QUALITY ASSESSMENT OF HERBAL MEDICINAL PRODUCTS IN BENIGN PROSTATE HYPERERTROPHY: CHEMICAL MARKERS AND FINGERPRINT ANALYSIS

Huda Mando¹,²*, Ahmad Hassan², Ghassan Abo Chameh³ and Nathalie Moussa⁴

¹Department of Pharmaceutical Chemistry and Drug Control, Faculty of Pharmacy-Damascus University, Damascus, Syria
²Department of Pharmaceutical Chemistry and Drug Control, Faculty of Pharmacy, Damascus University, Damascus, Syrian Arab Republic
³Department of Chemistry, Faculty of Science, Damascus University, Damascus, Syrian Arab Republic
⁴Department of Pharmaceutical Chemistry and Quality Control of Medicaments, Faculty of Pharmacy, Manara University, Latakia, Syria

Herbal products with beneficial influence on BPH have been widely used. These herbal preparations include the extracts of Serenoa repens and/or Pumpkin seed oil. Chemical markers are crucial in the quality control of herbal medicines and construct with chromatographic fingerprint a powerful tool for quality assessment. The aim of this study was to evaluate the quality of herbal formulations used to treat BPH in Syrian local market by identifying fatty acids profile and tocopherols, besides analyzing fingerprint of methanol extracts. For this purpose, GC/MS analysis has been applied. The fatty acids content was consistent with the quality requirements in four samples. α and γ tocopherol content was in agreement of previous studies between 181-875 µg/g with the exception of one sample reached 77.5 µg/g for both α and γ tocopherol. Chromatographic fingerprint revealed the shortage of ∆7 phytosterols in pumpkin seed oil samples and the presence of parabens in one of them. The absence of Luaric acid in one sample was confirmed with chromatographic fingerprint. The results have highlighted on the importance of combining active markers with chromatographic fingerprint as a suitable technique for quality evaluation of herbal products as well efficiency and safety.

Keywords: BPH; herbal products; GC/MS; official markers; analytical markers; chromatographic fingerprint

INTRODUCTION

Benign prostate hypertrophy (BPH) occurs in about 50% of men over 50s, and 50% of all aging men over 80 years¹. Phytotherapy is commonly used to treat BPH. Treatment with botanical medicines improves symptoms in most of patients, which makes treatment with botanical pharmaceutical products the preferred choice over chemotherapy in men with BPH. A variety of herbal preparations are available on the market where Serenoa repens, Urtica dioica and Cucurbita pepo construct most of them³.

The historical use of Serenoa repens to treat BPH has been common for centuries in Asia and in native cultures in America. The medicinal part is the fruit, and the dried ripe fruit is used in dietary supplements in several forms of extracts, with the lipid extract being the most common. Alternative names as Saw palmetto and Sabal serrulata are also in use³.

Pumpkins including Cucurbita pepo belong to the genus Cucurbita. They were primarily used by native Americans and later
adopted by European and other countries for their traditional nutritional and medicinal values. Fruits, flowers, seeds, and seed oil possess a range of biological activities characterized by antibacterial, antiviral, cytotoxic and antitumor activities. Clinical studies suggested that pumpkin seed oil improves the symptoms of BPH and effective as alternative medicine8. The complex nature of constituents of herbal drugs necessitates applying extensive quality control tests. Ensuring consistency, safety and efficacy are the main objectives of quality control of herbal medicines5. Chemical markers whether compendial or analytical are effective tools to evaluate the quality of herbal products. Chromatographic fingerprinting also has proven to be a powerful quality control technique for herbal medicines. Consolidation between chemical markers and chromatographic fingerprinting will provide a unique pattern that elucidates the presence or absence of multiple constituents within the sample6.

A variety of natural product preparations containing *Serenoa repens* and pumpkin seed oil are the most studied and used medicinal plants for prostatic hypertrophy7. Bioactive components include free fatty acids (lauric acid) and betasitosterol in *Serenoa repens*8. Other constituents are carbohydrates such as Mannitol and polysaccharides with Galactose and Arabinose, aromatic acids like Ferulic and Vanillic acid, β-carotenes, the vitamin E derivates γ-tocopherol and δ-tocopherols, Lipase, Tannin, and Anthranilate acid10. Fatty acids, tocopherols, phytosterols, in addition to β-carotenes are the main constituents in pumpkin seed oil11.

Previous analytical studies were conducted mostly on preparations containing *Serenoa repens*, as they constitute the largest proportion of the total plant medicines used for BPH. The studies documented the use of different analytical techniques to identify and quantify fatty acids, phytosterols12, and flavonoids13, in order to assess the quality of preparations and to compare between different commercial varieties of origin, source and method of preparation.

Based on the previous studies, we will monitor the quality of the preparations of plant origin in the local Syrian market used to treat BPH by determining the fatty acids content, tocopherols and analyzing chromatographic fingerprint.

So far, to our knowledge, there is no study on a chromatographic fingerprint for neither preparations of the *Serenoa repens* nor pumpkin seed oil, and what was mentioned in the studies of the term chromatographic fingerprint was limited to determining the profile of fatty acids.

**MATERIALS AND METHODS**

**Chemicals**

Potassium hydroxide, hexane, anhydrous sodium sulfate, methanol, reference standards of γ and α tocopherol were purchased from Sigma Aldrich.

**Instruments**

Gas chromatographic analysis (GC) were conducted using Agilent 7890A GC/MS instrument with an HP5 column of 30 m length, 250 mµ diameter and 0.25 m µ film thickness.

**Samples**

Five samples were supplied from the local market retail pharmacies. The first sample Sawbal (S) is tablets containing 360 mg of dry fruit extract of *Serenoa repens*, while the second sample Mivolis (SP) contained *Serenoa repens* extract, Pumpkin seeds and Pumpkin seed oil. Sample Pepon (P1) was a soft gelatin capsule containing 300 mg of Pumpkin seed oil (*oleum cucurbita pepo*). Sample P2 was a glass bottle containing 95 ml of cold-pressed Pumpkin seed oil and did not contain additives. As for the sample P3, it is a glass bottle of 60 ml capacity, claimed to contain 100% natural pumpkin seed oil extracted in ideal way to preserve active ingredients without altering chemical structure.

**Techniques**

**Determination of fatty acids**

The content of fatty acids was determined by slightly modification of the method described by Khandro *et al*14. Fatty acids were converted to their corresponding methyl esters by transesterification with Hexane and 2N methanolic KOH and analysed by gas chromatography (GC) Agilent 7890A equipped
with a mass spectrum detector (MS). Separation was obtained using non polar HP-5 capillary column (30 m x 250 µm x 0.25 µm column HP5-MS). The thermal program, started at a temperature of 100 °C for a minute and raised by 40 °C per minute up to 160 °C and then rise by 2.5 °C per minute to 180 °C, followed by a rise of 2 °C per minute to 185 °C, followed by a rise of 5 °C per minute up to 250 °C and the elevation continued at 50 °C per minute up to 300 °C. Helium was used as carrier gas at a flow rate of 1.2 ml/min, and the analysis time was 34 min. The mass spectrometer was operated in the electron impact (EI) mode at 70 eV in the scan range from 50 to 600 m/z.

Identification of tocopherols

The tocopherol content in the studied samples was determined by scarcely modification of the method described by Rui Zhang et al for better resolution of the four tocopherols α, β, γ and δ without interference of other components. Methanolic extract has been obtained by adding 10.0 ml of methanol to 1.5 g of the herbal samples. Ultrasound apparatus for 2 hours has been used for extraction. After centrifugation, methanol extracts were withdrawn and subjected to evaporation till dryness. The residue was dissolved in 1.0 ml of hexane and injected into GC/MS. The work was carried out according to the thermal program, starting with a temperature of 60 °C, rising at a rate of 3 °C per minute up to 300 °C. Helium was used as carrier gas with a flow rate of 1 ml/min, and analysis time was 56 min. The four tocopherols were identified by the National Institute of Standards and Technology (NIST) library. Each of the herbal samples was screened for the four isoforms.

Assay of α and γ tocopherol

The method described by Rui Zhang et al was applied for quantification of α & γ tocopherol using gas GC/MS operated in selected-ion monitoring (SIM) mode. Molecular specific ions were selected for each compound as quantifiers. Samples were prepared in a similar way for the identification of tocopherols with the final solvent methanol replacing hexane. A mixed standard solution containing α & γ tocopherol was prepared from a stock solution of a concentration 100 µg/ml. Calibration curve of concentrations 10, 20, 50, 100, 200, 500 and 1000 ng/ml was obtained by diluting the working standard 10 µg/ml with methanol. Each of the examined samples was screened for the relative amounts of the two isoforms.

RESULTS AND DISCUSSION

Determination of fatty acids

Sample S

The chromatogram showed the presence of 6 peaks, four of which are methyl esters and two are ethyl esters, as shown in Fig. (1). Fatty acids were defined by comparison with the mass spectrum of the reference library of the National Institute of Standards and Technology (NIST) where they were confirmed by the presence of mass fragment ions at m/z 87, 74, 55. The ion of m/z 74 denotes the presence of a methyl ester of an acid, and collectively these ion fragment are most abundant in saturated fatty acids such as Palmitic, Stearic, Caprylic, Caproic, Lauric, and Myristic acid.

Fig. (2) shows the spectrum of Palmitic acid methyl ester in which all the previous fragments appear. Table (1) shows the identity, peak area and retention time estimated in min for methyl esters in the chromatogram.

The results revealed the dominance of Linoleic acid as the main fatty acid. Serenoa repens extracts predominantly consist of fatty acids (~85%) and are unique compared with other extracts and oils in that they are a rich source of the saturated, medium-chain fatty acids laurate (12:0) and myristate (14:0), however, there was neither saturated, medium-chain fatty acids, nor lauric acid in this sample
Fig. 1. Chromatogram of fatty acids methyl esters in sample S by GC/MS.

Fig. 2: Spectrum of Palmitic acid methyl ester by GC/MS.

Table 1: The percentage of fatty acids in samples S, SP, P1, P2, P3.

<table>
<thead>
<tr>
<th></th>
<th>Caprylic</th>
<th>Lauric</th>
<th>Myristic</th>
<th>Palmitic</th>
<th>Linoleic</th>
<th>Oleic</th>
<th>Stearic</th>
<th>Arachidic</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5.9</td>
<td>54.061</td>
<td>23.1</td>
<td>1.6</td>
<td>-</td>
</tr>
<tr>
<td>SP</td>
<td>1.0</td>
<td>12.6</td>
<td>5.1</td>
<td>15.2</td>
<td>38.6</td>
<td>23.3</td>
<td>4.2</td>
<td>-</td>
</tr>
<tr>
<td>P1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>14.7</td>
<td>50.3</td>
<td>30.5</td>
<td>4.4</td>
<td>-</td>
</tr>
<tr>
<td>P2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>14.4</td>
<td>57.7</td>
<td>22.6</td>
<td>5.0</td>
<td>0.3</td>
</tr>
<tr>
<td>P3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>11.6</td>
<td>53.8</td>
<td>29.2</td>
<td>4.0</td>
<td>0.3</td>
</tr>
</tbody>
</table>

The U.S. P Pharmacopoeia contains four monographs describing the fruits, powder, extract, and capsules of *Serenoa repens* content. According to the U.S.P 35 version, the capsules must contain at least 22% of Lauric acid, and the ratio of Lauric acid to Caprylic acid should be between 8.5-17.5%, and between 2.2 and 2.8% for Myristic acid. The pharmacopoeia also specified a qualitative range for the ratio of 9 fatty acids to Lauric acid that can be relied upon to detect adulteration by adding vegetable oils unless they were added in proportions that mimic the original distribution ratios. The USP formulary contains general tests for quality assurance and specific tests for three types of phytosterols which are Betasitosterol (not less than 0.1%), Stigmasterol, and Campesterol. The European Pharmacopoeia stipulated that Lauric acid area should not be less than 20% of the total area of the total fatty acids and the same applies to the British Pharmacopoeia.
According to the laboratory guideline issued by the American Botanical Society, the most adulterated vegetable oils for the saw palm plant are pistachio oil, canola oil, coconut, olive, sunflower and fatty acids of animal origin, and the ratios we obtained for methyl esters do not correspond to any of the previous oils.

Sample SP
The chromatogram showed the presence of 7 peaks (Fig. 3), expressing the methyl esters of Caprylic, Lauric, Myristic, Palmitic, Linoleic, Oleic and Stearic acid. Table (1) shows the peak area and retention time of the methyl esters of fatty acids that were identified in the SP sample. Unsaturated fatty acids, i.e. Linoleic acid and Oleic acids were predominant with 62% of total fatty acids. Lauric acid constructs 12.56% of the total fatty acids, which is less than the required percentage according to the American and European Pharmacopoeia. The ratio of Lauric acid to Caprylic acid of 12.35 can be compared to being within the range 8.5-17.5 mentioned in the U.S.P Pharmacopoeia, and the ratio of Lauric acid to Myristic acid is 2.49 and it is within the range 2.2-2.8 specified in the previous pharmacopoeia, and these three fatty acids are specific components of *Serenoa repens*. Peaks 4,5,6,7 in the chromatogram are correspondent to Palmitic, Linoleic, Oleic and Stearic acid, which in turn are due to the presence of seed and pumpkin seed oil in the sample.

Anthony Booker and colleagues used two analytical techniques, gas chromatography and 1H nuclear magnetic resonance (NMR) spectroscopy followed by principal component analysis (PCA) to compare 57 commercial preparations of *Serenoa repens*. These preparations contained the *Serenoa repens* as a single ingredient or as a combination of multiple ingredients. This study relied on fatty acids as markers and on NMR as a suitable, robust and rapid method for evaluating the quality of these preparations. Prior to this study, Feifer AH and his colleagues documented a difference between what is labeled on the preparations and what is present, and that what is present of the ingredients is 25% less than the amount labeled in half of the preparations.

In a study published in 2013 and conducted on 20 commercial preparations in the form of liquid, powder, dry fruits and dye, the content of fatty acids was analyzed by gas chromatography method equipped with a gas chromatography with flame-ionization detector (GC/FID), and phytosterols by gas chromatography method supplied with a mass spectrometry (GC/MS) detector. The results of the analysis showed the presence of additives to some forms, and this was inferred from the high percentage of lauric acid and Myristic acid. The low percentage of these two acids indicates the presence of these additives. Some preparations contained a high percentage of oily acid, and others contained a higher amount of fatty acids than the labeled amount. Penugonda K and Lindshield compared these results with the results of a 2002 study and found that 83% of the liquid formulations contained more fatty acids than the labeled ones, and that this percentage was 33% higher than the percentage found in the 2002 study.

In the latest study on *Serenoa repens* preparations in 2021, four samples of Korean preparations from the Korean market were analyzed and compared with a reference extract and to ensure their compliance with the specifications contained in the United States Pharmacopoeia (U.S.P), and it was found that the content of fatty acids matched the constitutional regulations and the low content of Long-chain fatty acids exceed the minimum constitutional limit of 0.15%, in contrast, the level of sterols is 1.4-2.0 times higher than the constitutional limit. It is worth mention that despite the many and varied benefits of pumpkin seed oil resulting from its content of unsaturated fatty acids, carotenoids, tocol and phytosterols, the pharmaceutical market lacks preparations containing it, and what is found is almost numerical. In a study conducted on Bioprost suppositories containing 25% of pumpkin seed oil in addition to Thymol and used to treat BPH, it was found that they contain carotenoids in an amount of 48 mcg per suppository, 6 mg of α tocopherol, 50 mg of γ tocol, and 630 mcg of Betasitosterol.
Sample P1

The chromatogram (Fig. 4) shows the presence of four peaks corresponding to Palmitic, Linoleic, Oleic and Stearic acid. The results insured the dominance of Linoleic and Oleic acid. The content of *Cucurbita pepo* seed oil in sample P1 makes this result satisfied in terms of the presence of the four previous fatty acids stated in the literature, in addition to the percentage of unsaturated fatty acids being 80.8 within the range 78.34-95.33%. Table (1) shows the peak area and retention time for the four fatty acids in the sample P1, from which we see that the percentage of Palmitic acid is 14.7%, which is the saturated fatty acid with the largest percentage, and the percentage of Linoleic acid (50.3%) within the range 44.3-51.58, which is greater than the percentage of oleic acid (30.5%), which ranges from 20.4 to 37.8*26,27*.

Sample P2

The chromatogram (Fig. 5) showed the presence of five peaks, which are the same as those that appeared in the sample P1 in addition to the Arachidic acid. Table (1) shows: Peak area and time of fatty acid retention in sample P2. The fatty acids in this sample, in terms of their four main types, the percentage of saturated ones, and the individual percentages of each of them are in match with previous studies. Arachidic acid was found in a small percentage of 0.3%. In details, the percentages of Palmitic acid (14.35%), Linoleic acid (57.74%), Oleic acid (22.57%), and Stearic acid (4.96%) are similar to the study of Bardaa *et al*28.
Sample P3

The chromatogram (Fig. 6) showed the presence of five peaks expressing five fatty acids similar to those that appeared in sample P2. The percentage of unsaturated fatty acids (82.8%) is in agreement with the published studies, as well as is the percentage of Arachidic acid and saturated fatty acids\(^{28,26}\).

Identification of tocopherols

A Modified method of Rui Zhang et al. was applied for identification of tocopherols. GC equipped with MS is convenient in identifying and quantifying the tocopherols\(^{29}\). \(\gamma\) and \(\delta\) tocopherol are predominant in *Serenoa repens*\(^{30}\). Tocopherol profile is helpful in assessing the authenticity of pumpkin seed oil\(^{15}\). The tocopherol isomers were identified by (NIST) library where retention time was 38.5, 39.7, 40.5 for \(\delta\), \(\gamma\) and \(\alpha\) tocopherol respectively. It is worth noting to report that each tocopherol is unique in its fragmentation pattern where the degree of methylation and position of methyl substituents on the chromanol ring constitute the base of differentiation between each individual tocopherol. For instance, the fragmentation pattern of \(\alpha\) tocopherol is distinctive by its molecular ion \(m/z\) 430, besides its daughter ion \(m/z\) 165 as a result of a loss of side chain followed by breaking the bonds in the chromanol ring resulting in the loss of propyne fragment\(^{29}\). This mechanism is suggested in a similar way for the formation of selective marker ions \(m/z\) 151, and \(m/z\) 137 for \(\beta\), \(\gamma\) and \(\delta\) isomers, respectively enabling confirmation for tocopherol isomers identification\(^{31}\).
Based on the previous studies we utilized tandem mass spectrometry analysis in the full scan mode to identify the four tocopherol isomers α, β, γ and δ in our herbal preparations. Modification made on Rui Zhang et al method although with longer run time, allowed efficient separation of tocopherols from the large number of herbal constituents. The results showed the presence of γ, α and γ, γ and δ plus γ tocopherol in samples SP, P1, P2 and P3 respectively. Full scan mode shows the absence of tocopherols in sample S. **Table (2)** illustrates the presence of α, β, γ and δ tocopherols in the tested samples. **Fig. (7)** represent the spectrum of α tocopherol obtained by GC/MS, and **Fig. (8)** illustrates the chromatogram where γ, α, and α tocopherol acetate were detected.

**Quantification of λ & α tocopherol**

It is interesting to note that among the four tocopherol isomers, α tocopherol has the greatest bioavailability owing to α-tocopherol transfer protein (α-TTP) that is selective for α-tocopherol\(^{12}\).  

**Table 2:** Tocopherol isomers in samples S, SP, P1, P2, P3.

<table>
<thead>
<tr>
<th></th>
<th>α tocopherol</th>
<th>β tocopherol</th>
<th>γ tocopherol</th>
<th>δ tocopherol</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>SP</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>P1</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
<td>P2</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>P3</td>
<td>+</td>
<td>+</td>
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<td>+</td>
</tr>
</tbody>
</table>

**Fig. 7:** Spectrum of alpha αtocopherol obtained by GC/MS.

**Fig. 8:** Chromatogram of γ, α, and α tocopherol acetate by GC/MS.
SIM enables monitoring a subset of fragments with their relevant mass values in a definite retention time (RT) scale. Here in our study, we used the most unique molecular fragments for quantification. The correlation coefficient ($R^2$) constructed by plotting area versus concentration was 0.9902 and 0.9993 for linear series of $\alpha$ and $\gamma$ tocopherol respectively. The retention time is 9.3 for $\gamma$ tocopherol and 9.9 min for $\alpha$ tocopherol.

Results extracted from quantification of $\lambda$ & $\alpha$ tocopherol in tested herbal medications are listed in Table 3. The overall content of $\alpha$ tocopherol in samples containing saw palmetto extract, and/or pumpkin seed oil was between 3.5-49.9 µg/g in accordance with previous studies. The content of $\alpha$ tocopherol in P3 is as low as 0.7 µg/g. This low content may give rise to doubt with other oils like sesame oil where the content of $\alpha$ tocopherol is 20 to 60 times less than other oils. $\alpha$ tocopherol content in Cucurbita pepo which is one of the 10 top popular types of vegetables in the world is between 36.6 to 43.9 µg/g while in sample P1 is 19.03 µg/g where the pharmaceutical company declares on the label that the species of Cucurbita is pepo.

Table 3: $\alpha$ & $\gamma$ tocopherol concentration in the tested herbal pharmaceutical products.

<table>
<thead>
<tr>
<th></th>
<th>$\alpha$ tocopherol µg/g</th>
<th>$\gamma$ tocopherol µg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>3.59111</td>
<td>23.02621</td>
</tr>
<tr>
<td>SP</td>
<td>49.93164</td>
<td>633.6396</td>
</tr>
<tr>
<td>P1</td>
<td>19.03232</td>
<td>445.3681</td>
</tr>
<tr>
<td>P2</td>
<td>13.65129</td>
<td>382.9741</td>
</tr>
<tr>
<td>P3</td>
<td>0.736403</td>
<td>76.71312</td>
</tr>
</tbody>
</table>

All tested samples were characterized by a high content of $\lambda$ tocopherol ranging between 23.02 and 633.63 µg/g with the highest content 633.63 in sample SP containing both of Serenoa repens & pumpkin seed oil in agreement with literature. No previous studies reported to assay tocopherols in Serenoa repens pharmaceutical products. The sum of the isomers $\alpha$ and $\lambda$ is from 181 to 875 µg/g in fulfillment of previous publications with the solely significant difference in sample P3 being 77.4 µg/g. Table (3) represents the concentration of $\alpha$ & $\gamma$ tocopherol in the tested samples expressed in µg/g.

Chromatographic fingerprint

Chromatographic fingerprint provides a rational approach for assessing the quality of botanical medicines containing one or more plants, which in turn contains many components where no single component is responsible for the overall efficacy. Selection of active ingredients or markers for the identification and quality assessment of botanical medicines. Chromatographic fingerprint can be obtained by analytical methods such as GC, HPLC, HPTLC. These methods provide the possibility of knowing the presence or absence of the active ingredient or chemical marker, an integrated set of ratios for all detectable analytes, as well as the knowledge of adulteration. Accordingly, chromatographic fingerprinting is a comprehensive qualitative approach to ascertaining the authenticity of plant species, evaluating quality, and confirming sustainability and stability across batches and stability. It comprises with complementary methods rational evidence for the presence of contaminants and adultrants. Fig. (9) illustrates the percentage of components in the tested samples.

Fig. 9: Contents distribution of herbal product by GC/MS fingerprint analysis.
As for S sample, the largest percentage in the extract was for Palmitic acid (15.5%) and Loranol (dodecanol alcohol) 15.5%. The percentage of total fatty acids in the extract was 65%, of which 64.1% were free fatty acids and 0.9% esterified fatty acids. The percentage of alcohols was 21.4%, including 15.5% for lauranol, 6% hydcarbons, 0.2% aldehydes, 0.17 ether, 1.6% sulfur compounds, and 5% other compounds. Neither tocopherols were identified in the sample nor phytosterols.

The largest component in sample SP (31.3%) was for a steroid compound (5-chloro-6beta-cholestan-3-one), followed by glyceryl dilaurate (dodecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester) 26%. The percentage of free fatty acids and their esters and glycerides was 40%, steroids 32%, aldehydes 11%, hydrocarbons 3.2% and triterpenes 0.16%. Betasitosterol was not found neither Campesterol or Stigmasterol, which are the predominant phytosterols in Serenoa repens. Also, phytosterols of type Δ7 were not seen, which are characteristic of pumpkin seeds and oil, and constitute more than 80% of the total sterols. γ tocopherol was also identified within the sample (0.25%) at a retention time of 39.726 min.

Concerning Methylparaben and Propylparaben which were found in sample SP in percentages of (11.5%) and (4.3%) respectively, they are preservatives that are not mentioned among the ingredients in the label or leaflet and may increase the risk of breast cancer and tumor cell proliferation in breast cancer. Again, there was not any of Δ7 phytosterols characteristic of pumpkin seed oil. Cucurbita pepo has positive effects on health and has been the subject of many studies aimed at determining primarily the content of fatty acids and tocopherols, and fatty components such as sterols, and phenolic acids. γ tocopherol was identified in the sample at the time 39.68, and alpha-tocopherol, at 40.51 as to alpha-tocopherol acetate at 41.06 min, were identified.

The percentage of the compounds in sample P2 was distributed among fatty acids 84%, aromatic compounds 2%, triterpenes 6%, sterols 3%, γ tocopherol 0.3% and aldehyde 0.36%. Δ7 sterols appeared in this sample with a percentage of 0.8%, which is less than the percentage of Δ5 sterols (2%). The Squalene (characteristic of pumpkin seed oil) also appeared, but no alcohols were detected. The high levels of Betasitosterol and some types of phytosterols, especially Δ5, indicate the presence of adulteration because they are found naturally only in small amounts.

The aldehydes normally present in Cucurbita pepo are C6-C7, which give off its aromatic odor and are produced by a series of enzymatic reactions of lipoxygenase on linoleic and linolenic acid. The presence of derivatives of these aldehydes is a result of roasting the seeds (in the essential oil), and the presence of aldehydes can be considered as a marker to determine the roasting conditions before extracting the oil from the seeds, and what appeared in the sample P2 is 2,4-Decadienal by 0.36%. As for the compounds containing sulfur, furan and pyrazine, they increase according to the degree of roasting.

The methanol extract of sample P3 contained aldehydes 5.8%, alcohols 2.9%, and fatty acids 67.5%, of which 3% were methyl esters, the largest percentage of which was Linoleic acid 40%, Oleic acid 10%, hydcarbons 6%, triterpenes 2.2%, and tocopherols 1.3% (δ tocopherol 0.72%, γ tocopherol 0.6%) and 8.5% sterols are all of the Δ5 type and there is no Δ7, knowing that the Δ7 sterols are dominant in pumpkin seed oil and the content of Δ5 sterols is very low compared to other oils and the presence of Betasitosterol in a high amount is considered an indicator for the addition of other cheaper oils. The GC-phytosterol profile is therefore a method for testing the authenticity of pumpkin seed oil. Fig. (9) illuminates contents distribution of the tested samples among chemical groups.

**Conclusion**

In this study, we determined fatty acid composition in 5 herbal pharmaceutical products used to treat BPH in Syrian local market. Lauric acid is the major fatty acid in Serenoa repens extract, yet it did not appear in sample S. Fatty acids profile was in agreement with official and quality requirements in samples SP, P1, P2, and P3. Detection and quantification of tocopherols in herbal preparations in the market to treat BPH is influential in quality assessment. Identification of tocopherols by GC/MS/full scan mode
showed the presence of one or more isomers in all tested samples except sample S. However, α & γ isomers appeared in SIM mode in all samples. δ isomer was found in one sample containing pumpkin seed oil similar to published studies. Assignment of the concentration of α & γ tocopherols is advantageous in ensuring the quality and authenticity of pharmaceutical herbal preparations claimed to contain *Serenoa repens* and/or pumpkin seed oil. Tocopherols profile will help in such quality assurance.

Our results showed that α tocopherol content ranged from 3.59111 to 49.93164 µg/g. one sample contained considerable low amount of 0.7 µg/g. the amount of γ tocopherol was as high as expected ranging from 23.02621 to 633.6396. The sum of α & γ tocopherols fall in the range of 181-875 µg/g suggesting excellent nutritional antioxidant properties. Our findings proved an overall good quality for the studied samples.

Tocopherols as potential active nonsteroidal lead inhibitors for 5α-RII are nominated for further in vitro investigation.

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تقييم جودة المستحضرات النباتية المستخدمة لعلاج ضخامة البروستات الحميد:
تحليل الواسطات الكيميائية والبصمة الكروماتوغرافية

هدي مندو1 – غسان أبو شامه2 – ناتالي موسى3 – أحمد حسن

1 قسم الكيمياء الصيدلية ومراقبة الأدوية، كلية الصيدلة، جامعة دمشق، دمشق، الجمهورية العربية السورية
2 قسم الكيمياء الصيدلية ومراقبة الأدوية، كلية الصيدلة، الجامعة العربية الدولية، درعا، الجمهورية العربية السورية
3 قسم الكيمياء، كلية العلوم، جامعة دمشق، دمشق، الجمهورية العربية السورية
4 قسم الكيمياء الصيدلية ومراقبة الأدوية، كلية الصيدلة، جامعة المنارة، اللاذقية، الجمهورية العربية السورية

تستخدم المستحضرات النباتية التي تملك تأثيراً مفيداً في مرض ضخامة البروستات الحميد على نطاق واسع. من ضمن هذه المستحضرات، تلك التي تحتوي على خلاصة نبات النخيل المنشاري و/أو زيت بذر القرع، تلعب الواسطات الكيميائية دوراً حاسماً في ضبط جودة المستحضرات النباتية، وتشكل إلى جانب البصمة الكروماتوغرافية أدناة مهمة في تقييم جودة هذه المستحضرات. تهدف هذه الدراسة تقييم جودة المستحضرات النباتية المستخدمة لعلاج مرض ضخامة البروستات الحميد الموجودة في السوق السورية المحلية عن طريق تحديد مرتسم الحموض الدسمة والتوكوفيرولات، إلى جانب تحليل البصمة الكروماتوغرافية للمخللات الميتانولية لهذه المستحضرات. ومن أجل ذلك تم إجراء تحليل باستخدام الكروماتوغرافيا الغازية المزودة بمكتشف طيفي الكتلة. جاءت نتائج تحليل الحموض الدسمة مطابقة لمتطلبات الجودة في أربع عينات. وكان المحتمل أن الألفا و غاما توكوفيرول متواجداً مع الدراسات السابقة وضمن المجال 85-181 مكغ/غ باستثناء عينة واحدة احتوت على 77 مكغ/غ لكلهما. أظهر تحليل البصمة الكروماتوغرافية عدم وجود الوفينيسيرولات من نوع 78 في العينات الحاوية على زيت بذر القرع، كما احتوت إحداها على البارابينات. تبين غياب حمض الغار في إحدى العينات وتأكد عدم وجوده من خلال البصمة الكروماتوغرافية. أظهرت النتائج السابقة أهمية الجمع بين الواسطات العالمة والبصمة الكروماتوغرافية كتقنية مناسبة لتقدير جودة المستحضرات النباتية وكذلك فعاليتها إلى جانب المامونية.