P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com

Research Article

Phytochemical Constituent, HPLC Profiling and Antioxidant Activity of *Passiflora incarnata* and *Arctium lappa* Leaves Extracts

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Article History: 19-664 Received: September 07, 2019 Revised: October 26, 2019 Accepted: October 30, 2019

ABSTRACT

Chemical evaluated of some bio extracts as follows: Phytochemical screening, Total Polyphenols and Flavonoids content were determined in *Passiflora incarnata* and *Arctium lappa* leaves. Bio extracts were shown through color analysis containing phytochemical compounds such as (Terpens, Tannins, Flavonoids, Saponins, Alkaloids, Glycosides, Ph. Glycosides and Resins). *Passiflora incarnata* leaves have the highest of total polyphenols and flavonoids contents, which were 133.7mgGAE/g and 19.03mgQE/g, followed by *Arctium lappa* leaves, which were 128.5mgGAE/g and 16.05mgQE/g, respectively. Antioxidant activity of aqueous and methanolic extracts were determined using (FRAP and DPPH), The aqueous and methanolic extract of *Passiflora incarnata* leaves, have the highest of reducing power which were 198.79 and 210.51%, at the concentrations of 40mg/ml, respectively. Followed by aqueous and methanolic extract of and *Arctium lappa* leaves, which were 159.31 and 177.06%, at the same concentration. Also, when using (DPPH), of the same plant leaves, the IC_{50} value were 0.006 and 0.009 of aqueous extract, at the concentrations of 40mg/ml, respectively. While, the IC_{50} value were 0.004 and 0.007 of methanolic extract, at the same concentrations, respectively.

Key words: Plant extracts, Phytochemical analysis, HPLC and Antioxidant activity

INTRODUCTION

Passiflora incarnata L. belongs to Passifloraceae family and is one of widely used medicinal plants in traditional medicine. P. incarnata extracts have been recorded for their variety of pharmacological activities including antitussive, analgesic, anticonvulsant, anti-inflammatory, anti-asthmatic, anticarcinogenic, anticancer, antimicrobial, antioxidant and aphrodisiac activity. Suvarna and Sanjay (2012).

Arctium lappa L. belonging to the Compositeae family, commonly found in Europe, North America and Asia. In traditional medicine, burdock is famous for a wide variety of medical benefits. It has the ability to decrease the organism's quantity liquids, treat skin illnesses. Previous studies have connected Arctium lappa to several therapeutic operations. It has a more powerful antioxidant impact than other fruits or vegetables. Plant extracts are able to exhibit antitumor activity against human cancer cell lines of leukaemia, kidney and mammary. Andrada *et al.* (2016).

Free radical damage is one of the most prominent causes of disturbing illnesses that are responsible for murdering millions of individuals around the globe and this can patent as heart attacks and cancers. Naturally, free radicals happen in the body as a consequence of chemical reactions during ordinary cellular procedures, such as adapting food to the body's energy. Numerous researches on antioxidants in biological systems have inveterate their neutralizing effects on oxidative stress that predispose the human body to lethal diseases and thus, making keen notice in valuation of antioxidant potentials of replaceable food compounds antioxidants and contain a number of chemical compounds Ahiakpa *et al.* (2010).

Medicinal plants with different phytochemicals and bioactive elements such as trace metal ions, vitamins, alkaloids, carotenoids, polyphenols, fats, carbohydrates and proteins are engaged in the improvement of long-term health advantages. Antioxidant activity of plants have been observed, using FRAP, DPPH, ABTS and scavenge and suppress the formation of (ROS) reactive oxygen species Sravanthi and Rao (2014).

Cite This Article as: Elghobashy KA, Eldanasoury MM, Elhadary AA and Farid M, 2020. Phytochemical constituent, HPLC profiling and antioxidant activity of *Passiflora incarnata* and *Arctium lappa* leaves extracts. Int J Vet Sci, 9(1): 42-49. www.ijvets.com (©2020 IJVS. All rights reserved)

MATERIALS AND METHODS

Plant materials

Plants samples of *Passiflora incarnata* and *Arctium lappa* were kindly achieved from ARC, Egypt. Samples were air dried in the shade and converted into a fine powder.

The powdered air dried leaves which were divided into dry part and extracts. The dry part: It was used to estimate polyphenol and flavonoid content, as well as the separation of compounds using HPLC technique.

Aqueous extract: the plant powdered leaves (500gm) were extracted with distilled water by boiling for 3 hours at temperature from 80 to 100° C in reflux. The extract was filtered to room temperature. Finally, the extract was lyophilized and conserved at −20°C until further use Kim *et al.* (2011).

Methanolic extract: the plant powdered leaves (500gm) of the plant were extracted by soaking at room temperature for six times with methanol (5 L), then the successive extraction was carried out by using methanol. Extract was obtained and then it was concentrated to approximately dryness under vacuum using rotary evaporator at 45ºC, which kept under 4ºC, until use Farid *et al.* (2016).

The yield of aqueous extract was 24.66 and 22.09%, of *Passiflora incarnata* and *Arctium lappa* leaves, respectively. While, the yield of methanolic extract was 26.05 and 23.30%, of the same plant leaves, respectively. All experiments were carried out in (SAER), Egypt.

Phytochemical tests of plant leaves extracts

Preliminary phytochemical tests were agreed out on the aqueous and methanolic extracts, to detect the presence of some chemical compounds: terpenes, tannins, flavonoids, saponins, alkaloids, carbohydrates and/or glycosides, phenolic glycosides and resins. Detected according to the method adopted by Harborne, (1988).

Detection of terpenes: A small quantity of plant extracts was liquefied in chloroform, then a few drops of concentrated sulfuric acid were carefully added to the test tube wall to form two separate layers, the resulting yellow ring changed to orange and then red indicating terpenes.

Detection of tannins: A few milliliters of aqueous extract were added to distilled water and filtrate, then ferric chloride solution (5 percent) was added to the filtrate. The presence of yellowish green tannins has been acquired.

Detection of flavonoids: A small amount of crude plant extract was macerated overnight in hydrochloric acid (1%), then added to the filtrate sodium hydroxide solution (10%), the yellow color appearance indicates the presence of flavonoids.

Detection of saponins: The plant extracts were forcefully shaken developing a voluminous froth which persisted for almost one hour specify the presence of saponins.

Detection of carbohydrate and/or glycosides: Some drops of α-naphthol in ethyl alcohol were added to 1ml of crude boiling water extract, then closely added 1ml of concentrated sulfuric acid without shaking, a purple ring showing the existence of carbohydrates and/or glycosides in crude plant extract emerged.

Detection of alkaloids: Add 2ml of hydrochloric acid diluted to 1ml of plant extract. Then, after adding each drop, five drops of Wagner's reagent were added to 1ml of the solution and shaking. The precipitate created after leaving for sometimes indicates the existence of alkaloids.

Detection of phenolic glycosides: By the following method: a few drops of concentrated sulfuric acid have been added to 1ml of extract, producing a red color that disappears when water is added.

Detection of resins: The extracts were boiled on water bath for 20 minutes and added distilled water to extracts, a white precipitate was formed in presence of resins.

Total phenolic contents

Total phenolic contents of air dried leaves were determined by using Folin–Ciocalteu reagent method according to Lin and Tang, (2007). Approximately 0.1 g of dried air sample was dissolved in 1 ml of distilled water individually. Aliquots of 0.1 ml from the prior solution were drawn and blended with precisely 2.8 ml of distilled water, 2.0 ml of sodium carbonate (2% w/v) and lastly 0.1 ml of 50% (v/v) of Folin – Ciocalteu reagent. Mixture was incubated at room temperature for 30 minutes and the absorbance of the resulting color was measured at 750 nm against distilled water as blank, using a Spekol 11 (Carl Zeiss -Jena) spectrophotometer. A normal curve using gallic acid (0- 200 mg/l) was prepared in the same way for quantitative determination. Total polphenolic contents were expressed as milligram gallic acid equivalent (GAE/g) based on dry weight.

Total flavonoid contents

Total flavonoid content of air dried samples using aluminum chloride was calorimetrically determined as described by Chang *et al.* (2002), About 0.1 g of dried air sample in 1ml of distilled water was dissolved. The resulting solution (0.5 ml) was mixed with 1.5 ml 95% ethyl alcohol, 0.1 ml 10% aluminum chloride (AlCl3), 0.1 ml 1M sodium acetate (CH3COOK) and 2.8 ml distilled water. After 40 minutes of room temperature incubation, the absorbance of the reaction blend was evaluated as blank at 415 nm against distilled water, using a Spekol 11 (Carl Zeiss-Jena) spectrophotometer. Quercetin was elected as a standard for creating the normal curve (0–50mg/l) of flavonoids. Total flavonoid content levels were displayed as an equal milligram quercetin (QE)/g based on dry weight.

Analysis of phenolic compounds by HPLC technique

The phenolic compounds were determined by HPLC technique according to the method of Goupy *et al.* (1999), as follow; 5 g of plant powder sample was mixed with methanol and centrifuged at 10000 rpm for 10 min and the supernatant was filtered through a 0.2µm Millipore membrane filter then 1-3 ml was composed in a vial for injection into the HPLC Agilent 1200 series armed with auto sampling injector, solvent degasser, ultraviolet (UV) detector set at 280 nm and quarter HP pump (series 1050).

The column type was ODS column with measurement of 5µm x4mm, the column temperature was kept at 35°C. Methanol and acetonitrile gradient separation was performed as a mobile phase at a flow rate of 1 ml / min. Standard phenolic acid from sigma Co. were dissolved in a mobile phase and injected into HPLC. Retention time and peak zone were used by HEWLLET packed software to calculate the concentration of phenolic compounds.

Analysis of flavonoid compounds by HPLC technique

The flavonoid compounds were determined by HPLC technique according to the method of mattila *et al.* (2000), as follow; 5 g of sample was mixed with methanol and centrifuged at 10000 rpm for 10 min and the supernatant was filtered through a 0.2µm Millipore membtrane filter then 1-3 ml was composed in a vial for injection into the HPLC Agilent 1200 series armed with auto sampling injector, solvent degasser, ultraviolet (UV) detector set at 254 nm and quarter HP pump (series 1050). The column type was ODS column with measurement of 5µm x4mm, the column temperature was kept at 35°C. Methanol and acetonitrile gradient separation was performed as a mobile phase at a flow rate of 1 ml/min. Standard Flavonoid from sigma Co. were dissolved in a mobile phase and injected into HPLC. Retention time and peak zone were used by HEWLLET packed software to calculate the concentration of flavonoid compounds.

Determination of Reducing power, (FRAP)

It was determined the reducing power of aqueous methanolic extracts was reduced according to the method of (Oyaizu, 1986), Extract (0–100 mg) from each sample was added to 2.5ml potassium ferricyanide (10mg / ml) in a 0.20mol phosphate buffer, pH 6.6 (2.5ml), blend incubated at 50oC for 20min. Trichloroacetic acid (TCA) (2.5ml, 100mg/ml), was added to the mixture after 10 minutes of centrifugation at 650g. The supernatant (2.5ml) was blended with distilled water (2.5ml) and 0.5ml (1mg / ml) ferric chloride solution. and the resulting color absorption was measured using a 700 nm spectrophoto-meter from Spekol 11 (Carl Zeiss -Jena). Higher absorbance of the reaction mixture showed greater reduce. The free radical scavenging activity (% antiradical activity) was calculated using the following equation:

$$\text{Increase in reducing power (\%)} = \frac{\text{(A Test–A Blank)}}{\text{(A Blank)}} \times 100$$

Determination of ($DPPH^+$) radical scavenging activity

The $DPPH^+$ free radical scavenging activity of aqueous and methanolic extracts at various concentrations was measured by lightening the purple color of (2.2 Diphenyl -1-picryl hydrazyl) using the procedure Pratap *et al.* (2013), Exactly 0.1 ml solution of different concentration of extract was added to 1.4 ml of $DPPH^+$ 0.1mM and kept in dark for 30 min. The absorbance was measured at 517 nm, using a Spekol 11 (Carl Zeiss-Jena) spectrophotometer. And the inhibition coefficient was calculated using the current equation.

$$\text{Inhibition (\%)} = \text{(A Blank − A Test) / A Blank)} \times 100$$

Statistical analysis

Using the statistical software package, information collected were evaluated (CoStat, 2005). All comparisons were first subjected to one-way ANOVA and important distinctions between treatment means were determined using the multi-range test of Duncan at $P<0.05$ as the meaning point (Duncan, 1955).

RESULTS AND DISCUSSION

Preliminary phytochemical screening of crude aqueous and methanolic leaves extracts

Table 1 characterized the phytochemical constituents of *Passiflora incarnata* and *Arctium lappa* aqueous and methanolic leaves extracts. The aqueous and methanolic extracts of investigated plant leaves, were rich in terpenes, tannins, flavonoids, alkaloids, carbohydrate and/or glycosides and phenolic glycosides within the acceptable limits. While, the same plant extracts were poor in resins. Moreover, the methanolic extract of the same leaves contains a high ratio of flavonoid and ph. Glycosides, respectively.

Our data about *Passiflora incarnata* leaves were in agreement with those reported by Sita *et al.* (2009), they stated that the phytoconstituents content of alkaloids, phenols, glycosyl flavonoids and cyanogenic compounds were reported to be known in the genus (*Passiflora incarnata*).

Phamiwon and Sheila (2017), who found that the phytochemical analysis of *Passiflora edulis* leaves extract reflected the presence of flavonoids, tannins, polyphenols, terpenoids, steroids and alkaloids. Chitra *et al.* (2009), presented that the alkaloids, carbohydrates, proteins, flavonoids, glycosides, saponins and terpenes in methanol extract are present in aerial parts of *Passiflora incarnata*.

Our results agree with Talele *et al.* (2012), they revealed that the main active principle of *Arctium lappa L.* as follows Flavonoid Hexasaccharide, tannin and volatile oil. Al-Snafi (2014), it was discovered that active chemical isolated groups of Arctium lappa included unstable oils, lignans, sesquiterpene lactones, polyynes, polysaccharides, phytosterols, tannins, flavonoids, amino acids, trace components and other substances.

Total polyphenols and total flavonoids content of investigated plant leaves

Total polyphenols comprise several classes of phenolic compounds that are metabolites of secondary plants and the main portion of human and animal diets. Flavonoids are a large group of phenolic compounds that primarily consist of flavonols, flavanols and anthocyanins. Phenolic compounds can play a major role in stopping hydrogen peroxide damaging to body cells and organs, damaging lipid peroxides and scavenging or neutralizing free radicals Sroka and Cisowski, (2003).

Free radical scavenging and antioxidant activity of several medicinal plants have been revealed to be responsible for their therapeutic effects on cancer, diabetes, inflammatory tissue and cardiovascular diseases Cai *et al.* (2004).

Table (2) showed the total polyphenols (mg GAE/g) and total flavonoids (mg QE/g) contents of *Passiflora incarnata* and *Arctium lappa* leaves. Data in table (2) showed that, *Passiflora incarnata* and *Arctium lappa* leaves have the highest of total polyphenols concentration, which were133.7 and 128.5mgGAE/g, consecutively.

Table 1: Phytochemical tests (Qualitative).

Plants Leave extracts		Terpens	Tannins	Flavonoids	Saponins	Alkaloids	Glycosides	Ph. glycosides	Resins
Passiflora	*Aqueous*	++	++	++	++	++	++	+++	-
incarnata	*Methanolic*	++	++	+++	++	++	++	+++	-
Arctium	*Aqueous*	++	++	++	++	++	++	++	-
lappa	*Methanolic*	++	++	+++	++	++	++	+++	-

= negative ++ = positive +++ = strongly positive

Table 2: Total polyphenols and total flavonoids content.

Samples of plants leaves	Total polyphenols (mg GAE/g)	Total flavonoids (mg QE/g)
Passiflora incarnata	133.7	19.03
Arctium lappa	128.5	16.05

Table 3: Identification of polyphenols of investigated plant leaves by HPLC technique.

Phenolic compounds	*Passiflora incarnata*		*Arctium lappa*	
	R_{time}	ppm	R_{time}	ppm
Gallic	7.667	5.55	7.627	1.67
Pyrogallol	7.760	204.96	7.725	218.35
4-Aminobenzoic	8.767	3.65	8.793	2.17
Protocatechuic	9.156	151.05	--	--
Cataehein	9.320	201.71	9.220	93.76
Chlorogenic	9.596	284.67	9.628	124.64
Catechol	9.901	134.74	9.929	59.82
Caffiene	10.215	59.44	10.248	20.35
P.oH.benzoic	10.374	221.52	10.393	93.41
Caffeic	10.746	21.47	10.773	12.81
Vanillic	10.821	24.80	10.841	9.06
P-coumaric	11.855	84.30	11.820	24.35
Ferulic	12.000	97.30	12.013	15.48
Iso-Ferulic	12.327	48.42	12.353	4.07
Alpha-coumaric	13.111	20.11	13.200	7.83
Ellagic	13.280	165.12	13.300	46.20
Benzoic	13.413	361.03	13.417	256.99
3,4,5-methoxy-cinnamic	13.973	162.03	13.953	55.92
Salicylic	14.340	1170.02	14.345	1665.06
Cinnamic	14.716	20.01	14.607	44.04

Moreover, *Passiflora incarnata* and *Arctium lappa* leaves contained average values of total flavonoids, which were 19.03 and 16.05mgQE/g, singly.

Our results of *Passiflora incarnata* leaves were in agreement with those conveyed by Saravanan and Parimelazhagan. (2014), who found that, the total phenolic and flavonoid contents of methanolic extract, were (137.90mg GAE/g extract) and (233.33mg RE/g extract), of *Passiflora ligularis,* respectively. Kim *et al.* (2015), they investigated that total phenolic and flavonoid content of *Arctium lappa*, were (144.0mg GAE/g) and (395.1 mg QCE/g), respectively. *Arctium lappa* leaves were discovered to have the largest complete polyphenols content compared to what Park *et al.* (2016), who found that, the total polyphenol contents of dried burdock roots, ranged from (25.46 to 38.75mgGAE1/g) while the results were consistent of total flavonoid, which ranged from (09.28 to 25.33mg QE2/g), after from 5 to 30min, when extracted under temperature 100°C, of the same plant.

Noor *et al.* (2017), who found that, the total phenolic and flavonoid contents, which were (82.09mg GAE/g) and (205.59mg QE/g), of *Passiflora foetida* methanolic extract respectively. Paşca *et al.* (2016), who revealed that, the total phenolic and flavonoid content, were (14.01 and 5.00mg QE/g), for Arctium lappa acids.

Table 4: Identification of flavonoid of investigated plants using HPLC technique.

Flavonoid compounds	*Passiflora incarnata*		*Arctium lappa*	
	R_{time}	ppm	R_{time}	ppm
APig. 6-rhamnose 8-glactose	11.847	3628.45	11.757	1634.98
APig. 6-rhamnose 8-glucose	12.063	268.15	12.020	158.57
Narengin	12.427	576.36	12.287	142.12
Hisperidin	12.533	10672.2	12.420	4063.70
Rutin	12.607	2068.19	12.507	633.74
Apig.7-o-neohespiroside	12.875	137.90	12.936	57.18
Kamp.3,7-dirhamoside	13.210	158.06	13.115	42.05
Quercetrin	13.433	176.39	13.373	89.77
Apigenin-7-glucose	13.663	203.44	13.600	55.07
Acacetin.7 -neohespirside	14.362	311.96	14.317	32.61
Kamp3,(2-comaroyl) glactose	14.398	1198.03	--	--
Acacetin. neo rutinoside	14.465	326.55	14.472	122.85
Querceitin	14.560	163.08	14.554	51.73
Naringenin	14.642	37.39	--	--
Hispirtin	14.707	264.37	14.721	72.83
Kampferol	15.367	41.17	--	--
Rhamentin	15.487	262.49	15.433	54.69
Apegnin	15.600	37.63	15.633	25.95

Identification of polyphenolic fractions of investigated Plants using HPLC technique

High performance liquid chromatography (HPLC) method has been used to analyze *Passiflora incarnata* and *Arctium lappa* Leaves. Twenty polyphenolic compounds as dependable samples namely: Gallic, Pyrogallol, 4-Aminobenzoic, Protocatechuic, Cataehein, Chlorogenic, Catechol, Caffiene, P.oH.benzoic, Caffeic, Vanillic, P-coumari, Ferulic, Iso-Ferulic, Alpha-coumaric, Ellagic, Benzoic, 3,4,5-methoxy-cinnamic, Salicylic and Cinnamic acid. These standard samples were used to identify the corresponding components of plant polyphenols.

From Table 3, it could be noticed that gallic acid was the main identified component of *Passiflora incarnata* and *Arctium lappa* leaves*,* at low concentrations which were (5.55 and 1.67ppm), respectively. While, the pyrogallol compound found at high concentration, which were (204.96 and 218.35ppm), of the same plants, respectively. The 4-Aminobenzoic content of *Passiflora incarnata* and *Arctium lappa* leaves*,* which were (3.65 and 2.17ppm), respectively. Although, Protocatechuic content of *Passiflora incarnata* leaves, which was (151.05ppm), while not found in *Arctium lappa* leaves. Also, the cataehein content of the same plants, was (201.71 and 93.76ppm), respectively.

From Table 3, it could be observed that chlorogenic was the predominant identified component in both *Passiflora incarnata* and *Arctium lappa* leaves*,* at high concentrations, which were (284.67 and 124.64ppm), respectively. Followed by, Catechol content of the same plants, which were (134.74 and 59.82ppm), respectively.

Table 5: Reducing power of crude aqueous and methanolic leaves extracts.

Concentration mg/ml of plant leaves	Optical density at 700nm and % of Inhibition							
	Passiflora incarnata				*Arctium lappa*			
	Aqueous		*Methanolic*		*Aqueous*		*Methanolic*	
5	0.924	59.31	0.990	70.68	0.792	36.55	0.834	43.79
10	1.211	108.79	1.380	137.93	0.978	68.62	1.171	101.89
20	1.529	163.62	1.553	167.75	1.223	110.86	1.369	136.03
40	1.733	198.79	1.801	210.51	1.504	159.31	1.607	177.06

Table 6: Antioxidant capacity of aqueous and methanolic extracts using ($DPPH^{+}$) radical.

Concentration mg/ml of plant leaves	IC_{50} of Extracts			
	Passiflora incarnata		*Arctium lappa*	
	Aqueous	*Methanolic*	*Aqueous*	*Methanolic*
5	0.032	0.022	0.045	0.028
10	0.019	0.013	0.022	0.17
20	0.011	0.009	0.014	0.011
40	0.006	0.003	0.009	0.007

While, the Caffiene content of *Passiflora incarnata* and *Arctium lappa* leaves*,* at average concentrations (59.44 and 20.35ppm). Though, the P.oH.benzoic content of the same plants, which were (221.52 and 93.41ppm), respectively. Also, the P-coumaric content of plants leaves, which were (84.30 and 24.35ppm), of the same plants, respectively. Similarly, Ferulic and Iso-Ferulic content of *Passiflora incarnata* at medium concentration (97.30 and 48.42ppm), while the content for the same compounds, was very low (15.48 and 4.07ppm), of *Arctium lappa* leaves.

From Table 3, it could be noticed that ellagic was the predominant identified component in both *Passiflora incarnata* and *Arctium lappa* leaves*,* at concentrations of (165.12 and 46.20ppm), respectively. Likewise, the content of Benzoic compound, it was in high proportions (361.03 and 256.99 ppm), of the same plants, respectively. Also, 3,4,5-methoxy-cinnamic content of the same line, were (162.03 and 55.92ppm), respectively. The Salicylic acid content of the same plants Was the highest concentration (1170.02 and 1665.06ppm), respectively. While, the following compounds, (Caffeic, Vanillic, Alpha-coumaric and Cinnamic), have medium values of all plants.

The researchers identified some polyphenolic derivatives from the *Passiflora incarnata* for instance Ruta *et al.* (2008), who established that, the main components of Passiflora ethanolic extracts, were 284, 178, 4963, 175, 7, 3299, 21, 164, 42, 6102 and 1436µg/mL, of chlorogenic acid, hyperoside, isovitexin, caffeic acid, quercetin, luteolin, orentin, rutin, scutelarein, vicenin and vitexin, respectively. While, the main components of Passiflora aqueous extracts, were 26, 14, 410, 11, 0.2, 0.75, 287, 12, 0.0, 465 and 96µg/mL, of the same component, respectively. Saravanan *et al.* (2016), who found that, the bioactive compounds such as hyperin, chlorogenic acid, rutin and caffeic acids were identified in *P. leschenaultii* leaves using HPLC analysis.

The some data were similar with that recorded by Dhawan *et al.* (2004), who found that, The Phytoconstituents of *Passiflora edulis* containing, Phenols (4-Hydroxy-ionol, 4-oxo-ionol, 4-hydroxy-7,8-dihydro-ionol, 4-oxo-7,8-dihydro-ionol, 3-oxo-ionol, isomeric 3-oxo retro-ionols, 3-oxo-7,8-dihydro-ionol, 3-hydroxy-1,1,6-trimethyl-1,2,3,4-tetrahydronaphthalene vomifoliol and dehydrovomifoliol).

Our results agree with Tang *et al.* (2014), who detected that, the UPLC-MS data identified five active compounds, namely: caffeic acid, p-coumaric acid, cynarin, quercetin and luteolin, at retention time 3.13, 3.75, 4.33, 5.44 and 6.19T_r, respectively. Tonea *et al.* (2016). who presented that, the phenolic compounds of Arctium lappa, were 114.529, 84.062, 95.942, 186.706, 323.886, 104.601, 181.077, 111.676, 85.165, 133.115 and 87.042(mg/100g), for Chlorogenic acid (5-caffeoylquinic acid), caffeoylquinic acid isomer, caffeoylquinic acid isomer, lappaol A, arctiin, isolappaol A, lappaol F, arctignan E, matairesinol, arctignan D and arctigenin, respectively.

Our results agree with Ionescu *et al.* (2014), the fractionation polyphenol compounds using HPLC of *Arctium lappa*, were (caffeic, chlorogenic, ferulic, cichoric and cinaric. Pirvu *et al.* (2014), They detailed that the leaves extract of Greater burdock, containing o caffeoylquinic acids (such as caffeic, chlorogenic, isochlorogenic, and other isomers),

Identification of Flavonoid fractions of investigated plants using HPLC technique

High performance liquid chromatography (HPLC) procedure was used for qualitative and quantitative analysis of flavonoids compounds of *Passiflora incarnata* and *Arctium lappa* Leaves. Eighteen Flavonoids fractions as authentic samples namely: APig. 6-rhamnose 8-glactose, APig. 6-rhamnose 8-glucose, Narengin, Hisperidin, Rutin, Apig.7-o-neohespiroside, Kamp.3,7-dirhamoside, Quercetrin, Apigenin-7-glucose, Acacetin.7-neohespirside, Kamp3, (2-comaroyl) glactose, Acacetin. neo rutinoside, Querceitin, Naringenin, Hispirtin, Kampferol, Rhamentin and Apegnin. Various levels were used compared to conventional compounds.

Obtained data revealed that eighteen compounds with different retention times were recognized in HPLC chromatogram of *Passiflora incarnata* leaves, and sixteen compounds with different retention times were recognized in HPLC chromatogram of *Arctium lappa* leaves.

From Table 4, it could be noticed that APig. 6-rhamnose 8-glactose, Hisperidin, Rutin and Kamp3,(2-comaroyl) glactose, were the predominant identified component in *Passiflora incarnata* leaves at the highest concentrations (3628.45, 10672.2, 2068.19 and 1198.03ppm), followed by *Arctium Lappa* Leaves*,* were (1634.98, 4063.90 and 633.74ppm), of the same compounds, but Kamp3,(2-comaroyl) glactose compound, not found. The APig. 6-rhamnose 8-glucose was the main component of flavonoids in *Passiflora incarnata* and *Arctium lappa* Leaves, at concentrations (268.15 and 158.57ppm). While, Narengin content was (576.36 and 142.12ppm), of the same plants.

From Table 4, it could be noticed that medium value for Apig.7-o-neohespiroside, Kamp.3,7dirhamoside, Quercetrin, Apigenin-7-glucose and Acacetin. 7-neohespirside, of *Passiflora incarnata* leaves, which were (137.90, 158.06, 176.39, 203.44 and 311.96ppm), respectively. While the low value of *Arctium lappa* Leaves, were (57.18, 42.05, 89.77, 55.07 and32.61ppm), of the same compounds, respectively. Also, the, Querceitin, Hispirtin and Rhamentin content, was medium concentrations (163.08, 264.37 and 262.49ppm), of *Passiflora incarnata* leaves. Followed by *Arctium lappa* Leaves, were (51.73, 72.83 and 54.69ppm), of the same compounds, respectively.

From Table 4, it could be noticed that, the low values of Naringenin, Kampferol and Apegnin compound, which were (37.39, 41.17 and 37.63ppm), of *Passiflora incarnata* leaves. While, *Arctium lappa* Leaves contains the Apegnin by (25.95ppm), but not contain Naringenin, Kampferol compounds.

Several authors identified some flavonoid derivatives from the *Passiflora incarnata,* for instance, Elsas *et al.* (2010), who established that, the likely identity of flavonoid peaks in *Passiflora* extract, were Isoorientin-2″-O-β-glucopyranoside, Vicenin-2, isoschaftoside, schaftoside, isoorientin and isovitexin-2″-O-β-glucopyranoside, at retention time 14.40, 15.87, 17.65, 17.93, 18.59 and 19.69, respectively. Dhawan *et al.* (2004), who stated that Phytoconstituents of P. incarnata containing, Flavonoids (Apigenin, luteolin, quercetin, kaempferol, C-glycosyl flavonoids, vitexin, isovitexin, orientin and isoorientin-2-O-gluco-pyranoside),

Our results agree with Ionescu *et al.* (2014), who established that, the fractionation flavone compounds using HPLC of *Arctium lappa*, were (rutin, quercetin, quercetrin, luteolin and Apigenin).

Tang *et al.* (2014), who found that, the identified by UPLC-MS data five active compounds, namely: caffeic acid, p-coumaric acid, cynarin, quercetin and luteolin, of *Arctium lappa* at retention time 3.13, 3.75, 4.33, 5.44 and 6.19R_t.

Reducing power of plant leaves extracts

Efficiency of methanolic and aqueous leave extracts to reduce Fe^{+++} to Fe^{++} has been determined by the technique outlined by Sroka and Cisowski *et al.* (2003). The optical reaction mixture density was measured at 700 nm wavelength using a spectrophotometer from Spekol 11 (Carl Zeiss-Jena). The information acquired are shown in table (5), the absorption showed the reduction energy for various levels of crude aqueous and methanolic extracts of *Passiflora incarnata* and *Arctium lappa* leaves. Data expressed as absorbance at 700nm for producing color as a result and % inhibition for using four concentrations (5, 10, 20 and 40 mg/ml) for each sample. From Table 5, some points could be inferred: The reducing power capacity increased with increasing the aqueous and methanolic extracts concentrations for all samples.

Passiflora incarnata leaves have the largest proportion of reduction energy ranging from 70.68 to 210.51%, for methanol extract at 5 and 40mg/ml levels, respectively. Followed by the aqueous extract of the same plant, which were ranged from 59.31 to 198.79%, at the same concentration. While, *Arctium lappa* leaves have the average percentage of reducing power which was ranged from 36.55 to 159.31%, for aqueous extract at concentrations of 5 and 40mg/ml, respectively. Moreover, the methanolic extract of *Arctium lappa* leaves had a similar effect to the Aqueous extract of *Passiflora incarnata* leaves, which was 177.06% at the concentration of 40mg/ml.

High rates of reduction indicated the existence of certain compounds that could be regarded as donors of electrons and could respond with free radicals to make them more stable Arabshahi and Urooj. (2007). Different studies indicated that, the electron donation capacity which reflecting the reducing power of bioactive compounds was connected with high antioxidant activity Siddhuraju *et al.* (2002).

The results were in the same trend with those reported by Silva *et al.* (2013), who found that, the antioxidant activity using (FRAP) of *Passiflora edulis* leaves aqueous extract, was 19.2μmol TE g^{-1}. Silva *et al.* (2016), They found that, the antioxidant activity using (FRAP), of *Passiflora edulis* extracts, ranged from (1.02 to 1.89 mmol Fe^{2+} g -1). Noor *et al.* (2017), who estimated that, the antioxidant activity of *Passiflora foetida* methanolic extract determined by (FRAP) was (0.41mM FE).

Our results agree with Malinowska. (2013), who found that, the antioxidant capacity using (FRAP and TEAC), were [6.95μmol Fe^{2+}/g of *Arctium lappa* extract] and [4.91μmol Trolox/g of extract], respectively.

Antioxidant activity using ($DPPH^+$) radical

The antioxidant activity of the two studied plant species of both methanolic and aqueous extracts is recorded in table (6). The antioxidant concentration required to reduce the original DPPH concentration by 50 percent (IC_{50}) is a commonly adjusted parameter for measuring antioxidant activity. Sanchez *et al.* (1998). The lower EC_{50} pointed to the higher antioxidant activity.

The antioxidant activity of the confirmed extracts was measured using DPPH radical scavenging activity. The antioxidants scavenging activities by DPPH are accredited to their hydrogen-donating abilities Biswas *et al.* (2010). Vitamin C was used as the reference compound.

From Table 6, it is clear that, the scavenging effect (IC_{50}) of aqueous and methanolic extracts for *Passiflora incarnata* leaves have the most effective of inhibition percentage (0.006 and 0.003), at a concentration 40mg/ml, followed by *Arctium lappa* leaves of the same extracts, which were (0.009 and 0.007), at the same concentrate. Whereas, the scavenging effect (IC_{50}) of *Passiflora incarnata* leaves aqueous and methanolic extracts, have the effective of inhibition percentage ranged from (0.019 to 0.011 and 0.013 to 0.009), at a concentration 10 and 20mg/ml respectively**.** Followed by *Arctium lappa* leaves of the same extracts, ranged from (0.022 to 0.014 and 0.017 to 0.011), at the same concentration, respectively**.** Though, the scavenging effect (IC_{50}) of *Passiflora incarnata* leaves aqueous and methanolic extracts, have the lowers effective of inhibition percentage, which were (0.032 and 0.022), at a concentration 5mg/ml respectively**.** Followed by *Arctium lappa* leaves of the same extracts, which were (0.045 and 0.028), at the same concentration, respectively.

The present data of *Passiflora incarnata* leaves for antioxidant capacity are agreed with those attained by Saravanan *et al.* (2016), who revealed that, the antioxidant scavenged using the ($DPPH^+$), which was (IC_{50}-29.14µg/mL), of *Passiflora leschenaultii* leaves acetone extract. Tatiana *et al.* (2016), Passiflora ethanol extract of leaves showed the highest antioxidant activity using (DPPH-IC_{50}), was (54.01µg/ml).

Our results agree with Saravanan and Parimelazhagan. (2014), who found that, the IC_{50} value of DPPH and the scavenging activity of superoxide radical, were (26.37µg/mL) and (75.18%), respectively. Silva *et al.* (2016), who found that, the antioxidant activity using (DPPH), of *Passiflora edulis* extracts, ranged from (84.23 to 99.92 IC_{50}µg ml^{-1}).

The results were in the same line with those reported by Koleckar *et al.* (2008), who found that, the antioxidant activity using DPPH radical scavenging activity IC_{50} value, were 0.245 and 0.221mg/ml, *Arctium lappa* flower and leaves, respectively.

Conclusions

Medicinal plants extract contained high percentage of active compounds such as polyphenols and flavonoids of *Passiflora Incarnata* and *Arctium Lappa* leaves. The effect of natural extracts as antioxidant tested using (FRAB and DPPH radical) showed these plants ' elevated capacity to scavenge laboratory free radicals.

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