



QUALITATIVE, QUANTITATIVE, AND MICROSCOPIC EVALUATION OF SYRIAN PANAX GINSENG PRODUCTS

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The use of Panax Ginseng (PG) products is continuously expanding around the world. The popular belief said herbal products are always “safe”, because of its natural source. However, evidences that indicate its quality before marketing are very limited. Although PG products have shown promising potential with the efficacy, many of these products remain untested. This work aims to evaluate some quality aspects of PG products in regard with Qualitative, Quantitative (total Ginsenosides content (TGC)), microscopic, Loss On Drying (LOD) tests and in comparison with PG crude plant. The qualitative test revealed the presence of flavonoids, alkaloids, glycosides, and saponins with the exception of tannins. The TGC ranged from (31.16 to 102.58 mg/g). Microscopic findings revealed the presence of parenchyma cells with gelatinous content in product A. Moreover, Most of the products exceeded the reference limits for LOD. This process is becoming increasingly important, along with ‘quality control/assurance’, as a means of ensuring a consistent supply of high-quality herbal medicines.

Keywords: Panax Ginseng, Qualitative, Quantitative, microscopic, LOD.

INTRODUCTION

Nowadays, The safety of herbal products (HP) has become a cause of concern to all of the general public and the national health authorities¹. The popular belief said HP are always “safe”, however, HP are not free of risk and many studies suggest potential adverse reactions. Yet, the information which are available about its safety, purity and effectiveness is insufficient, thus raises a great concern about its quality and safety². Even though the media around the world frequently try to persuade us otherwise, not all HP are free of adverse effects³, and that is the reason why there is always a need for ensuring the safety profiles and stringent quality control systems for better authentication and standardization of HP⁴. Panax ginseng (Araliaceae) is a very used medicinal herb, it was reported that PG had the ability to enhance immune system and it is widely used as an adaptogenic factor⁵. Actually,

the chemical composition of PG is quite complicated, as ginsenosides constitute are the largest groups of bioactive substances isolated from PG⁶. Furthermore, the content of ginsenosides is considered as an important index for evaluating PG products quality^{5,6}. A variety of analytical methods can be used to quantify these compounds, however UV/Vis spectrophotometric determination became one of the most widely used methods for quantification of total ginsenosides due to its simplicity and low cost of implementation. This approach becomes more critical due to the high cost or absence of reference substance needed for determination of individual ginsenosides⁷. On the other hand, the Qualitative and microscopic evaluation are essential steps in verifying HP effectiveness, purity and safety. The current work aims to evaluate the total ginsenosides content (TGC), Qualitative, Microscopic, and LOD testes of PG products, as they are the most important parameters for

quality evaluation of HP⁸. After all adhering GMP to all production stages is very important to obtain high quality products with no harmful effects.

MATERIAL AND METHODS

Chemical and Equipment

Automatic mill, Ethanol Solution 70%, aluminum chloride (10% w/v), potassium acetate (1M), distilled water, Ginseng Extract RS standard (Aldrich Chemical), Spectrophotometer (Shimadzu), chloral hydrate solution 60%, iodine solution 1%, Micros Optical Microscope (Austria, Precisa-XB220), Switzerland. All solvents were of analytical grade.

Samples

Three different batches of four PG products (A, B, C and D) were purchased from local Syrian pharmacies. PG crude plant was kindly gifted from Syrian Pharmaceutical Industries (Homs, Syria). The crude plant was grinded into powder using the automatic mill. A and B products were capsules with dried roots powders. C products were capsules with dried roots extract where D products were syrups with liquid roots extracts. The batches were coded 1,2,3 respectively.

Qualitative chemical examination

Qualitative Chemical tests for the screening and identification of bioactive constituents such as flavonoids, tannins, alkaloids, glycosides, and saponins in the PG samples were carried out. The method of Harborne was adopted for the preliminary phytochemicals screening⁹.

Estimation of Total Ginsenosides Content (TGC)

The total ginsenosides content of PG samples were determined using colorimetric assay. The PG samples were extracted under reflux according to GHP10¹⁰. Briefly, to 1 mL of extracted sample, 4 ml of acetic acid – sulfuric acid mixture (50:50, v/v) were added. After 15 min of incubation the mixture turns to yellow whose absorbance was measured at 520 nm using a spectrophotometer. Obtained results were expressed in mg/g Ginseng Extract RS equivalents.

Microscopic Evaluation Method

Samples were tested by adding few drops of chloral hydrate solution 60% and the powders was studied using light microscope with 10 and 40 lenses. An iodine solution was used to detect the presence of starch grains in the studied samples. PG crude plant was used as comparative material.

Loss on Drying Test (LOD)

It was conducted by taking 1 g of product A, B and PG crude plant, followed by heating in an oven at 105 °C for two hours. The percentage of LOD is calculated by the equation¹¹.

The percentage of LOD = $\frac{\text{Weight of the material before drying} - \text{Weight of the material after drying}}{\text{Weight of the material after drying}} \times 100$

RESULTS AND DISCUSSION

Qualitative Evaluation

In this study, qualitative chemical analysis of PG samples revealed the presence of flavonoids, glycosides, alkaloids and saponins as shown in Table 1. This confirms that PG medicinal herb has the ability to synthesize these compounds and store them within its tissues. However, the qualitative test for the presence of tannins in PG samples was negative. Thus indicates that PG medicinal herb may not synthesize tannins, and this matches with Alqethami's study on ginseng roots of plant where the results of the qualitative test were positive for these components (flavonoids, alkaloids and glycosides, and saponins) with the exception of tannins, as the result of their qualitative test was negative in ginseng as in our study¹², confirming that ginseng did not manufacture or store tannins in this studied part of the plant. In fact, the presence of these active components confirms their medicinal value and their versatility as medicinal plants, as plants produce many natural compounds as secondary metabolites and play an important role in plant defense, for example, flavonoids protect plants from UV damage, while alkaloids protect plants from insects and herbivores, due to their bitter taste and high toxicity. These compounds also have many important physiological activities for humans, where tannins are used for their astringent effects in cases of diarrhea and hemorrhage, and as an antidote for cases of alkaloid or metal poisoning.

Table 1: Qualitative Evaluation test on PG samples

| sample | Crude plant | A1 | A2 | A3 | B1 | B2 | B3 | C1 | C2 | C3 | D1 | D2 | D3 |
|--------|-------------|-------|-------|-------|-------|-------|-------|--------|-------|-------|-------|-------|-------|
| TGC% | 86.04 | 94.37 | 94.19 | 94.91 | 86.69 | 88.83 | 87.94 | 102.58 | 99.19 | 95.80 | 34.37 | 33.66 | 31.16 |

(+): positive, (-): negative

Estimation of Total Ginsenosides Content (TGC)

The ginsenosides content was calculated using the following linear equation based on the Ginseng Extract RS calibration curve: $Y = 0.0056 C + 0.0265$, $R^2 = 0.9993$, where Y is the absorbance and C is the ginsenosides content in mg/g. The standard curve of Ginseng Extract RS clarified in Fig.1, as the results of TGC included in table 2. The TGC in the studied PG samples ranged from 31.16 to 102.58 mg/g, as the lowest value was found in product D1 (31.16 mg/g), while the highest value was found in product C1 (102.58 mg/g). Moreover, TGC values vary between the batches of each product. These results are corresponding to the study of Kevers on PG products as TGC values in solid capsules products were 88%¹³. While the TGC values in the liquid products were low in our study and in the study of Kevers (8%)¹³, this variation between the solid and liquid products may be due to the used extraction methods which directly affect the percentages of TGC. Actually, the spectrophotometric assay is the best and optimal method as well as the it is one of the easy, quick and economical ways to determine the percentage of TGC in solid ginseng products, while it may be insufficient in determining it in liquid products, moreover, the used extraction method may not permit a good extraction of ginseng from liquid products, and this may explain the low percentage of TGC in liquid products¹³. These results are also corresponding to the results of Kurkin's study that determined the percentage of TGC in ginseng syrups using a spectrophotometric assay, and the percentage of TGC was also low and ranged from 0.047% to 0.051%¹⁴. It was observed that the TGC ratios in the products containing the dried root extract (D) were higher than in the products containing the dried root powders (A,B). These results are corresponding to Brown's study¹⁵.

On one hand, one of the extraction steps is the plant exposure to high temperatures thus, ginsenosides chemical structure become

unstable and cause decline in its content in the plant samples¹⁶. On the other hand, the climatic and geographical conditions, environmental factors, soil as well as the age of the plant¹⁷⁻¹⁸⁻¹⁹, in addition, the extraction and drying methods play a major role in the TGC variation in samples^{20&21}. Moreover, places with relatively low temperatures are matches with the highest content of ginsenosides, usually PG grows in shady forests and prefers relatively low temperatures ranging between 10°C and 20° C, and growth at relatively high temperatures exceeding 30°C causes the cessation of the metabolism process in the PG and may cause the occurrence of mold.²² Furthermore, references indicates that there is a strong relationship between TGC values and the root age, as it was found that TGC increase with increasing the root age¹³, where the Chinese Pharmacopoeia uses TGC index to determine the approximate age of the root, as the highest percentages of TGC correspond to roots with ages not less than four years, and the results of our study may agree with this definition, which makes us guess that the PG roots used in our study are of at least four years old¹³. On the other hand, the variation of TGC in our study may be due to the used part of the root, as it was reported that there is a variation in TGC between the outer part of the root and the inner part of it, where the concentrations of ginsenosides are higher in the outer parts of the root, contrary to the belief that says the inner part is the richest in ginsenosides, and this is due to the outer part of the root has relatively wide surface areas, and thus the concentrations of ginsenosides are higher compared to the inner part, and that extracts prepared from roots with many side branches are very rich in ginsenosides²³. In addition to the what mentioned above, the different environmental factors, soil factors, humidity, harvest, the use extraction methods, the type of solvent used in the extraction processes, and the used drying methods played a major role in the variation of TGC in ginseng roots^{24&25}.

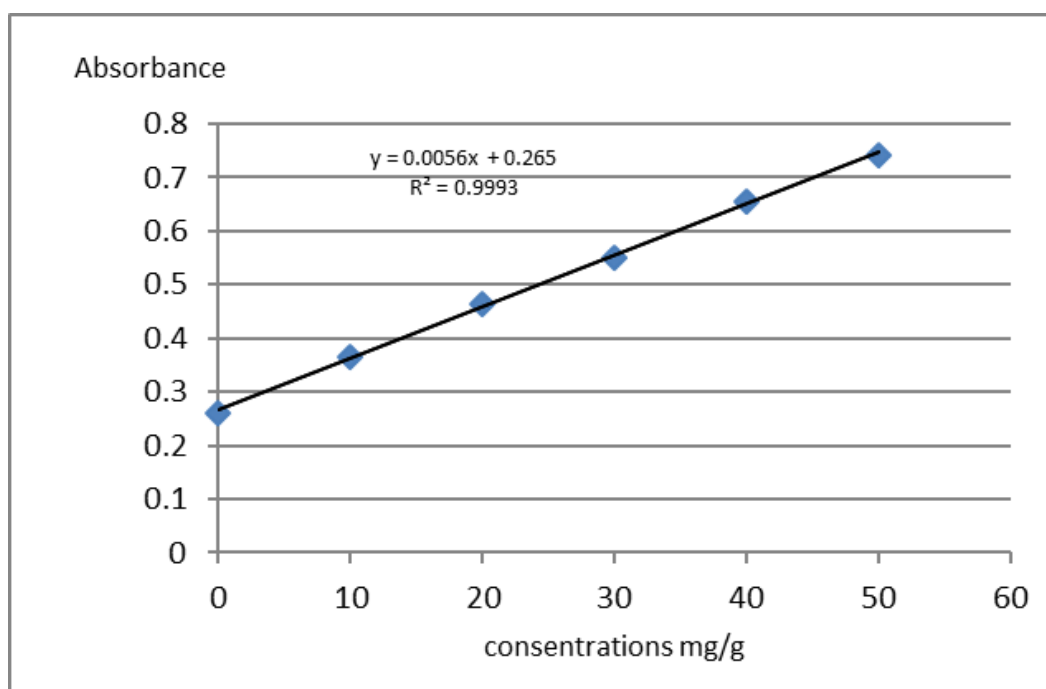


Fig. 1 Standard calibration curve of Ginseng Extract RS

Table 2: Total Ginsenosides Content (TGC) in PG samples

| A1 | A2 | A3 | B1 | B2 | B3 | C1 | C2 | C3 | D1 | D2 | D3 | Crude plant | |
|----|----|----|----|----|----|----|----|----|----|----|----|-------------|------------|
| + | + | + | + | + | + | + | + | + | + | + | + | + | Flavonoids |
| - | - | - | - | - | - | - | - | - | - | - | - | - | Tannins |
| + | + | + | + | + | + | + | + | + | + | + | + | + | Alkaloids |
| + | + | + | + | + | + | + | + | + | + | + | + | + | Glycosides |
| + | + | + | + | + | + | + | + | + | + | + | + | + | Saponins |

Microscopic Evaluation

This test was conducted on products (A, B) and PG crude plant which is used as a comparative material. The results of microscopic tests were included in (fig.2,3). In fact, Ginseng has become a subject of adulteration for economic reasons, and since ginseng is expensive, adulteration may occur by replacing it with cheaper products, hence there is a demand to ensure its quality. Ginseng is somewhat similar to licorice root (*Glycyrrhiza glabra* L) in appearance and anatomical structures, but they differ from each other by calcium oxalate crystals, which take the prism shape in licorice, while they are

clustered in ginseng. Ginseng can also be adulterated by replacing it with a lower-priced herb similar to real ginseng in effectiveness, such as the root of *Codonopsis pilosula*, which is called poor man's ginseng, but it differs from PG microscopically by the presence of the lacteal vessels characteristic of the root of *Codonopsis*²⁶. Commercially, *Panax ginseng*, *Panax quinquefolius*, and *Pseudo Ginseng* are not often mixed together²⁶. So, The microscopic evaluation showed characteristic microscopic features of PG roots, which are fragments of parenchyma cells with thin walls associated with the presence of cluster crystal of calcium oxalate in surface view. In addition to cork cells, secretory canals, starch grains, and xylem vessels with annular thicknesses

(Fig.2,3). The American Herbal Pharmacopeia (AHP) referred to untreated Panax and American ginseng and pseudo-ginseng, which are anatomically identical and have identical diagnostic features under the microscope, but it could be distinguished with one diagnostic difference between treated and untreated Panax ginseng, where starch granules are absent in treated Panax ginseng and the content of parenchyma cells turn into gelatinous due to steaming process before undergoing drying. However, pseudo-ginseng is characterized by the presence of larger grains of starch, with the absence of cluster crystal of calcium oxalate²⁶, and this was appeared in A3, B1, B2, and B3 as the negative results of iodine reagent confirmed these results (absence of starch grain), thus, these samples had undergone steaming before drying. Moreover, the presence of calcium crystals was also observed in all products and in very small sizes, which supports the absence of pseud ginseng and

confirms the presence of PG, Furthermore, the absence of prismatic crystals denies mixing licorice with PG. On one hand, the presence of Codonopsis root was denied due to the absence of lacteal vessels, thus, supports The presence of PG according AHP²⁶. On the other hand, aromatic cavities and secretory canals were also observed in frontal view, which are large and full of yellowish-brown content. Although some references do not classify it as a characteristic diagnostic component of PG roots, but it was clearly observed in all samples. Actually, starch grains were presented in abundance in the products A1 and A2, as studies mentioned the presence of starch as a diagnostic element for the root, but its presence in abundance suggests using it as an diluent excipient, however, this cannot be confirmed. This microscopic test findings are corresponding to PG comparative crude plant and AHP²⁶.

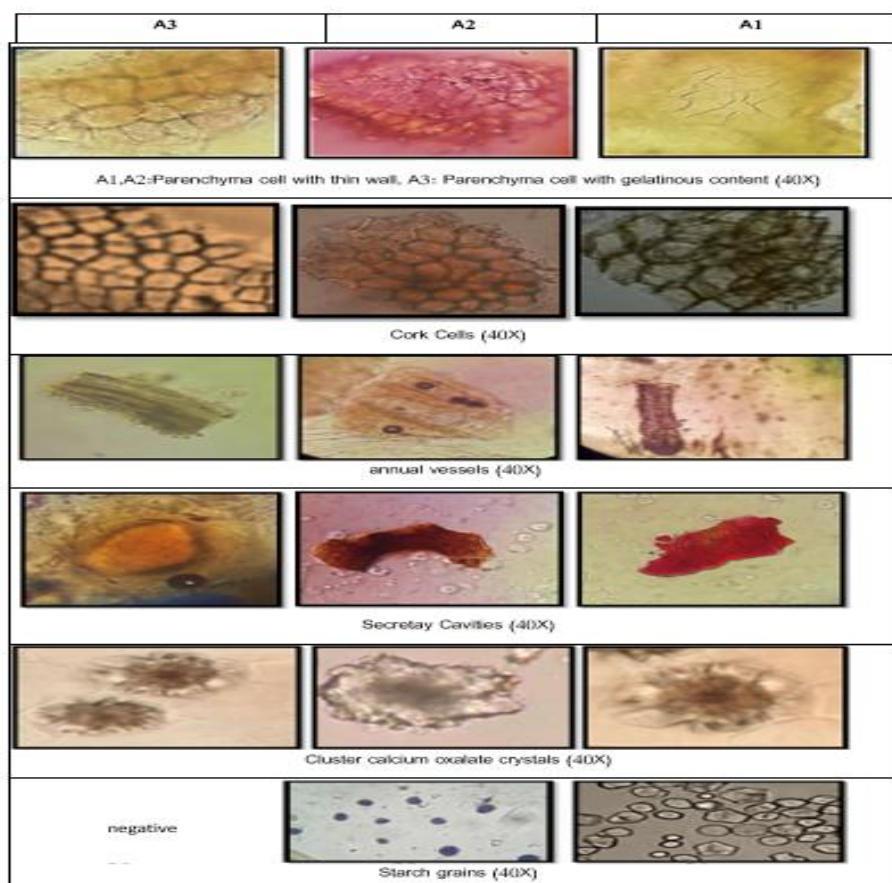


Fig. 2: The microscopic results of PG samples (product A)

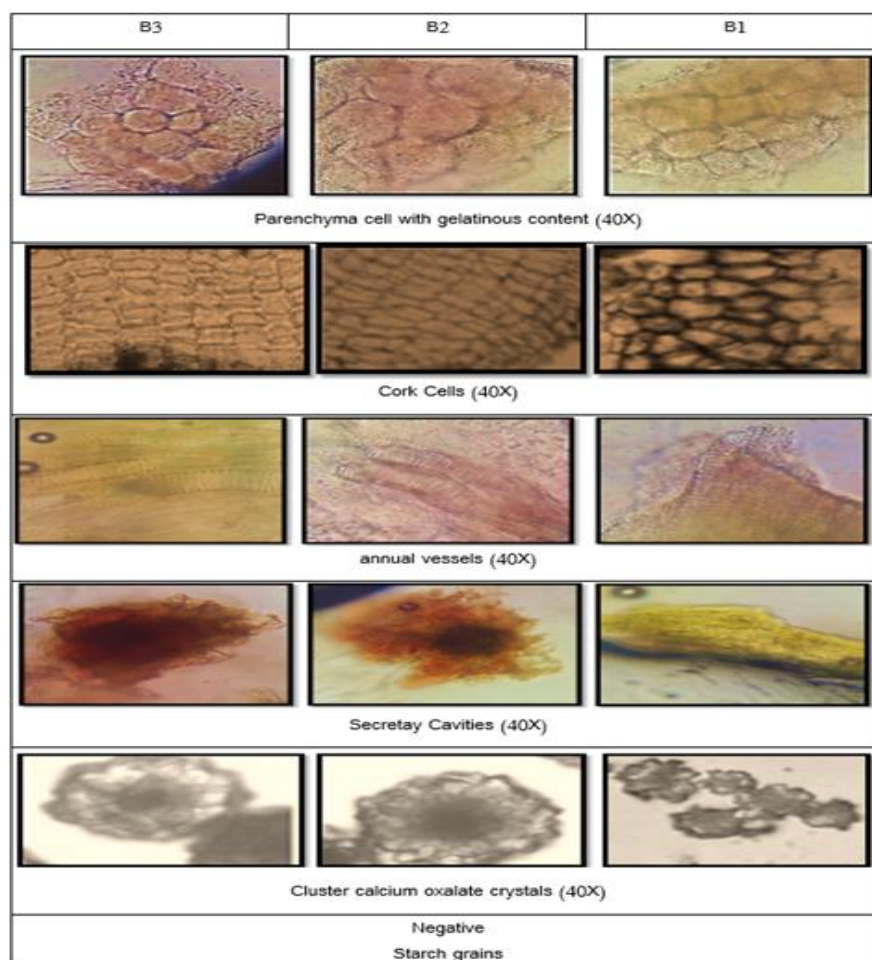


Fig. 3: The microscopic results of PG samples (product B)

Loss on Drying (LOD) Results

The LOD results is included in Table 3. It was observed that LOD average values were higher in crude plant (17.42%) than the finished products. As product B had the lowest LOD average value of (13.68%). Moreover, all average values of the samples exceeded the permissible limits for LOD which indicates a high moisture content. As the preserving and drying methods can affect LOD results²⁷.

Table 3: The results of Loss On Drying test (LOD)

| A% | B% | PG Root% | Reference limits (27) |
|-------|-------|----------|-----------------------|
| 13.74 | 13.68 | 17.42 | NMT 12% |

NMT: Not more than

Conclusion

The use of herbal products has increased rapidly with no applying of quality control in the same acceleration of products spread. In

this research, the Qualitative, Quantitative (TGC), microscopic, and LOD of Syrian PG products were conducted. Ginsenosides are key candidate compounds for evaluating the quality of PG products, so we highly recommend using TGC index measured by spectrophotometric analysis as an accurate, fast, economic, and simple way for evaluating the quality of ginseng herbal products. The qualitative test revealed the presence of flavonoids, alkaloids, glycosides, and saponins with the exception of tannins. The Quantitative test showed a variation in TGC content between products. The results confirmed the presence of PG roots which is stated on the product label. Parenchyma cells with gelatinous content were observed in some batches which indicates that the root had undergone steaming before drying. Large amounts of starch grains were observed in some products. In fact, it is necessary to determine most of the phytochemicals of herbal products in order to ensure the reliability and repeatability of pharmacological and clinical research, to

understand their bioactivities, and possible side effects of active compounds, and to enhance product quality control. The WHO recommends to store fresh medicinal plants at low temperatures, and therefore farmers should be trained for GMP, planting practices, Good agriculture and Good collection practice (GACP) and appropriate storage. All countries should make efforts to build consumers trust in herbal products by ensuring their safety and following GMP regardless of where it is manufactured and purchased.

Conflict of interest

The authors declare that they have no conflicts of interest.

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REFERENCES

1. D. Shaw, L. Graeme, D. Pierre, W. Elizabeth and C. Kelvin, "Phytovigilance of herbal medicine", *Journal of Ethnopharmacology*, 140(3), 513–518 (2012).
2. F. Shaikh, A. Salunkhe and S. Kadam, "An Outlook Towards the Microbial Quality of Marketed Herbal Medicinal Formulations", *American journal of cancer research*, 9 (02). 171-187 (2019).
3. E. Ernst, "Challenges for phytovigilance", *Postgraduate Medical Journal*, 80(943), 249–250 (2004).
4. WHO. "WHO Guidelines on Safety Monitoring of Herbal Medicines in Pharmacovigilance Systems", Geneva, Switzerland, World Health Organization. (2004).
5. T. Healthcare, "PDR for herbal medicines, 4th Edition", Thomson Reuters Corporation, Canada", 990 (2007).
6. H.J. Park, D.H. Kim, S.J. Park, J. M. Kim, and J. H. Ryu, "Ginseng in Traditional Herbal Prescriptions", *Journal of Ginseng Research*, 36(3), 225–241 (2012).
7. R. Lysiuk and N. Hudz, "Differential Spectrophotometry: Application for Quantification of Ginsenosides in Herbal Drugs and Nutraceuticals", *International Journal of Trends in Food and Nutrition*, 1, e102 (2017).
8. WHO, "WHO guidelines on good manufacturing practices (GMP) for herbal medicines", WHO Library, Switzerland, 92p (2007).
9. K. R. Khandelwal, "Practical pharmacognosy, techniques and experiments", 17th ed. Pune: Nirali Prakashan Publishers, , p. 149-56 (2007).
10. "German Homoeopathic Pharmacopoeia GHP, 2 Monographs E-Z", 2001 (2001).
11. WHO, "Quality control methods for herbal materials", World Health Organization, Geneva, (2011).
12. A. Alqethami, Y. Amal, and Y. A. Aldhebiani, "Medicinal plants used in Jeddah, Saudi Arabia: Phytochemical screening", *Saudi Journal of Biological Sciences*, 28 (1), 805–812 (2021).
13. C. Kevers, P. Jacques, T. Gaspar, P. Thonart, and J. Dommès, "Comparative Titration of Ginsenosides by Different Techniques in Commercial Ginseng Products and Callus Cultures", *Journal of Chromatographic Science*, 42(10), 554-558 (2004).
14. V. A. Kurkin, and A. S. Akushskaya, "Determination Of Saponins In Ginseng Syrup", *Pharmaceutical Chemistry Journal*, 48(4), 276-278 (2014)
15. P. N. Brown, and R. Yu, "Determination of Ginsenoside Content in Panax ginseng C.A. Meyer and Panax quinquefolius L. Root Materials and Finished Products by High-Performance Liquid Chromatography with Ultraviolet Absorbance Detection: Interlaboratory Study", *Journal of AOAC International*, 96(1), 12–19 (2013).
16. G.C. Yen and C. Y. Hung, "Effects of alkaline and heat treatment on antioxidative activity and total phenolics of extracts from Hsian-tiao (*Mesona procumbens* Hemsl.)", *Food Research International*, 33(6), 487-492 (2013).
17. L. Rimkiene, A. Kubiliene, A. Zevzikovas, D. Kazlauskienė and V. Jakstas, "Variation in Composition and Radical-Scavenging Activity in Ginkgo biloba L.

- due to the Growth Location and Time of Harvest", *Journal of Food Quality*, 2017(4), 1–8 (2017).
18. B. Sun, D. L. Dilcher, D. J. Beerling, C. Zhang, D. Yan and E. Kowalski, "Variation in Ginkgo biloba L. leaf characters across a climatic gradient in China", *Proceedings of the National Academy of Sciences*, 100(12), 7141–7146 (2003).
 19. G. wang, F. Cao and Y. El-Kassaby, "Role of Temperature and Soil Moisture Conditions on Ginsenosides Production and Biosynthesis-Related Genes in Ginkgo (Ginkgo biloba L.) Roots", *Natural Products Chemistry & Research*, 3(1), 1000162 (2015)
 20. J. Kobus, E. Flaczyk, A. Siger, M. Nogala-KaÅ, ucka, J.A. Korczak and R.B. Pegg, "Phenolic compounds and antioxidant activity of extracts of Ginkgo roots", *European Journal of Lipid Science and Technology*, , 111(11), 1150–1160 (2009).
 21. I. D. Boateng and X.M. Yang, "Effect of different drying methods on product quality, bioactive and toxic components of Ginkgo biloba L. seed", *School of Food & Biological Engineering, Jiangsu University, Zhenjiang*, 101(8), 3290- 3297 (2013).
 22. K. R. Ryu, M. H. Yeom, S. S. Kwon, H. S. Rho, D. H. Kim, & K. W. Yun, "Influence of air temperature on the histological characteristics of ginseng (Panax ginseng C. A. Meyer) in six regions of Korea", *Australian journal of crops science*, 6(12), 1637-1641 (2012).
 23. S. M. Lee, B. S. Bae, H. W. Park, N. G. Ahn, B. G. Ch., Y. L. Cho, and Y. S.Kwak, "Characterization of Korean Red Ginseng (Panax ginseng Meyer): History, preparation method, and chemical composition", *Journal of Ginseng Research*, 39(4), 384–391 (2015).
 24. J. E. Choi, X. Li, Y. H. Han, & K. T. Lee, " Changes of Saponin Contents of Leaves, Stems and Flower-buds of Panax ginseng C. A. Meyer by Harvesting Days", *Korean Journal of Medicinal Crop Science*, 17(4) , 251 – 256 (2009)
 25. C.Lu, and Y. Yin, "Comparison of Antioxidant Activity Ginseng (Panax Ginseng CA Meyer) Root Extraction between Ultrasound and Microwave Processing", *Advances in Biological Sciences Research*, 5, 107-112 (2018).
 26. R. Upton, A. Graff ,G. Jolliffe, R. LÄnger and E. Williamson, "American Herbal Pharmacopoeia Botanical Pharmacognosy-Microscopic Characterization Of Botanical Medicines", USA, Taylor & Francis Group, 774p (2011).
 27. United States Pharmacopoeia, USP 41-NF36.



نشرة العلوم الصيدلانية جامعة أسيوط



التقييم النوعي والكمي والمجهري لمنتجات باناكس الجنسج السوري

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يزداد استخدام مستحضرات الباناكس جنسج باستمرار في جميع أنحاء العالم، حيث يسود اعتقاد شائع بأن المستحضرات العشبية "آمنة" دائمًا، وذلك بسبب مصدرها الطبيعي. ومع ذلك، فإن الأدلة التي تشير إلى جودتها قبل التسويق محدودة للغاية. على الرغم من أن مستحضرات الباناكس جنسج قد أظهرت إمكانات واعدة فيما يتعلق بالفعالية، إلا أن العديد من هذه المستحضرات لا تزال غير مختبرة. يهدف هذا العمل إلى تقييم بعض معالم الجودة لمنتجات الباناكس جنسج من ناحية الاختبارات الكيفية والكمية (محتوى الجينسينوسيدات الكلي (TGC) والمجهرية والفقء بالتجفيف (LOD) ومقارنة هذه النتائج مع العقار الخام من الباناكس جنسج. أظهر الاختبار الكيفي وجود مركبات الفلافونويدات والقلويدات والجليكوزيدات والصابونينات باستثناء مادة التانينات. تراوح TGC من (٣١.١٦ إلى ١٠٢.٥٨ مجم / جم). أظهرت النتائج المجهرية وجود خلايا برانشيمية ذات محتوى هلامي في المنتج A علاوة على ذلك، تجاوزت معظم المنتجات الحدود المرجعية لـ الفقء بالتجفيف. LOD تزداد أهمية هذه العملية، جنبًا إلى جنب مع "مراقبة ضمان الجودة"، كوسيلة لضمان إمدادات ثابتة من الأدوية العشبية عالية الجودة.