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Standardization of Unani Drugs with Modern Analytical Parameters: A Necessary Step

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ABSTRACT

Mankind have been always utilized their native flora, not only as a source of food but also use for shelter, fuel, clothing, chemical production and healthcare products since ancient time. Traditional medicine (Ayurveda, Naturopathy, Unani, Siddha, and Homeopathy) and Yoga are gaining more and more attention all over the world, because of their long historic clinical practice and minimum side effects. Unani system of medicine also a part of traditional medicine, it also gaining more acceptability around the world, but to get the quality of the crude drugs and Unani formulations is still facing lacking of standardization. In this respect internationally recognition related with the quality is necessary. This can be achieved only if the herbal products are evaluated and analyzed using modern scientific tool and technologies of standardization. In this article discuss the various standardization parameters like morphological characters, microscopic evaluation, fluorescence analysis, physicochemical evaluations-proximate analysis (Moisture, ash content), solubility value (alcohol soluble, water soluble and ether soluble), heavy metal analysis and chromatography of botanicals are included. In the present review, various sensory parameters of the plant material (such as colour, odour, size, shape, and taste) were also mentioned of organoleptic evaluation method.

Keywords: *Herbal medicine, Standardization, Quality control, Chromatography*

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Introduction:

Plant has been used since the evolution of life, as a natural source of medicine in human ailments of various health problems. Medicinal plant has been playing a key role since ages for maintaining the health and it also serve as a valuable reservoir for drug development[1].This mean that new drug development has enhanced therapeutic properties and other pharmacological activities.

According to World Health Organization (WHO), globally 70-80% herbal based drugs are the mainstay for primary health care especially in developing world[2], as it has better acceptability, compatibility with the human biological system and its lesser side effect. While about half of the population in industrialized countries uses it.[3] The WHO has estimated the demand for medicinal plants is approximately $14 billion per annum (2006) and the demand is growing at the rate of 15–25% annually. The WHO estimates that by 2050 the trade will be up to US$ 5 trillion.[4]

In the past decade, there have been gain more and more attention and interest in the use of traditional medicine (Ayurveda, Naturopathy, Unani, Siddha, and Homeopathy) and Yoga globally, and become an important source of health management and some places it is an important source of care, especially in rural and tribal areas of the country.[5]

Under the shade of traditional medicine systems, the Unani system of medicine is also gaining global acceptance due to the amazing clinical efficiency of the formulations.[6] In Unani system of medicine is mainly based on naturally occurring drugs, mostly herbal in origin. Drugs of animals and mineral origin are also be used.[7] A number of drugs, both single and compound preparations are used widely in Unani Tibb (Unani medicine) in the management of various ailments, and It is a fact that many herbs have been reported with its diverse medicinal effects. The use of herbal medicines has increased remarkably in line with the global trend of people returning to natural therapies. Herbal medicine products may use as a dietary supplements that people take to

improve their health and are sold as tablets, capsules, powders, teas, extracts and fresh or dried plants etc.

Due to the long historical practice and less toxicity of Unani medicines, they are gaining more and more attention all over the world. Even today there is increase acceptability for traditional system of medicine for maintaining human heath quality. To full fill this necessity, the herbal plant materials are replaced by some unauthentic substituents or adulterants material and also not follow the proper guideline for the preparation of formulation etc. So, the safety and quality of crude medicinal drugs and finished herbal products have become a foremost concern for health authorities, pharmaceuticals and the public.[8]

To prevent this adulteration, unethical practice, some sort of uniformity in the manufacture of Unani medicine, the proper standardization process to be needed. This article deals with various techniques engaged in characterization and standardization of herbal medicines.

Standardization

It is a general phenomenon that nature has always helpful to overcome the harmful effects naturally. So, due to this tag the Unani products are increasingly being sought out as medicinal products, nutraceuticals and cosmetics.[9] In order to have a good coordination between the quality of raw materials, in process materials and the final products, it has become essential to develop reliable, specific and sensitive quality control methods using a combination of classical and modern instrumental method of analysis.

"Standardization of drugs means conformation of its identity and determination of its quality and purity and determination of nature of adulterant by various parameters like morphological, microscopical, physical, chemical and biological observations."

Used Unani drugs correctly, can help to treat a variety of disease conditions, and in some cases, may have fewer side effects than some conventional medications. Never assume that because herbs are "natural," they are safe. Some herbs may be inappropriate for people with certain medical conditions. Because they are unregulated, herbal products are often mislabeled and may contain additives and contaminants that are not listed on the label. Some herbs may cause allergic reactions or interact with conventional drugs, and some are toxic if used improperly or at high doses. So the effectiveness of Unani medicine is affected. Thus it is necessary that Standardization is an essential measurement for ensuring the quality control of the herbal drugs.[10]

Standardization of the herbal medicine is not an easy task. Numerous causes are present which influence the bio efficacy, reproducible therapeutic effect. In order to obtain quality oriented herbal product care should be taken right from the proper identification of plants, season, area of collection, their extraction and purification and rationalizing the combination in case of herbal drugs.[11] Standardization of the herbal drug begins from the collection of the herbal drug to its packaging/use as medicine.[12]

Need of standardization

Unani system of medicine is claim that mostly it is effective and safe, use to cure wide range of ailments, with the help of single drugs and compound formulations of *Adwiya Nabātiyya* (Plant orgin), *Adwiya Ḥaywāniya* (animal origin) and *Adwiya Ma'daniyya* (Mineral Origin), but mainly herbs are used. Unani compound preparations are commonly used in four forms viz. Solid, Semi Solid, Liquid and Vapours. Solid dosage forms are pill (Ḥabb), tablet (Qurṣ), powder (Safūf), Kushta etc., Semi Solid dosage forms are various type Jawārish, Ma'jūn, La'ūq, Iṭrīfal, khamīra Marham etc., Liquid dosage forms are decoction ('Arq), Syrup (Sharbat), drops(Qaṭūr) etc., and Vapours dosage forms are fumigation(Bakhūr), steam inhalation(Inkibāb), Lakhlakha, perfumes etc.[13,14]

It is well known fact that Unani drugs have been used since a long. In older times, Unani practitioners used to treat the patient on an individual basis with the single drugs and also formulate the compound formulation of medicine as need of the patients. They also mentioned a lot from their experience regarding the identification of crude drugs, their authentication methods and about their clinical efficacy but despite these the documentation of standardization on Unani drugs is negligible.

Traditional system of medicine including Unani system of medicine is popular worldwide and these drugs are being used by all community of the people, so there is increase the general acceptability of the use of herbal medicine in today medical practice. The increase in the demand of the drugs leads the shortage of crude drugs. To fill this necessity the herbal plant material are substituted by some adulterants and inferior products, also not follow the proper guideline for the preparation of formulation etc. which is responsible to wide range of abuse and adulteration of the products leading to 'consumer and manufacturer' disappointment and in some fatal concerns. To prevent this practice there should be some sort of uniformity in the manufacture of Unani medicines is needed to ensure quality control and quality assurance of the Unani drugs.

The World Health Organization (WHO) has appreciated the importance of medicinal plants for public health care in developing nations and has evolved guidelines to support the member states in their efforts to formulate national policies on traditional medicine and to study their potential usefulness including evaluation, safety and efficacy.[15]

Now, today the Unani medicinal products are manufactured on a large scale in mechanical units. Where manufacturers come across many problems such as non-availability of good quality raw materials and proper methodology for standardization, etc., there is also lacking of availability of SOP (Standard Operational Procedure) for manufacturing units on large scale production.[16] Thus to ensure to develop the quality of Unani drugs, the standardization of the single and compound drugs on modern analytical parameter is the basic requirement of Unani system of medicine now days.

Environmental Conditions Reflecting the Quality of Crude Drugs [17]

1. Metabolites affected by temperature, rainfall, length of day (including the quality of light) and altitude. Such effects have been studied by growing particular plants in different climatic areas and observing variations.

2. Temperature is a major factor controlling the development and metabolism of plants. Although each species has become adapted to its own natural environment, plants are frequently able to exist in a considerable range of environment.

3. Certain drugs now obtained almost exclusively from cultivated plants. These include cardamoms, Indian hemp, ginger, peppermint and spearmint for oil production. Other includes Ceylon cinnamon, linseed, fennel, cinchona and opium. In other cases, both wild and cultivated plants are used. Some plants have been cultivated sometimes immemorial (opium poppy and coca). Others are now grown

because supplies of the wild plants are insufficient to meet the demand are because owing to sparse distribution or inaccessibility, collection is difficult. Cultivation is essential in the case of the drugs Indian hemp & opium, which are subject to government control and recently for those plants in danger of over exploitation. In many cases, cultivation is advisable because of the improved quality of the drugs, which it is possible to produce. The improvement may be due to following-

i. The power to confine collections to species, varieties or hybrids which have desired phytochemical characters (e.g. Aconite, Cinnamom, Cinchona)

ii. The better development of the plants owing to improve conditions of the soil, pruning and the control of the insects, pests, fungi etc.

iii. The better facilities for treatment after collection are drying at a correct temperature in the cases of digitalis, colchicum, belladonna and valerian and peeling of cinnamon and ginger.

For success in cultivation, it is necessary to study the conditions under which the plant flourishes in the wild state and reproduce the conditions are improving on them. Small changes in ecology can affect plant products.[18,19]

Standardization of Unani drugs with modern analytical parameter

Process and Procedures of crude drugs- Standardization of herbal medicine is the process involved in the physicochemical evaluation of crude drugs like selection and handling of crude material, safety, efficacy and stability assessment of finished product, documentation of safety and rish based experience, provision of information to consumer and product promotion.[20, 21]

Authentication- Authentification of raw material is the basic starting point in developing of natural product. Each and every step has to be authenticated such as, area of the collection, parts of the plant collection, the regional situation, as phytomorphology botanical identity, harvesting, storage, processing and formulations.[22]

Pharmacognostic evaluation[23, 24]

Foreign organic matter- It is not possible to collect a herbal ingredient without small amounts of related parts of plant or other plants. Standards should be set in order to limit the percentage of such unwanted plant contaminants.

Macroscopic Evaluation- It identifies the botanical materials by the parameter like size, shape, color, odor, taste, texture of the drugs.

Microscopic Characters- it is a simple, inexpensive and widely used method for the authentification of herbal medicine with the help of light microscope. it analysis the characteristic feature of cell walls, cell contents, starch grains, calcium oxalate crystals, hairs, fibers, vessels etc.

Physico-chemical parameters- It includes foreign matter, total ash, acid-insoluble ash, swelling and foaming index, assay, successive extractive values, moisture content, viscosity, PH, Disintegration time, friability, hardness, flow capacity, flocculation, sedimentation, alcohol content. Chemical parameters it includes limit tests, chemical tests etc.

Ash values- Incineration of a herbal ingredient produces ash which constitutes inorganic matter. Treatment of the ash with hydrochloric acid results in acid-insoluble ash which consists mainly of silica and may be used to act as a measure of soil present. Limits may be set for ash and acid-insoluble ash of herbal ingredients.

Total Ash Value-The method of total ash is designed to determine the amount of material that remains after ignition. Ash is classified as physiological ash which is derived from the plant tissue itself and non-physiological ash which is the residue after ignition of extraneous matter (e.g. sand and soil). It is carried out at low temperatures possibly because alkali chlorides, which are volatile at low temperatures, may be lost. The total ash consists of carbonates, phosphates, silicates and silica.[25]

Acid insoluble ash- Sometimes, inorganic variables like calcium oxalate, silica, and carbonate content of the crude drug affects 'Total cash value'. Such variables are removed by treating with acid (as they are soluble in hydrochloric acid) and acid insoluble ash value is determined. Acid insoluble ash, water soluble ash and sulphated ash are also evaluated.

Swelling Index- The swelling index is the volume in ml taken up by the swelling of 1 g of plant material under specified conditions. Its determination is based on the addition of water or a swelling agent as specified in the test procedure for each individual plant material (either whole, cut or pulverized). It gives an idea about the mucilage content of the drug; hence it is useful in the evaluation of crude drugs containing mucilage.

Pesticides- Medicinal plant, particularly those grown as cultivated crops, may be contaminated by DDT (dichlorodiphenyl trichloroethane) or other chlorinated hydrocarbons, Benzene hexacloride (BHC), organo-phosphates, carbamates or polychlorinated biphenyls Inorganic pesticides: calcium arsenate, lead arsenate, Miscellaneous: ethylene dibromide, ethylene oxide, methyl bromide etc. Limit tests are necessary for acceptable levels of pesticide contamination of herbal ingredients.

Toxic metals- Lead, cadmium, mercury, thallium and arsenic have been shown to be contaminants of some Limit tests for such toxic metals are essential for herbal ingredients. Radioactive contamination: There are many sources of ionization radiation, including radionuclides, occurring in the environment. Hence, a certain degree of exposure is inevitable.

Microbial contamination- Aerobic bacteria and fungi are normally present in plant material and may increase due to faulty growing, harvesting, storage or processing. Herbal ingredients, particularly those with high starch content, may be prone to increased microbial growth. Pathogenic organisms including Enterobacter, Enterococcus, Clostridium, Pseudomonas, Shigella and Streptococcus have been shown to contaminate herbal ingredients. It is essential that limits be set for microbial contamination. The Unani Pharmacopoeia also specifies that E. coli and Salmonella spp. should be absent from herbal preparations.

Alfatoxins- Aflatoxins are naturally occurring mycotoxins produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus*. It is highly dangerous and extreme care should be exercised at the time of analysis.

Other contaminants- As standards increase for the quality of herbal ingredients it is possible that tests to limit other contaminants such as endotoxins and mycotoxins will be utilized to ensure high quality for medicinal purposes.

Extractive values- It is useful for evaluation of a crude drug. It gives an idea about the nature of the chemical constituents present in a crude drug. Useful for estimation of constituents

extracted with the solvent used for extraction. Employed for material for which as yet no suitable chemical or biological assay exists. It can be done by following methods: Cold maceration, hot extraction and ethanol.[26]

Chromatography

Thin Layer Chromatography(TLC)- It is a simple method and can employed for multi sample analysis-TLC is commonly used for the analysis of herbal drugs before the chromatographic instrument such as HPLC and GC were establish .But now a days , some pharmacopoeia are used it frequently. TLC gives the first characteristic finger prints of herbs and their constituent.it has the advantage to detect the many compound available in the drugs. The advantages of using TLC to construct the fingerprint of herbal medicines are its simplicity, versatility, high velocity, specific sensitivity, simple sample preparation and its economy. Therefore TLC is a convenient method to resolve of the adulteration of the Unani herbal product.

High Performance Thin Layer Chromatography (HPTLC) - HPTLC technique is widely employed in pharmaceutical industry in process of development, identification and detection of adulterants in herbal product and helps in identification of pesticide content, mycotoxins and in quality control and quality assurance of Unani drugs.

High Performance Liquid Chromatography (HPLC)- *HPLC* is commonly used to separate and analyze the concentration of molecules based on their polarity or lipophilicity. Preparative and analytical HPLC are widely used in pharmaceutical industry for isolating and purification of herbal compounds**.** There are basically two types of preparative HPLC: low pressure HPLC (typically under 5 bar) and high pressure HPLC (pressure >20 bar).[27,28] The important parameters to be considered are resolution, sensitivity and fast analysis time in analytical HPLC whereas both the degree of solute purity as well as the amount of compound that can be produced per unit time i.e. throughput or recovery in preparative HPLC[29] therefore this technique can also be helpful in the quality of Unani drugs.

Liquid Chromatography- Nuclear Magnetic Resonance (LC-NMR)- LC-NMR improves speed and sensitivity of detection and found useful in the areas of pharmacokinetics, toxicity studies, drug metabolism and drug discovery process [30,31,32]. The identification of adulterants in a Chinese herbal medicine was done by LC-NMR technique[33], so this procedure can be adopted for different Unani formulations.

Gas Chromatography (GC) and Gas Chromatography-Mass Spectroscopy (GC-MS) - GC-MS instruments have been used for identification of large number of components present in natural and biological systems[34], hence this technique, maybe useful for Unani drugs which have multi-ingredients.

Supercritical Fluid Chromatography (SFC) - SFC permits the separation and determination of a group of compounds that are not conveniently handled by either gas or liquid chromatography. SFC has been applied to a wide variety of materials including natural products, drugs, food and pesticide[35]. SFC enables the resolution of unknown components and known markers such as azadirachtin A and B, salannin, and nimbin in neem seed extracts [36.]

Capillary Electrophoresis (CE) - Researchers evaluated the importance of CE for quality control of herbal medicinal products. [37] Several CE studies dealing with herbal medicines have been reported and two kinds of medicinal compound i.e. Alkaloids and flavonoids have been studied extensively.[38]

The methodology of CE was established to evaluate one herb drug in terms of specificity, sensitivity and precision, and the results were in agreement with those obtained by the HPLC method. Furthermore, the analysis time of the CE method was two times shorter than that in HPLC and solvent consumption was more than 100-fold less.[39]

Conclusion and Perspectives- In the global view, there is a shift towards the use of Unani medicine. It is accepting as a natural and gentle alternative to the chemical based synthetic drug. The quality of Unani drugs is the sum of all factors which contribute directly or indirectly to the safety, effectiveness and acceptability of the product. It is the fundamental responsibility of the regulatory authorities to ensure that consumers get the medication, which guarantees purity, safety, potency and efficacy. However, we still face many problems in this areas.[40]

This can be achieved only if the Unani drugs to evaluate and analyze by using these sophisticated modern analytical parameters for standardization such as microbial load determination, Aflatoxins analysis, UV-visible, TLC, HPLC, HPTLC, GC-MS, spectrofluorimetric and other methods. It is a need of this modern age that Unani drugs should be safe and effective for the masses.

References-

1. Padalia RC, Medicinal and Aromatic plants: Chemical Goldmines. Medicinal Aromatic plants, 2012; 1:1-3.

2. http://www.who.int/research/en.[acessed 25-6-2019]

3. Kanwal H, Sherazi BA, Herbal medicine: Trend of practice, perspective, and limitations in Pakistan. Asian Pacific Journal of Health Sciences. 2017; 4(4): 7

4. Booker A, Johnston D, Heinrich M. Value chains of herbal medicines—Research needs and key challenges in the context of ethnopharmacology, Journal of Ethnopharmacology, 2012; 140: 625.

5. Shaik BT, Hatcher J. Complementary and alternative medicine in Pakistan: prospective and limitations, Evid Based Complement Alternat Med. 2005; 2(2): 139–142.

6. Ajazuddin SS, Evaluation of physicochemical and phytochemical properties of Safoof-E-Sana: A Unani polyherbal formulation. Pharmacognosy Research, 2010; 2(5): 318-322.

7. Mohd M, Sifi G, Jahan N, Baig Z, Unani approaches to the method of formulating a Murakkab (compound drug formulation) : Review Article, Journal of Ayurveda and Integrative Medicine,2017; xxx: 1-6

8. Jitubhai PP., Patel NM., Patel PM., WHO guidelines on quality control of herbal medicines*:* IJRAP ; 2011, 2 (4): 1148-1154

9. Kumari DR, Kotecha DM, A Review on the Standardization of herbal medicines, International journal of pharma sciences and research, 2016; 07: 98

10. Patel PM, Patel NM, Goyal, RK. Quality control of herbal products, The Indian Pharmacist, 2006; 5(45): 26-30.

11. Archana A, Anubha K, Standardization of herbal drugs: an overview, International research journal of pharmacy, 2011; 12(2): 56-60.

12. Khandelwal KR, Practical Pharmacognosy. 2nd ed. Pune: Nirali Prakashan; 2013. P. 23.1-23.4.

13. Afzal M, Khan NA, Ghufran A, Iqbal A, Inamuddin M. Diuretic and nephroprotective effect of Jawarish Zarooni Sada-a polyherbal unani formulation., Journal of Ethnopharmacology, 2004; 91: 219-223.

14. Unani System Of Medicine, The Science of Health And Healing. New Delhi: Ministry of AYUSH, Govt. of India; 2016. P 47-49.

15. National policy on traditional medicine and regulation of herbal medicines-Report of a WHO global survey, Essential Medicines and Health Products Information portal, A WHO resource, Geneva; 2005: iii.

16. Afaq SH, Tajuddin, Ahmad S, Abdullah, Rahman A. Standardization of Unani Ointments, Marham Kafoor, Hippocratic Journal of Unani Medicine, 2012; 12-25.

17. Husain A, Sofi GD, Dang R, Rani s, RH, Ahmed M. Production of crude Drugs in Unani System of Medicine, Pharmacognosy Journal, 2010; 2(16): 1-4

18. Hamdani K, Tahaffuz-e-Advia in Usoole Tib, 2nd ed. New Delhi: National Council of Promotion of Urdu Language; 2001. P. 37-38.

19. Grainger. N (2001) Herbal Druga And Phytopharmaceutials, 2nd edition, Med Pharm Scientific Publishers, Stattgart.

20. Quality Assurance of Pharmaceuticals: A Compendum of Guidelines and Related Materials, Good Manufacturing Practices and Inspection. 2nd ed. Geneva: World Health Organization; 2007; 2: 1-4.

21. Guidelines for the Assessment of Herbal Medicines. WHO Technical Report Series, No.863- Thirty-fourth report, Essential Medicines and Health Products Information portal, A WHO resource, Geneva; 1996: 1-4.

22. Quality Control Methods for Medicinal Plant Materials. World Health Organization, Geneva. 1992.

23. Quality control methods for herbal medicine, World Health Organization Geneva; 2011: 09-85.

24. The Unani pharmacopoeia of india, 1st ed. New Delhi: Ministry of Health & Family Welfare, Government of India, Department of Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homoeopathy (AYUSH), 2009. P. 160-200.

25. Shinde VM, Dhalwal K, Potdar M, Mahadik KR. Application of Quality control of herbal drugs. International Journal of Phytomedicine, 2009; 1: 4-8.

26. Ansari S.H. 2006. Essentials of Pharmacognosy. 1sted. New Delhi: Birla Publications; P. 581- 596.

27. Chimezie A, Ibukun A, Teddy E, Francis O. HPLC analysis of nicotinamide, pyridoxine, riboflavin and thiamin in some selected food products in Nigeria. Afr J Pharm Pharmacol 2008; 2(2):29-36.

28. Saravanan J, Shajan A, Joshi NH, Varatharajan R,Valliappan K. A simple and validated RP-HPLC method for the estimation of methylcobalamin in bulk and capsule dosage form. Int J Chem Pharm Sci 2010; 1(2):323-324.

29. Rao Udaykumar B, Anna NP. Stability- indicating HPLC method for the determination of efavirenz in bulk drug and in pharmaceutical dosage form. Afr J Pharm Pharmacol 2009; 3(12):643-650.

30. Dachtler M, Frans HM, de Put V, Frans V, Stijn Christiaan M, Fritsche BJ .On-line LC-NMR-MS characterization of sesame oil extracts and assessment of their antioxidant activity. Eur J Lipid Sci Technol 2003; 105(9): 488–496.

31. Pasch H, Heinz LC, Macko T, Hiller W. High-temperature gradient HPLC and LC-NMR for the analysis of complex polyolefins. Pure Appl Chem 2008; 80(8): 1747–1762.

32. Patil PS, Rajani S. An advancement of analytical techniques in herbal research. J Adv Sci Res 2010; 1(1):8-14.

33. Kestling JR, Haung J, Serensen D .Identification of adulterants in a Chinese herbal medicine by LC-HRMS and LC-MS SPE/NMR and comparative *in vivo* study with standards in a hypertensive rat model. J Pharm Biomed Anal 2010: 51(3):705-711.

34. Binit DK, Sunil K, Nayak C, Mehta BK. Gas chromatography mass spectrometry (GC-MS) analysis of the hexane and benzene extracts of the Piper beetle from India. J Med Plant Res 2010; 4(21): 2252-2255.

35. Matthew C, Henry R. Supercritical fluid chromatography, Pressurized liquid extraction, and supercritical fluid extraction. Anal Chem 2006; 78: 3909.

36. Agrawal H, Kaul N, Paradkar AR and Mahadik KR. Standardization of crude extract of neem seed kernels (Azadirachta Indica A. Juss) and commercial neem based formulations using HPTLC and extended length packedcolumns SFC Method. Chromatographia 2009; 62(3):183-195.

37. Wen HG, Lin SY, Jia L, Guo XK, Chen XG, Hu ZD. Analysis of protoberberine alkaloids in several herbal drugs and related medicinal preparations by non-aqueous capillary electrophoresis. J Sep Sci 2005; 28(1): 92-97.

38. Pietta P, Mauri P, Rava A, Sabbatini G. Application of micellar electrokinetic capillary chromatography to the determination of flavonoid drugs. J Chromatogr 1991; 549: 367-373.

39. Sombra LL, Gómez MR, Olsina R, Martínez LD, Silva MF. Comparative study between capillary electrophoresis and high performance liquid chromatography in 'guarana' based phytopharmaceuticals. J Pharm Biomed Anal 2005; 36: 989–994.

40.Dorđević S, Dickov A, Pavkov S, Tadić V, Arsić I, Žugić A. Manufacturing process of high quality phytopreparation on example of herbal sedative. Medicinski Pregled. 2013; **66(3–4)**:170–176.