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ORIGINAL ARTICLE

Phenolic Compounds and Terpenes in the Green Parts of Glycine Hispida

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Kyslychenko, V., Karpiuk, U., Diakonova Ia. and Mohammad S. Abu-Darwish: Phenolic Compounds and Terpenes in the Green Parts of Glycine Hispida

ABSTRACT

A study was conducted to determine the phenolic compounds and terpenes in the chloroform and water extracts of the green parts of *Glycine hispida* collected during the flowering stage. The organoleptic and physico-chemical parameters were defined. The phenolic and terpenoied component of chloroform and water extracts was studied using gas-chromatograph/mass spectrometer (Hewlett Packard HP-6890) with a mass-selective detector (HP-5972). The results showed that, chloroform and water extracts from the green parts of the *Glycine hispida* were 2.69 and 32.12% of the mass, respectively. The Gc/Mass analysis of the samples revealed that, the chloroform extract had components that are belong to terpenes, including megastigmatrienone I, phytol, isophytol and alpha-pinene. Megastigmatrienone I, is the one of the main components of the terpenes that has been found. The quantitative content of Megastigmatrienone I is 84.19 mg/kg. Megastigmatrienone I, has a specific tobacco-like odor and is the one of the main odor components of the chloroform extract. Pyrogallol and phenolic acids were found in the water extract of the green parts of *Glycine hispida,* .The phenolic acids in this water extract were ; isovanillic and salicylic acids (benzoic acid derivatives), and ferulic and m-coumaric acid (cinnamic acid derivatives). Salicylic acid has the greatest quantitative content in the water extract (71.43) mg/kg. The greatest quantitative content of the cinnamic acid derivatives is ferulic acid (77.9) mg/kg.

Key words: Glycine hispida, GC/MS, phenolic compounds, terpenes.

Introduction

The arsenal of herbal medicines and plant-based, biologically active supplements is growing in the pharmaceutical market in the world every year. *Glycine hispida* is frequently used in their ingredients. Their composition, both reactants and additives, contains *G.hispida* bean-based, biologically active substances and its processed products. *G.hispida* beans originated in the Orient. During the Chou Dynasty (1134-246 BC) the *G. hispida* beans were designated one of the five sacred grains, along with barley, wheat, millet and rice. Nowadays, the scope of *G.hispida* bean application is constantly expanding. *G. hispida* is used not only as a food product, but also is an integral part of the pharmaceutical, paint ,varnish, textile, paper manufacturing and animal-feed industries. Today *G.hispida* is cultivated not only for the beans, but also to obtain green forage. The *G. hispida* plant is used for feeding animals, for silage feedstock, grass flour, and processed

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granules [1,17-19,23]. *G. hispida* seeds contribute too many drugs and food supplements, because of its rich chemical composition and medicinal contents. It´s seeds are rich in protein, oil and polysaccharides. The major liposoluble components of *G.hispida* beans are saturated and unsaturated fatty acids, phospholipids and sterols. In addition, they are rich in phenolic compounds. The most widely studied components of *G.hispida* beans are the isoflavones. These compounds have estrogenic properties and can reduce the risk of breast cancer. Recent investigations show that isoflavones can be used in hormone-replacement therapy [3,7, 13,14,17-19,23].

The green part of *G.hispida* has been scarcely studied compared to *G.hispida* beans and *G.hispida* bean-based products. In an attempt to expand the raw-material base for the pharmaceutical industry, this study of *G. hispida* grass was conducted in its flowering stage, to determine the phenolic compounds and terpenes in the chloroform and water extracts in the green parts of the plant.

Materials and methods

Aerial green parts of *G.hispida* were collected during the flowering stage in June-July 2008 from the experimental farms of the Institute of Animal Science, Ukrainian Academy of Agrarian Sciences in Kharkov region. The collected plant material was dried in draughty place at about 20Cº. The dried green material of *G.hispida* was separately crushed and mild in to small pieces and sieved through (0,5mm) mesh sieve. Chloroform extract was obtained from the dried, crushed and sieved green parts of *G.hispida* by conducting an exhaustive extraction with chloroform in a Soxhlet apparatus. Water extract was obtained using hot water.

Analysis of water and Chloroform Extracts:

The obtained water and chloroform extracts were evaporated under reduced vacuum pressure . The residual extracts were removed for the determination of lipophilic and water complex contents. Organoleptic and physico-chemical parameters in the chloroform and the water fractions were also studied. Sample pretreatments were conducted for chemical analysis of extracts residuals. After saturation with hydrogen chloride, double methylation with acidified methanol and diazomethane was introduced. Also, in order to protect the functional groups of the components, each sample was siliconized by using BSTFA (bis-[trimethylsilyl]-trifluoroacetamide).

Analysis of chloroform and water extracts residuals were conducted on a gas-chromatograph/mass spectrometer (Hewlett Packard HP-6890) with a mass-selective detector (HP-5972). Separation of the mixture of the components was carried out using a capillary column HP-5MS (5% Diphenyl) 30 m long, with an inner diameter of 0.25 mm and stationary layer phase thickness of 0.25 μm. The carrier gas was helium, for temperature programming at a constant rate of flow. The flow rate of the carrier gas was 1 cm^3/min. The column was kept at a temperature 45°C for 2 min, then heated at a rate of 10°C/min to 315°C and then held at this final temperature for 5 min. The volume of the sample that was injected was 1 ml. The evaporator temperature was 250°C, the temperature of the detector 280°C, the temperature of the ionic source 280°C, the interface line temperature 280°C. The electron impact ionization mode had electron energy of 70eV, scanning in the range of mass numbers from 40 to 550. The identification and the quantification of the compounds was performed by comparing the retention times with similar indicators of standardized pure substances from the electronic libraries Nist 02 and Wiley 138k. The quantity of each component was calculated by the ratio of the area of the corresponding peak, to the sum of the areas of all peaks.

Results and discussion

Chloroform Extract:

The results showed that, chloroform extract from the green parts of the *G. hispida* was 2.69 % of the mass. The extract was viscous and pasty-looking; its color was dark green; its aroma was oleoresinous. The taste was astringent. The extract was insoluble in water but was very soluble in chloroform, hexane and ether, only slightly soluble in 96 % ethanol.

Using the GC/MS method, it was found that, the chloroform of green parts of the *G. hispida* extract had components belonging to terpenes, including megastigmatrienone I, phytol, isophytol and alpha-pinene, with the masses that are shown in table (1).

Megastigmatrienone I (Figure 1) is one of the components in the chloroform extract that gives its smell. Megastigmatrienones are C13 nor-isoprenoids, which are produced from carotenoids and are main compounds found in tobacco. [1,11,16,25]. These compounds are found in many plant families, including Solanaceae, Lamiaceae, Asteraceae, Moringaceae, Liliaceae,and Ericaceae [1,4,5,11]. The nor-isoprenoids can be formed by direct degradation of carotenoids, and they also frequently occur as bonded forms [1]. The

bonded glycosidically conjugated nor-isoprenoids can be mono-oxygenated conjugates, dioxygenated conjugates, or higher-oxygenated conjugates [8].

Nor-isoprenoids are important class of aromatic components of many species of plants. The megastigmatrienon that is found in blackberries is responsible for its aroma [8]. The essential oils *Stachys palustris* and *Bidens pilosa* contain megastigmatrienons [1]. Many nor-isoprenoids are found to be part of the aroma of red and white wines that are reminiscent of grassiness, tea, oak, honey, and pineapple [2,15,22]. Norisoprenoids in grapes arise from photochemical and enzymatic degradation of carotenoids present in the skin and pulp, such as β-carotene and lutein [15]. And so, with increasing sunlight on the grapes, there is an increase of the norisoprenoids. Furthermore, megastigmatrienones are present in different kinds of honey, where they constitute markers of floral origin [11,20]. A simple, but very important diterpene is phytol, an acyclic diterpene, which is a part of chlorophyll's molecule. Phytol forms the lipophilic side-chain of chlorophylls [6,12]. It is also the one of the main components of essential oils having antifungal properties [4]. Pinens are widespread natural compounds. They are bicyclic monoterpenes found in many plants, such as *Citrus limon, Juniperus communis, Rosmarinus officinalis, Pinus palustris, Coriandrum sativum,* and *Eucalyptus globulus*. They are the components that stipulate the flavour, antiseptic, aroma-therapeutic, and disinfectant properties of those plants [6]. The resulted odor components that have specific flavor in chloroform extract are shown in table (2). They give the chloroform extract its characteristic odor [16].

Water Extract:

Water extract was 32.12 % of the mass. The extract was a pasty looking mass, brown in color, with a pleasant smell. It was highly soluble in hot water 80-90 ^{o}C, only slightly soluble in 96 % ethanol, insoluble in organic solvents.

The phenolic compounds found in the water extract of the green parts of *G.hispida*, are shown in table (3).

The phenolic compounds, found in water extract of green parts of Glycine hispida, are pyrogallol and phenolic acids. The free phenols and phenolic acids are usually identified together during plant analysis. Phenolic acids may be present as simple glycosides, as esters, ethers or acetates. Phenolic acids are widely spread through many plants, but some free phenols are quite rare in plants. Pyrogallol, for instance, has been reported in only a few sources [12,24]. Phenolic acids are aromatic secondary plant metabolites. There are two widely spread groups of phenolic acids: benzoic-acid and cinnamic-acid derivatives. Isovanillic and salicylic acid (derivatives of benzoic acid) and ferulic and m-coumaric acid (derivatives of cinnamic acid) were found in water extract of the green parts of *G. hispida.*

The simple phenols and phenolic acids have various biological and pharmacological effects. They are precursors to the synthesis of many complex compounds such as flavonoids, tannins ,etc. They play an important role in the natural host defense mechanism of plants against infectious diseases and inhibit multiplication of plant pathogenic bacteria, viruses, and fungi. Phenolic acids are generally considered to be nontoxic and are often found in many traditional herbal medicines. Most of the benzoic-acid derivatives show antioxidant activity, which is depend on the number of hydroxyl groups in their molecule. Cinnamic-acid derivatives were found to be more active than benzoic ones, due to the presence of the –CH=CH-COOH group in the cinnamic-acid molecule.

Recent investigations have linked some phenolic acids with anticancer activity. Animal studies and in vitro studies suggest that ferulic acid may have antitumor activity against breast and liver cancer. Some of the phenolic acids, such as salicylic and ferulic acids, have anti-inflammatory and antirheumatic properties. Also salicylic acid has analgesic, antipyretic and keratolytic activities. Phenolic acids are considered to have potential as immunostimulating compounds. For example, salicylic acid has significant stimulatory influence on the production IgG antibodies, and system application of this acid could result in suppression of leukocyte accumulation, thus beneficial in the treatment of chronic inflammatory diseases [24].

Conclusion:

Megastigmatrienone I is the one of the main components of terpenes that have been found in green parts of *G.hispida*. The quantitative content of Megastigmatrienone I is 84.19 mg/kg. Megastigmatrienone I has a specific tobacco-like odor and is the one of the odor components of the chloroform extract. Pyrogallol and derivatives of benzoic and cinnamic acid have been found in the water extract of green parts of *G.hispida*. Salicylic acid is the derivative of benzoic acid which has the greatest quantitative content in the water extract: 71.43 mg/kg. The greatest quantitative content of the derivatives of cinnamic acid is ferulic acid: 77.9 mg/kg. The obtained

results show, that not only *G. hispida* seeds are the source of valuable compounds, but also green part of *G. hispida* can be raw material for obtaining important biological active compounds, for the treatment of some chronic diseases and also for preventive measures.

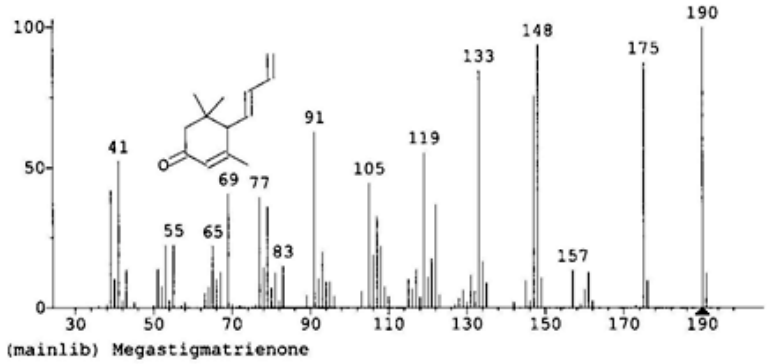

Fig.1: Mass spectrum of megastigmatienone I**.**

Table 1: Terpenes from chloroform extract of the green parts of *G.hispida*

No	Compound	Content, mg/kg
1	Megastigmatrienone I	84.19
2	alpha-Pinene	16.91
3	Phytol	9.63
4	Isophytol	15.06

Table 2: Odor compounds of the chloroform extract of the green parts of *G.hispida*

No	Constituent	Odor description
1	Megastigmatrienone I	Warm, Dry, Sweet, Tobacco-Like
2	alpha-Pinene	Resinous, pine odor
3	Nonanoic acid	Fatty, musty, sweaty sour "goaty"
4	Tetradecanoic acid	Very faint, waxy-oily; nearly odorless
5	9-Hexadecenoic acid	Weak fatty-tallowy fried odor
6	Dodecanoic acid	Weak, refreshing, fatty, waxy odor

Table 3: Phenolic compounds of the water extract of the green parts of *G. hispida*

No	Compound	Content, mg/kg
1	Pyrogallol	51.33
2	Salicylic acid	71.43
3	Isovanillic acid	28.66
4	Ferulic acid	77.99
5	m-Coumaric acid	57.50

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