21,223) can be observed, which indicates the presence of anthocyanins [10].

By noting Table (2), we find that the GCMS device has determined the type of anthocyanins, which is Kuromanine, which is C-3-G Cyanidin-3-o-Glucoside (Figure 4), with the chemical formula C21H21O11 and molecular weight = 449 molars.

His scientific name is: (2S,3R,4S,5S,6R)-2{2-(3,4-dihydroxyphenyl)-5,7-dihydroxychromenylium-3-yl} oxy-6-(hydroxymethyl) oxan-3,4,5-triol.)

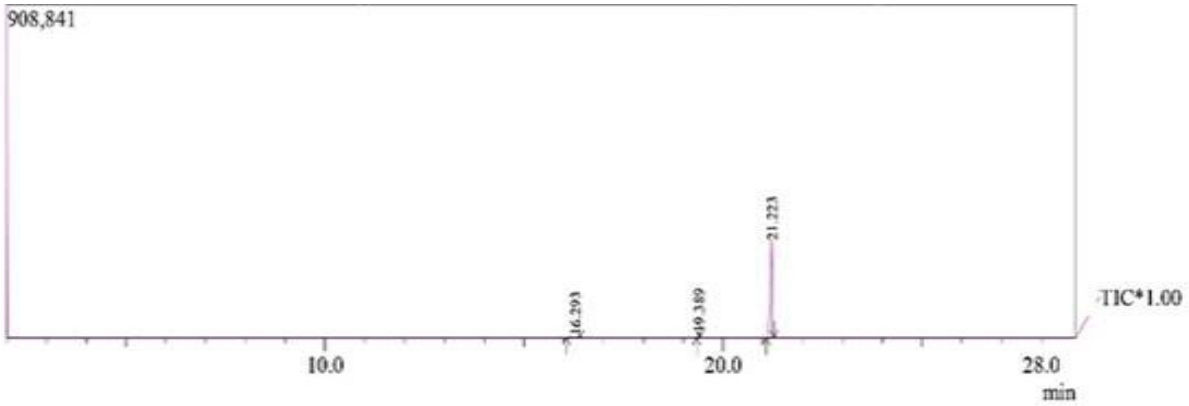

Figure (3): Peaks of anthocyanins and the compounds that make up white cowpea using GCMS and methanol as a solvent

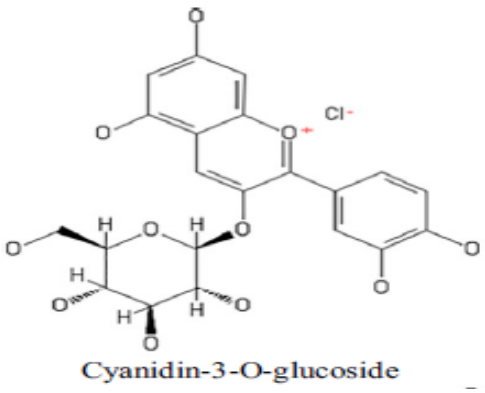

Figure (4): Cyanide-3-glucoside in white cowpea

Table (2): Type of anthocyanins and their compounds in white cowpea by GCMS device and the use of methanol as a solvent

Peak	Molecular weight	Chemical formula	Compound name	Area%	Area	Detention time
1	88	C4H8O2	Butanoic acid	5.31	52623	16.293
2	132	C5H8O4	Glutaric acid	3.11	30809	19.389
3	449	C21H21O11	Kuromanine	91.58	907230	21.223

3- Diagnosis of anthocyanins with red cowpea and ethanol solvent:

Figure (5) indicates the appearance of several peaks of the compounds that make up red cowpea (Al-Hawizawy) when dissolved in ethanol alcohol and placed in the GCMS device, and it can be observed that the second peak reading (detention time) 19.589 indicates the presence of anthocyanins.

By observing Table (3), we find that the GCMS device identified the type of anthocyanins, which is Kuromanine, which is C-3-G Cyanidin-3-o-Glucoside (Figure (6), with the molecular formula C21H21O11 and molecular weight 449 molars.

His scientific name is: (2S,3R,4S,5S,6R)-2{2-(3,4-dihydroxyphenyl)-5,7-dihydroxychromenylium-3-yl} oxy-6-(hydroxymethyl) oxan-3,4,5-triol.)

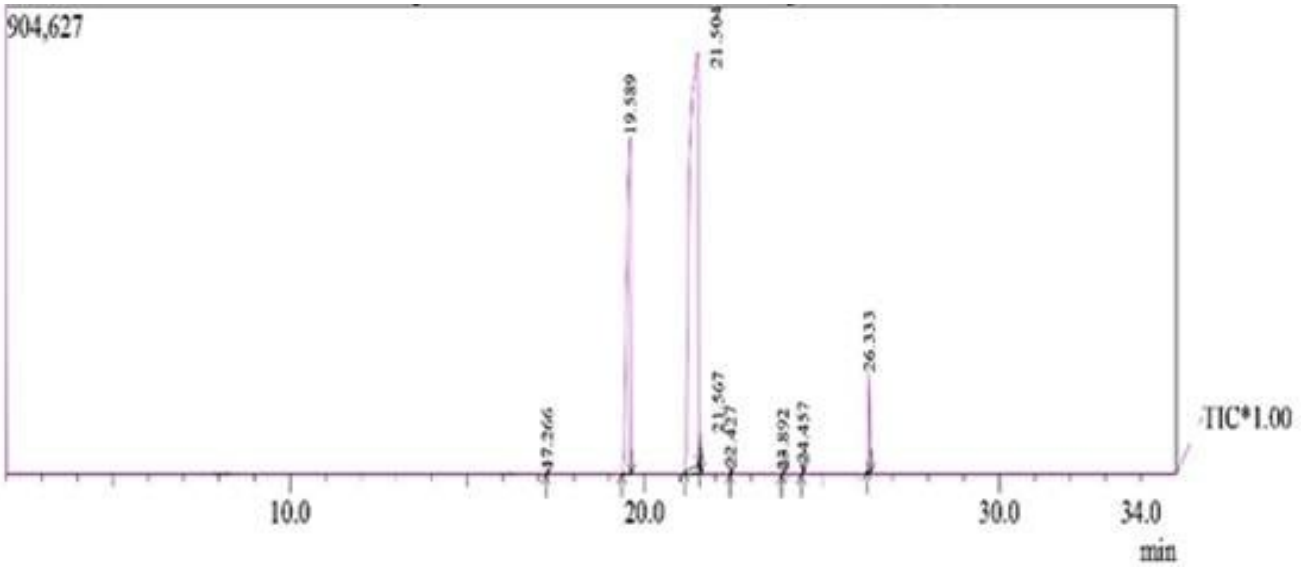

Figure (5): shows the peaks of the appearance of anthocyanins and some compounds that make up red cowpea using GCMS and ethanol as a solvent

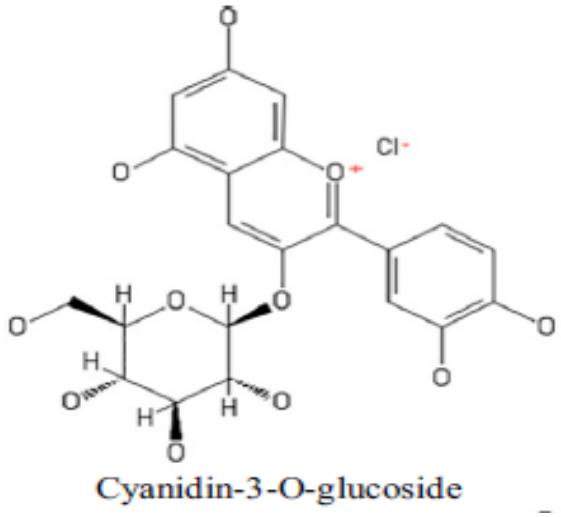

Figure (6): shows cyanidin-3-glucoside in red cowpea

The same figure (5) indicates the third peak reading (detention time) (21.504) to the presence of a second type of anthocyanin, and the device identified its type as Peonidin-3-o-Glucoside (P-3-oG) Figure (7), Its chemical formula is C22H23ClO11 and its molecular weight = 498 molars, while its scientific name is: (2S,3R,4S,5S,6R)-2-{5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl) chromenylium-3-yl} oxy-6-(hydroxymethyl) oxane-3,4 ,5-triol, chloride.

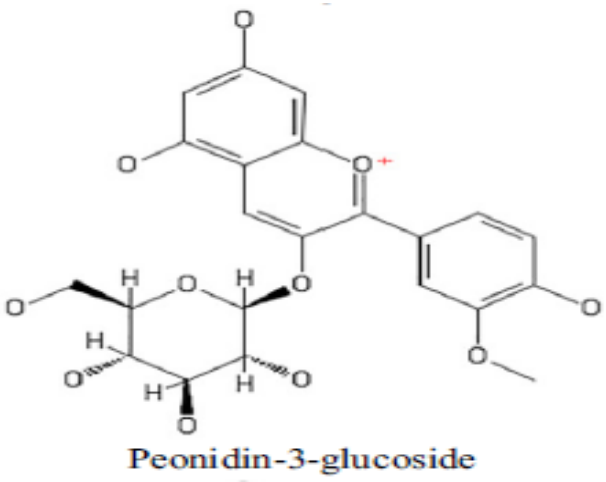

Figure (7): shows Biondine-3-glucoside in red cowpea

Table (3): Type of anthocyanins and some of the compounds included in red cowpea with GCMS device and the use of ethanol as a solvent

Peak	Molecular weight	Chemical formula	Compound name	Area%	Area	Detention time
1	256	C16H32O2	Palmitic acid	0.09	16324	17.266
2	449	C21H21O11	Kuromanine	24.41	4555193	19.589
3	498	C22H23ClO11	Peonidin 3-glucoside	72.11	13454821	21.504
4	284	C18H36O2	Stearic acid	0.39	14429	21.567
5	189	C9H19NO3	Nitric acid	0.08	14429	22.427
6	114	C7H14O	Heptanol	0.06	11343	23892
7	161	C7H15NO3	L-Carnitine	0.17	32444	24.457
8	266	C18H34O	Octadecadien-1-ol	2.68	500965	26.333

4- Diagnosis of anthocyanins with red cowpea and methanol solvent:

Figure (8) indicates the appearance of several peaks of the compounds that make up red cowpea (Al-Hawizawy) when dissolved in methanol alcohol and placed in the GCMS device.

It can be observed that the fourth peak with reading (detention time) 17,563 indicates the presence of anthocyanins.

By noting Table (4), we find that the GCMS device determined the type of anthocyanin, which is C-3-G Cyanidin-3-o-Glucoside (Figure 9), with the molecular formula C21H21O11 and a molecular weight of 449 mo.

His scientific name is: (2S,3R,4S,5S,6R)-2{2-(3,4-dihydroxyphenyl)-5,7-dihydroxychromenylium-3-yl} oxy-6-(hydroxymethyl) oxan-3,4,5-triol.

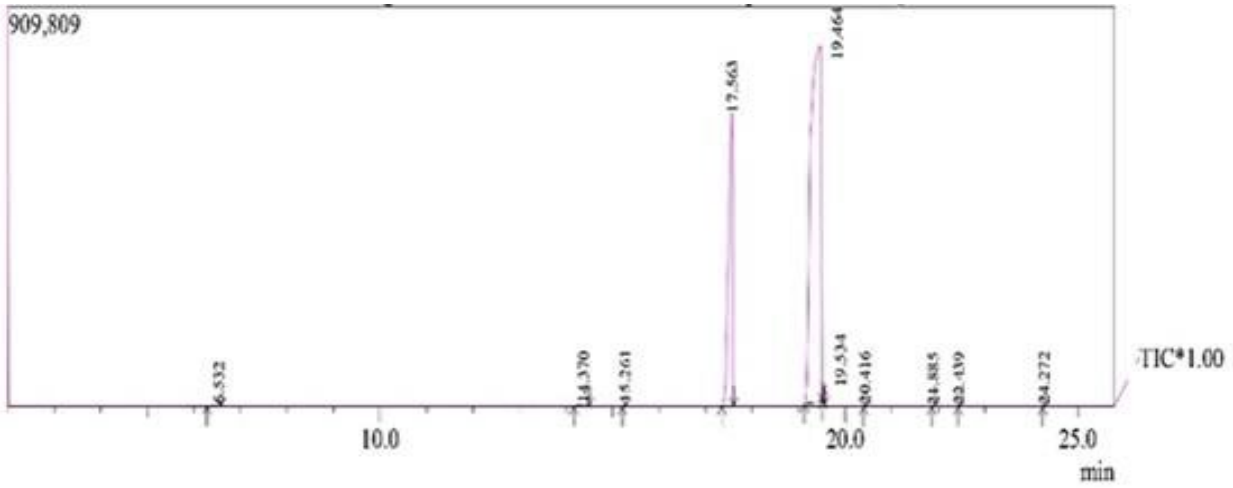

Figure (8): Peaks of anthocyanins and the compounds that make up red cowpea using GCMS and methanol as a solvent

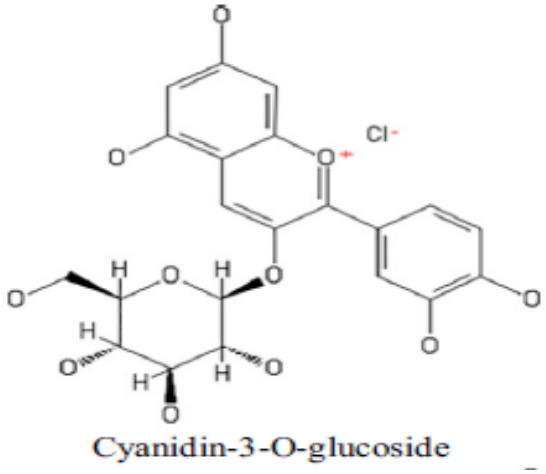

Figure (9): shows cyanidin-3-glucoside in red cowpea

The same figure (8) with the fifth peak reading (holding time) (19.464) indicates the presence of a second type of anthocyanin, and the device identified its type as Peonidin-3-o-Glucoside (P-3-oG) Figure (10), Its chemical formula is C22H23ClO11 and its molecular weight = 498 molars, while its scientific name is: (2S,3R,4S,5S,6R)-2-{5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl) chromenylium-3-yl} oxy-6-(hydroxymethyl) oxane-3,4 ,5-triol, chloride.

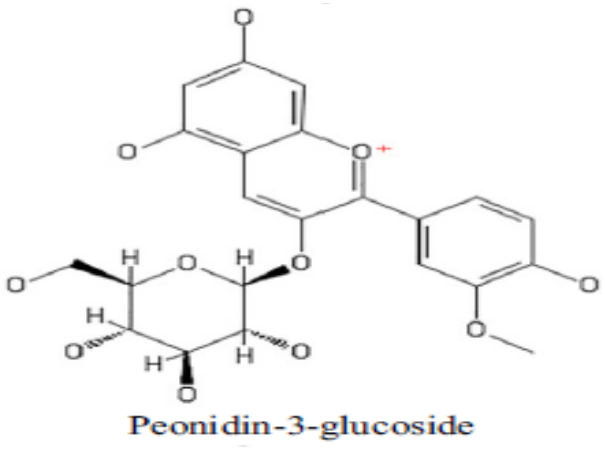

Figure (10): shows Biondin-3-glucoside in red cowpea

Table (4) Type of anthocyanins and some other compounds in red cowpea in GCMS and methanol as solvent

Peak	Molecular weight	Chemical formula	Compound name	Area%	Area	Detention time
1	90	C3H6O3	Lactic acid	0.17	28200	6.532
2	256	C16H32O2	Palmitic acid	0.29	47627	14.370
3	117	C5H11NO2	L-Valine	0.12	20485	15.261
4	449	C21H21O11	Kuromanine	23.98	3930800	17.563
5	498	C22H23ClO11	Peonidin3-glucoside	75.05	12301381	19.464
6	508	C34H68O2	Palmityl Stearate	0.24	39524	19.534
7	171	C9H17NO2	Butanoic acid	0.04	6759	20.416
8	58	C2H6N2	Azomethane	0.03	4155	21.885
9	188	C9H16O4	Azelaic acid	0.02	3314	22.439
10	83	C5H9N	Piperidine 1-	0.05	8141	24.272

The above results are in agreement with the studies of [11-16]. Studies indicate that white and red cowpea contain four Types of anthocyanins in a large way (cyanidin-3-o-glucose by 91%, and, peonidin-3-o-glucose (7%), cyanidin 3,5 diglucose (0.92%), and cyanidin-3-rutinoside (0.94%), but the main anthocyanins in white and red cowpea are C-3-oG). cyanidin-3-o-glucose and peonidin-3-o-glucose(P-3-oG) [17]. These results can be explained by the fact that anthocyanins are structurally related to glycosides with anthocyanidin (that is, anthocyanins are linked with sugar molecules and with a hydroxyl group OH), as there are more than 500 types of anthocyanins and 23 types of anthocyanins, but only six of them are found in abundance in plants, namely (Cyanidin, Bionden, Petundin, Delvendin, Bilrcundin, Malvindin [18].

The anthocyanin content of the anthocyanin portion of the Flvylium ion (2-phenyl benzo pyrilium) is attached to the methyl and hydroxyl groups, and anthocyanins are attached to one or more glycosidic units at position 3; 5; 7, where the glycosalation process occurs, and it is a widespread process in plants, especially the hexoses, which include (galactoside and glucoside), While the pentagram is less common, Pentose, which includes (rhamnose, xylose, arabinose), This process may be the reason why C-3-o-G and P-3-o-G are abundant in red and white cowpea, and this is supported by Kong et al. [18]. Note that the chemical composition of anthocyanins in grains and vegetables is more stable than in fruits (where it is not subjected to other processes that change its color), so we find that C-3-OG and P-3-oG are more prevalent because it is more stable. The reason for the presence of these two types of anthocyanins in red and white cowpea can be attributed to genetic factors [19]. PURPLE PERICARP A (Pp) and PURPLE PERICARP B (Pb), which are located on chromosomes 1 and 2, respectively [20, 21]. It was found that the C 3-OG dye with the Pp allele is completely dominant over the recessive pp allele, and since the white color appeared in the presence of C 3-OG, this means that the Pp allele was dominant, because the number of the hybrid Pp alleles determines the concentration of C 3-OG In white [22]. The cause could be the White hull 4 (Bh4) gene, which is located on the fourth chromosome [23]. As for red cowpea, it also needs two genes for expression, namely Rc and Rd, and the loss of the function of the two genes leads to a change in color to white and consequently, the absence of cyanidin and bayundin[24, 25].

Conclusion

Several compounds were identified in the anthocyanin extract, and the anthocyanin type was identified, as it was found that white and red cowpea contain Cyanidin 3-glucoside, while Peonidin 3-glucoside was found in red cowpea only.

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