Chemical Constituents and Biological Activity of *Hernaria Cinerea*a, Family Caryophyllacea

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Abstract: This research aims to detect the Phytochemical Screening and investigate chemical constituents of *Hernaria cinerea* in different months (March, April and May). Water content, total ash, organic matter, acid soluble and insoluble ash, water soluble and insoluble ash, crude fiber, total carbohydrates, soluble and insoluble carbohydrates, total nitrogen, total protein, total lipids, free amino acids, total phenolics, total flavonoids, total saponins, total tannins and total alkaloids were analyzed. Water content, organic matter, total carbohydrates, insoluble carbohydrates, total nitrogen, total protein, total phenolics, total flavonoids, total tannins and total saponins were increased in March while soluble carbohydrates and total alkaloids were increased in April, while total ash, acid soluble and insoluble ash, water soluble and insoluble ash and crude fiber were increased in May. Preliminary phytochemical screening in different months of *Hernaria cinerea* showed that Volatile oil is absent. Ld_{50} of methanol extract 70% of *Hernaria cinerea* was 5500 mg /Kg. two specific dose (275 mg/kg and 525 mg/kg) used for hepatoprotective activity of *Hernaria cinerea* evaluation and results showed that the total extract of *Hernaria cinerea* plant produced high effect for protection of hepatic cells.
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1. Introduction

The Egyptian deserts are rich in medicinal plants belonging to many families (Boulos, 1995). Family Caryophyllacea (commonly called the pink family or carnation family). The family includes over 75-104 genera and 2,000 individual species. Many members of the Caryophyllacea family are important for medicinal, ornamental, and economic purposes (Mueller *et al*., 2000). Plant natural products are involved in many aspects of human existence. These natural products may be used as purified compounds or as components of complex mixtures which serve as medicines, pesticides, flavorings, herbicides, etc. Family Caryophyllaceae (pink family) is one of the largest families in the plant kingdom known to be rich in medicinal plants. Many species of this family had medicinal values and used in folk medicine. The whole plant of *Polycarpaea repens* is used as an antidote for snake bite. The ash or crushed leaves are used to treat sarcoptic mange of camels. α-1-barrigenol, camelliagenin and stigmasterol have been isolated from Polycarpaea corymbosa (Ghazanfar, 1994). The roots of Dianthus deserti are used for sprains and as an ingredient in making soup. Glycosides and triterpenoid saponins are reported in Dianthus superbus (Evans, 1989; Schopen, 1983).

So it is of interest to choose *Hernaria cinerea* herbal plant belongs to this family. The aim of the study was evaluated some metabolomics parameter also biologically evaluation activity of the methanol extracts (70%) of *Hernaria cinerea*.

Hepatic damage is a global metabolic and epidemic disease, affecting essential biochemical activities in almost every age group.. Also, the spectrum of liver abnormalities caused by wrong drugs so that the broad Conventional drugs used of liver disorders treatment are often inadequate, In view of severely undesirable side effects of synthetic agents, it is necessary to search for alternative drugs for the treatment of liver diseases to replace the currently used drugs, which are of doubtful efficacy and safety, there is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activities (Jessy and Sneha, 2012). Hepatic disease is a term that affects the cells, tissues, structures, or functions of the liver remain to be serious health problems and the management of liver disease is still a challenge to the modern medicine (Pal and Manoj, 2011). Therefore, many folk remedies from plant origin are tested for its potential hepatoprotective liver damage in experimental animal model (Piyush *et al.,* 2012).

Herbal drugs are more widely used than allopathic drugs in hepatic disease as they are inexpensive, better cultural acceptability, better compatibility with the human body and minimal side effects. These herbal drugs have shown the ability to maintain the normal functional statues of the liver with or without fewer side effects; Liver protective plants contain a variety of chemical constituents like

phenols, Coumarins, glycosides, flavonoids, alkaloids and xanthenes (Anil, 2012).

A drug having beneficial effect on the liver is known as hepatoprotective drug such as silymarin, is a flavono-lignan mixture obtained from seeds of Silybum marianum (Saumendu *et al.,* 2012).

This study has been presented to evaluate the hepatoprotective effect of ethanol extract against CCl_4-induced hepatotoxicity in rats based on promising Phytochemical data.

2. Materials and Methods

2.1. Plant material

Hernaria cinerea was collected in March, April and May, 2013 from Sidi-Barrany.el-salome road. The taxonomic identification of plant materials was confirmed by Desert reserch center herbarium team.

2.2. Methods

2.2.1. Eco-physiological study including determination of the percentage of plant water content of *Hernaria cinerea* (Rowell, 1994). Determination of certain pharmacopoeial constants of plant material, including inorganic (ash) and organic matter (Brower and Zar, 1984), acid-soluble and acid-insoluble ash, water-soluble and water- insoluble ash (Askar and Treptow, 1993) and crude fibers (British pharmacopoeia, 1980).

2.2.2. Investigation of metabolic products including determination of total carbohydrates, soluble and insoluble carbohydrates (Chaplin and Kennedy, 1994).

2.2.3. Total nitrogen and protein content determined by using Kjeldahl method (James, 1995). Free amino acids and protein-amino acids were accomplished according to Pellet and Young (1980) using Amino Acid Analyzer (Beakman system 7300 High Performance analyzer).

2.2.4. Total lipids content according to British Pharmacopoeia (1993).

Phytochemical study including preliminary phytochemical screening, including steam distillation of volatile oils (Balbaa *et al.,* 1981), test for Alkaloids (Woo *et al.,* 1977), test for glycosides (Treare and Evan, 1985), test for cardiac glycosides (Treare and Evan, 1985), test for saponins (Kokate *et al.,* 2001; Kokate, 1994), test for phenols (Ahmad *et al.,* 2005), test for phytosterols (Brieskorn *et al.,* 1961; Fieser and Fieser, 1959), test for tannins (Treare and Evan, 1985), test for flavonoids (Geissmann, 1962; Khandeal, 2008).

2.2.5. Total phenolics were determined with the Folin Ciocalteu as described by Maurya and Singh (2010), concentrate more in leaf aerial part. They were known to show medicinal activity as well as exhibiting physiological activity (Sofowara, 1993).

Total flavonoids by Samatha *et al.* (2012), total tannins by Ali *et al.* (1991), total saponins according to Obadoni and Ochuko (2001) and total alkaloids as described by Woo *et al.* (1977).

2.2.6. Acute Toxicity and Median Lethal Dose (LD50) according to the method of Ecobichon (1997).

2.2.7. Experimental Design for hepatoprotective

Rats were divided into five groups (6 rats each). Rats of group I: Normal control (N.C), group II: CCl_4- intoxicated control received the vehicle in a dose of 5 mL/kg, group III: received standard drug silymarin at a dose of 50 mg/kg. Groups IV & V were treated with M1 (275 mg/kg) & M2 (525 mg/kg.), respectively. All treatments were given orally by gastric intubation for 8 days. On the last day of the treatment, rats of group I were given a single subcutaneous dose of corn oil (3 mL/kg), while animals of the groups II, III, IV &V were received a single subcutaneous dose of CCl_4 (3 ml/kg) after 1 h of the vehicle, extract or standard silymarin treatments.

3. Results and Discussion

Metabolomics parameter of *Hernaria cinerea* March, April and May contents are summarized in table (1). Results indicated that, March of *Hernaria cinerea* contained the highest water content and total carbohydrates return to photosynthetic process which performed inside leaves (plastids) needs water and CO_2 uptake. Fiber is the amount of cellulose and lignin present in the plant. Stem is the woodier in plant aerial parts, so it contain the highest crude fiber and ash content to support and transfer water to leaves. The stem of most plant species have greater fiber levels compared to the leaves (Buxon and Redfearn, 1997). *Hernaria cinerea* contain higher insoluble carbohydrates in March as compared to April and May. This result agreed with that observed in *Nerium indicum* by Vijayvergia and Kumar (2007). The study also showed that the plant contains a large quantity of total flavonoids and total phenolic especially in march which support its use as herbal tea. Flavonoids have been shown to exhibit their actions on membrane permeability and by inhibition of membrane-bound enzymes such as the ATPase and phospholipase (Li *et al.,* 2003). Phenols have antibacterial and anti-inflammatory activities (Vijayvergia and Kumar, 2007), also responsible for antioxidant and free radical scavenging effect of plant materials (Hasanuzzamn *et al.,* 2013; Seladji *et al.,* 2014). The studied plant have intermediate amount of total tannins, alkaloids and saponins which

Total nitrogen, total protein reached maximum values in March and minimum values in May for plant under investigation. Total lipid reached maximum values in March and minimum values in

May and this maybe the ability of leaf to storage water to completed metabolic process. The percentage of inorganic matter (ash), acid soluble ash, acid insoluble ash, water soluble ash, water insoluble ash and crude fibers reached maximum values in May and minimum values in March for plant under investigation.

This may be due to the increase of total ion accumulation as a result of increasing soil moisture stress and soil salinity, which agreed with the results obtained by Larcher (1995) and Alli Smith (2009).

Table 1. Metabolomics parameter of *Hernaria cinerea* different months

Item (%)	*Hernaria cinerea*		
	March	April	May
Water content	62.33±0.41	55±0.54	50.66±0.25
Total ash	16.7±0.26	19.2±0.25	20.3±0.16
Organic matter	83.2±0.26	80.4±0.16	79.6±0.16
Acid soluble ash	9.8±0.20	11±0.14	11.2±0.21
Acid insoluble ash	7.8±0.43	8.23±0.15	9.1±0.22
Water soluble ash	10.9±0.20	12.0±0.18	12.7±0.19
Water insoluble ash	5.9±0.16	6.8±0.34	7.6±0.18
Crude fibers	15.5±0.23	17.1±0.15	17.8±0.14
Total carbohydrate	31.7±0.27	31.2±0.17	29.8±0.23
Soluble carbohydrate	16.4±0.29	19.2±0.22	17.1±0.27
Insoluble carbohydrates	15.3±0.24	12±0.14	12.7±0.36
Total nitrogen	1.66±0.08	1.54±0.07	1.52±0.07
Total protein	10.4±0.15	9.64±0.17	9.52±0.17
Total lipids	1.55±0.10	1.31±0.03	1.25±0.07

Free amino acids of *Hernaria cinerea*

Table (2) showed that the separation of free amino acids in the different Months (March, April, and May) of the *Hernaria cinerea* was achieved using amino acid analyzer, and each component. The obtained results were calculated and tabulated in table (2), where fifteen free amino acids were presented in different months. It was obvious that glutamic, serin and Isoleucine was the highest amino acid of the separated free amino acids at different Months (March, April, and May) of the *Hernaria cinerea*, respectively.

The preliminary phytochemical screening of *Hernaria cinerea* plant of different months (March, April and May) to investigated alkaloids, glycosides, cardiac glycosides, saponins, phenol, sterol, tannins, flavonoids, amino acid and present in plant under investigation. Table (3) showed that Volatile oil was absent. Table (4) shows the percentages of total flavonoids and total phenolic acid, total tannins, total saponins has increased value in March and decreased value in May, The percentages of total alkaloid has increased value in April and decreased value in May.

Acute Toxicity & Median Lethal Dose (LD_{50}) of *Hernaria cinerea* extract in rats was 5500 respectively but high decrease by (3.26 & 6.48) in (TP & ALB), respectively when compared with as compared with CCl_4 group.

M1 group showed high increase by group showed increase by (92.1, 111.33, 1.8, 125 &199.3) in mg/kg. Therefore, the tested plant can be categorized as safe since substances possessing LD_{50} higher than 50 mg/kg are non toxic.

Effect on liver functions

The pretreatment of the normal rats with the M1 & M2 extracts for 35 consecutive days has no significant change in liver function parameters confirming its safety profile.

Hepatoprotective Activity

Table (5) showed the effect of *Hernaria cinerea* extract on some biochemical parameters in rats with CCl_4 induced–hepatotoxicity in table (1) clearly indicated that, CCl_4 group showed high increase by (104.32, 191.33, 5.1, 2.99, 1.7, 155.32, 215.33) in (ALT, AST, TP, TAG_S and Cholesterol), respectively but high decrease by (2.8 &4.98) in (TP & ALB), respectively when compared with (N.C) group. Silymarin group showed increase by (55.66, 63, 1.35, 117.33 &135.33) in (ALT, AST, TP, TAG_S and Cholesterol), respectively but high decrease by (3.26 & 6.48) in (TP & ALB), respectively when compared with (N.C) group. On the other hand Silymarin group showed high decrease by group showed increase by (55.66, 63, 1.35, 117.33 &135.33) in (ALT, AST, TP, TAG_S and Cholesterol), (ALT, AST, TP, TAG_S and Cholesterol), respectively but high decrease by (4.87, 3) in (TP & ALB), respectively when compared with (N.C) group. On the other hand, M2 group showed high decrease by (55.6, 93.4, 1.4, 125, 155.2) in (ALT, AST, TP, TAG_S and

Cholesterol), respectively, high increase by (9.26 & 12.12) in (TP & ALB), respectively as compared with CCl_4 group.

Table 2. free amino acids of *Hernaria cinerea* different months

Name of amino acids	*Hernaria cinerea*		
	March	April	May
Aspartic	1.386	0.578	0.086
Theronine	0.841	0.782	0.063
Serine	0.610	0.851	0.125
Glutamic	1.773	0.632	0.640
Proline	1.330	0.77	0.517
Glycine	0.698	0.446	0.444
Alanine	0.776	0.355	0.486
Valine	0.716	0.431	0.120
Isoleucine	0.214	0.089	1.0051
Leucine	0.602	0.141	0.452
Tyrosine	0.740	0.414	0.545
Phenyl alanine	0.571	0.223	0.345
Histidine	0.914	0.276	0.240
Lysine	0.342	0.124	0.111
Arginine	0.596	0.134	0.146

Table 3. Preliminary phytochemical screening in different months (March, April and May) of *Hernaria cinerea*

Groups	Tests	Methanol Extracts		
		March	April	May
Alkaloids	Wagner's reagent	+ve	+ve	+ve
	Dragendorrf's reagent	+ve	+ve	+ve
Glycosides	Glycosides test	+ve	+ve	+ve
	Modified borntrager's	+ve	+ve	+ve
Cardiac glycosides	Legal's test	+ve	+ve	+ve
Saponins	Foam test	+ve	+ve	+ve
	Haemolysis tests	+ve	+ve	+ve
Phenols	Ferric chloride test	+ve	+ve	+ve
Phytosterols	Liberman burchard's test	+ve	+ve	+ve
	Salkwaski reaction	+ve	+ve	+ve
Tannins	Gelatin test	+ve	+ve	+ve
	Lead acetate test	+ve	+ve	+ve
Flavonoids	Schinodar's test	+ve	+ve	+ve
	NaOH test	+ve	+ve	+ve
Amino Acids	Xanthoproteic test	+ve	+ve	+ve
	Ninhydrin test	+ve	+ve	+ve
Volatile oil's	Steam distillation	-ve	-ve	-ve

Table 4. Total active materials in different months of *Hernaria cinerea*

Months	Item	Total active materials in different months		
		March	April	May
Hernaria cinerea	Total flavonoids (mg/gm rutin)	298±0.57	277.3±1.02	250.7±0.78
	Total phenolic acids (mg/gm gallic acid)	379.7±0.68	363.7±0.97	338±0.76
	Percentage of Total Tannins (%)	1.63±0.13	1.51±0.06	1.38±0.09
	Percentage of total Saponins (%)	2.26±0.13	1.73±0.11	1.51±0.06
	Percentage of total Alkaloids (%)	2.1±0.17	2.16±0.15	1.48±0.10

Table 5. Effect of silymarin and methanol extract (M) in rats with CCl_4 induced hepatotoxicity

Test Groups	ALT	AST	Total protein	Albumin	Total bilirubin	TAGs	Cholesterol
	(U/L)		(g/dl)		(mg/dl)		
Normal control Range Mean ±SD	25-30 28.3±1.8	22-33 27.4±3	6.7-7.7 7±0.2	3.4-4 3.65±0.3	0.9-1 0.91±0.02	47-52 48±1.1	110-21 116.6±3.1
CCl_4 Range Mean ±SD	100-110 104.32±2.1	188-203 191.33±2.4	4.98-5.6 5.1±1.18	2.8-3.2 2.99±0.06	1.5-1.9 1.7±0.11	151-166 155.32±5.41	214-218 215.33±1.1
Silymarin+CCl_4 Range Mean ±SD	51- 60 55.66±1.68	57-66 63±3.15	6.12-6.82 6.48±0.02	3-3.6 3.26±0.17	1.2-1.54 1.35±0.07	116-129 117.33±2.6	130-140 135.33±2.6
M1+CCl_4 Range Mean ±SD	89-95 92.12±1.6	110-120 111.33±2.6	4.6-5.3 4.87±0.1	2.9-3.3 3±0.1	1.8-2.5 2.1±0.2	125-146 136±3.8	189-204 199.3±6.1
M2+CCl_4 Range Mean ±SD	55-61 59.3±1.4	87-98 93.4±2.6	5.74-5.9 5.8±0.1	3.6-3.9 3.7±0.1	1.4-2 1.7±0.2	115-136 125±6.3	150-162 155.2±4.2

M1: 275 mg/kg of the extract, **M2:** 550 mg/kg of the extract

The liver is one of the most important organs in the body. It plays a pivotal role in regulating various physiological processes. It is also involved in several vital functions, such as metabolism, secretion and storage. It has great capacity to detoxicate toxic substances and synthesize useful principles. It helps in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction. In addition, it aids metabolism of carbohydrate, protein and fat, detoxification, secretion of bile and storage of vitamins (Ahsan *et al.,* 2009).

Hepatic disorders remain one of the serious health problems. Numerous medicinal plants and their formulations are used for liver disorders in Ethnomedical practices as well as in traditional Indian medicines (Babu *et al.,* 2001). It is well documented that, CCl_4 induced hepatotoxicity model is widely used for the study of hepatoprotective effects of drugs and plant extracts. Hepatotoxicity occurs through biotransformation of CCl_4 in liver by cytochrome P_{450} enzymes to produce very reactive CCl_3 radical. This active CCl_3 radical produces trichloromethylperoxyl radical (CCl_3O_2) after reacting with oxygen, which is then covalently binds with cellular macromolecules and biomembranes to cause lipid peroxidation of the lipid membranes of the hepatic tissue. This whole cascade of biochemical events ultimately causes hepatic damage by disturbing of cellular integrity (Suja *et al.,* 2002).

In the current study, a marked elevation in serum levels of ALT, AST which are normally located in the cytosol indicated the cellular leakage and loss of functional integrity of the cell membrane, and used as an index of liver damage and as a complicating case of CCl_4 intoxification (Parmar *et al.,* 2010; Mandade, 2011). Consequently, the maintenance of these serum enzymes near the normal value in plant extract pretreated-groups is an indication of stabilization of plasma membrane thereby preserving the structural integrity of cell as well as repairing and regeneration of hepatic tissue damage that caused by CCl_4 (Ali *et al.,* 2012).

The hyperbilirubinemia that observed in the untreated CCl_4-intoxicated group may be attributed to blockage of biliary tract. As a result of blockage of the biliary tract there is a mass inhibition of the conjugation reaction and release of unconjugated bilirubin from damaged hepatocytes (Manjir *et al.,* 2012).

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