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Original Article

Evaluation of the Pharmaceutical and Microbial Safety, as well as the Reliability, of Some Natural Pharmaceutical Products Available in Iraq

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Abstract

The use of natural pharmaceutical products (NPPs) for the treatment or prevention of diseases is continuously increasing. The ease of obtaining them without professional observation, as well as the incorrect popular belief that natural products are completely safe, increase the possibility of harmful and toxic effects of such products. In this study, some of the widely sold NPPs in Iraqi markets were evaluated for their pharmaceutical and microbial legibility to be consumed by humans. The evaluation includes organoleptic properties, foreign matters, loss on drying, water content, total ash percentage, heavy metal tests, aflatoxins, as well as microbial limit tests. The results revealed that some of the evaluated products were contaminated with heavy metals, lead, mercury, and cadmium. Additionally, pathogenic bacterial growth, including *Salmonella* species and *E. coli*, was detected. A high percentage of loss on drying and water content was detected in some of the tested products. All tested samples showed negative results for aflatoxins. Some of the evaluated products were pharmaceutically and/or microbiologically unacceptable and not safe to be consumed by humans. Serious and fast measures must be taken by the Drug Regulatory Authority of Iraq to issue more rigorous standards for the quality of NPPs with continuous monitoring and control of the marketed NPPs.

Keywords: Herbal, Medicinal plant, Natural pharmaceutical products, Safety

1. Introduction

Medicinal uses of herbs and plants are as old as diseases in humans. The oldest written record of using herbs as medicine in Iraq dates back to 2600 BC proclaiming the existence of a sophisticated ‘medicinal system’ in Mesopotamia (Iraq), which consisted of about 1,000 medicinal formulas derived from plants (1). It is noteworthy that the history and many applications of natural medicinal products have been recorded in Egypt, Ayroda, and traditional Chinese medicine (2). The interest in natural pharmaceutical products (NPPs) has been regenerated by the discovery of novel biologically active phytochemicals (3). More than 88% of the UK population, 71% of the Canadian population, and up to 70% of the USA population consume natural products for the treatment and/or prevention of diseases (4). The market value of herbal and plant products, a subcategory of natural pharmaceutical products (NPPs), have increased from 4.8 to 8.8 billion United States Dollar in 10 years (from 2008 to 2018) with a constant annual increment (5). The ease of obtaining them without the need for professional supervision and less rigorous regulations educate a false popular idea that NPPs are safe and without side effects. Simply, as NPPs have bioactivity (effects), it is expected of them to have undesirable adverse effects as well.

Despite the long history of using NPPs, there are real concerns about the safety, reliability, and efficacy of such

products. There are insufficient data for most NPPs to guarantee their efficacy, safety, and quality (5). So far, few programs have been organized to evaluate the efficacy and safety of NPPs, as suggested by the World Health Organization (WHO) guidelines (6). Organizations, including the United States Food and Drug Administration, European Medicines Agency, Unique Selling Proposition (USP) Herbal Medicine Compendium (7), Hong Kong Chinese Materia Medical Standard, and Indonesian Herbal Pharmacopoeia, all provide general guidelines for evaluating the quality of NPPs (8).

In Iraq, due to a lack of effective and accurate quality control regulations, lax controls of the borderline, and fragile legislation, there is less rigorous control over the marketing, handling, and consumption of such products. Therefore, NNPs are not subject to the same strict standards applied to the evaluation of synthetic medicines. It is noticeable that most problems accompanying the consumption of NPPs arise mainly from classifying these products as foods or dietary supplements. Moreover, confirming the quality and safety of these products is not a prerequisite for its marketing. Accordingly, there is a high probability that the available NPPs do not meet international standards and are not suitable for human consumption.

In the present study, samples of herbal preparations available and sold in the Iraqi market were randomly selected and evaluated. Microbiological tests and heavy metal limits, in addition to other parameters, were tested aiming to evaluate the extent to which the tested NPP samples conform to international standards and their suitability for human consumption. This is the first study in its field to deal with NPPs available in Iraqi markets.

2. Materials and Methods

2.1. Samples Selection

Samples were selected from widely consumed NPPs. They were purchased from local markets in Baghdad, Iraq, with almost the same or very close manufacturing date and storage conditions. The samples were coded as shown in table 1.

2.2. Organoleptic Properties

The product's color, odor, taste, mouthfeel, and texture are the main parameters to be tested, which provide an indication of the product's quality. The product should be free of abnormal odor, discoloration, slime, or signs of deterioration. Microscopic and morphological inspections were not applicable in this study as all the samples were processed products of extract, powdered, or pulverized raw materials.

2.3. Determination of Non-specific Parameters and Foreign Matters

According to the USP (7), pharmaceutical herbal products should be completely free of any foreign matter, including stones sand, plastic, metals, glass, and any foreign inorganic material. Additionally, products should be visually inspected for contamination with insects, mold, and other animal contamination or excreta. Non-specific parameters include total ash percentage, residue on evaporation, residual solvents, foaming index, swelling index, tannin total, and starch content.

Table 1. The samples codes

Sample code	Origin	Description	Manufacture date	Expiry date
S1	USA	Hard gelatine capsules filled with a mixture of powdered dry leaves. Bulk capsules packaged in an amber plastic container	April, 2019	April, 2022
S2	USA	Tablet of a mixture of plant extracts. Bulk tablets packaged in an amber plastic container	May, 2019	May, 2024
S3	USA	Tablet of a mixture of plant extracts and the powdered plant's part. Bulk tablets packaged in a non-transparent white plastic container	April, 2019	April, 2022
S4	USA	Hard gelatine capsules filled with a pulverized dried plant leaf. Bulk capsules packaged in an amber plastic container	March, 2019	March, 2022
S5	India	Sachets of packed with a mixture of pulverized dead plant parts and different plant extracts	March, 2019	March, 2021
S6	Iraq	A bag of a mixture of pulverized plants parts packaged in a paper box rubbed with nylon	June, 2019	June, 2021

2.4. Determination of Loss on Drying

The test was conducted as described in the USP (7) loss on drying. An accurately weighed sample (W_1) of 1-2 g (a portion of not less than four capsules or tablets) of a freshly opened package was placed in the bottle, the cover was replaced, and the bottle, as well as the contents, were accurately weighed. The test sample was weighed, the bottle was placed in an oven at 105˚C for 5 h, and the lid was placed in the oven after opening. Upon opening the oven, the bottle was closed quickly and it was allowed to cool down to the room temperature in a desiccator before weighing (W_2). The procedure was repeated until the difference between two corresponding consecutive weighings was not more than 0.25%. The percentage of weight loss on drying was calculated using the following equation:

$$\%\ loss\ on\ drying = \frac{W1 - W2}{W1} \times 100$$

The acceptable limit was NMT 5% of its original weight.

2.5. Measurement of Water Content

Water content was determined according to the USP 43-NF38, <561> Articles of Botanical Origin, Water Determination, Method I (Titrimetric), and the titrimetric solution (Karl Fischer reagent). A sample of 200-500 mg was used to determine the moisture content. Four tablets/capsules/sachets were crushed/emptied, homogenized, and accurately weighed. The sample powder was transferred to the titration vessel, titrated to the endpoint, and its water content was calculated in mg taken according to the following formula:

Water content=V×F

In which *V* is the volume, in mL, of the Karl Fischer reagent consumed in the titration, and *F* is the water equivalence factor of the reagent. The test was repeated in triplicate, and the results were expressed as the percentage of average±SD.

2.6. Measurement of the Total Ash

The test was performed according to the USP (7). The 4 g sample was carefully weighed in a shredded plant. Gradually, as the temperature increased, the sample was burned to obtain a white mass (or about the same mass) and constant weight. It was then cooled in a dryer, and the weight (%) of the total ash was calculated. To determine the acid-insoluble ash, 10 mL of diluted HCl was carefully added to the collected ash, and then, the crucible was covered with a watch glass and heated in a water bath at 50˚C for 10 min. The rinse was collected from the crucible and filtered using ashless filter paper. The crucible and the ashless filter paper were washed several times with water, and after that, the filter paper was transferred to the tared crucible and was incinerated until a constant weight was obtained. The percentage of acid-insoluble ash was also calculated.

2.7. Heavy Metal Tests

Heavy metal analysis was conducted using the method described by Li, Schoneker (9). Inductively coupled plasma–atomic emission spectroscopy technique was used to determine the levels of lead, cadmium, arsenic, and mercury.

2.8. Measurement of Aflatoxins

The determination of the products' contamination with aflatoxins was carried out as described by Wacoo, Wendiro (10). Immune-dip sticks were used, which are immune-chromatographic assays as a rapid testing kit. The principle of the method was based on utilizing highly sensitive and specific antibody-antigen reactions for the detection of aflatoxins. The range of quantitation was 3-100 ppb, the limit of detection was 2 ppb, and the testing time was 6 min, as described by the manufacturer.

2.9. Microbial Content Determination

Microbial limit tests were conducted following the USP (7). The total number of aerobic microorganisms (TNAM), total yeasts and molds (TYM), bile-tolerant Gram-negative bacteria (BTGN), *Salmonella* species (spp.), *E. coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* were quantitatively estimated.

3. Results and Discussion

Herbal medicinal products selected for this study consisted of capsules, tablets, and sachets. Samples were within their shelf life at the time of the investigation. Products were examined visually for the detection of any discoloration, spots, or abnormal appearance. No signs of color change or deterioration were observed. Additionally, no abnormal odor was detected.

Ten capsules/tablets/sachets were emptied/crashed in a glass dish and visually examined for the presence of plastic, stones, glass, any foreign inorganic, possible live insects, and parts of dead insects or animals. Additionally, a piece of magnetic was passed close to the sample to check for the presence of any metals. All the examined samples were found to be free of any insects and organic/inorganic foreign materials. Raw materials (extract or plants parts) used in the formulation of the tested NPPs underwent several processing steps, including cleaning, washing, drying, pulverizing, sieving, and possible extraction, all of which help in removing foreign matters.

The results of loss on drying showed that products S1 to S5 were within the acceptable limit (Table 2) while the S6 product showed an outstanding result. Products formulated as tablets appeared to show a lower value of loss on drying. The findings of loss on drying test and water content for products S1-S5 showed acceptable values possibly due to the processing step for the manufacturing of tablets which usually involves drying the prepared mixed powder before compression to insure flowability. Moreover, products that were formulated using extracts (S2, S3, and S5) also showed a lower value of loss on drying, compared to products formulated using powdered plants parts. On the other hand, product S6 showed an out-of-limit value for loss on drying test and a high value for water content. It can be due to the type of packaging material (cellulose bag), which allows for adsorbing the moisture, in addition to using pulverized plant parts. Pulverized dry plant parts usually contain more moisture and volatile materials than related extracts due to the evaporation of such materials during the rotary or oven drying and powdering of the extract (11, 12).

Table 2. Loss on drying percentage for the six evaluated products

Sample	Initial weight (W1, mg)	Final weight (W2, mg)	% Loss on drying
S1	2,023	1954.3	3.4
S2	2,018	1974.8	2.14
S3	2,098	2033.4	3.08
S4	2,004	1911.2	4.63
S5	2,045	1974.8	3.43
S6	2,105	1949.9	7.37

Table 3 showed the percentage of water content for the tested products (S1-S6). The results showed that products formulated with pulverized plants parts, such as capsules, or packaged as sachet and cellulose bags contain more water than those formulated as tablets. Simply similar to the results of loss on drying, the effects of packaging and the nature of raw materials used in manufacturing the product were clearly associated with water content.

All tested products showed a total ash and acid-insoluble ash values within the acceptable limit (Table 4). Samples S5 and S6 showed slightly higher values of total ash and acid-insoluble ash, compared to other evaluated products. The high values of total ash test for products S5 and S6, in spite of being within the acceptable limit, may be related to the low quality of raw plant material, which was contaminated with heavy metals (as shown in heavy metal tests) and the use of plants parts instead of extracts. Usually, plants contain a substantial quantity of inorganic compounds and elements that affect the total ash test. A considerable quantity of these inorganic compounds and elements may be lost during the extraction depending on the type of solvent used and the condition of extraction.

Products S4 and S6 showed out-of-limit high levels of contamination with Lead (Table 5). Additionally, products S4, S5, and S6 showed a detectable level of contamination with Mercury or Cadmium in spite of being within the acceptable limit. The rapid urbanization and the continuous expansion of industrial projects very close to agricultural lands and rural areas increase the possibility of the contamination of

irrigation water, soil, and air with heavy metals. These contaminants are usually easily absorbed by plants and transferred to humans (13). The results of heavy metal tests pointed toward the possibility of toxicity with heavy metals especially with long-term consumption of such products.

A rapid test was used for the detection of aflatoxins. The results showed that the tested products were not contaminated with aflatoxins. The main source of aflatoxins was found to be fungi, especially *Aspergillus parasiticus, Aspergillus flavus, and Aspergillus nomius*. The physical removal of mold-damaged plants' parts from supplies has been detected to reduce aflatoxins up to 80% (14). Additionally, the level of aflatoxin is highly affected by processing products during manufacturing, such as the type of heat treatment and excipients (14).

The results revealed that products (S4 and S6) were contaminated with bacteria to variable degrees (Table 6). Product S4 showed growth of *E. coli* while product S6 showed growth of *Salmonella* spp. and *E. coli.* Other tested products appeared to be free of microbial contamination.

Table 3. Water content percentage determined by titrimetric method expressed as the percentage of average±SD for the tested products

Sample	S1	S2	S3	S4	S5	S6
Water content (% ± SD)	0.93±0.035	0.62±0.003	1.41±0.064	2.85±0.107	2.17±0.433	4.16±1.103

Table 4. Total ash percentage and acid-insoluble ash percentage for the six samples of natural pharmaceutical products

Test (Reference range)	S1	S2	S3	S4	S5	S6
Total Ash % (NMT 10%)	3.65	3.43	4.11	5.41	7.66	8.92
Acid-insoluble ash % (NMT 2.5%)	0.82	0.89	2.17	1.62	2.09	2.34

Table 5. Heavy metal tests for the evaluated products

Metal	Reference range	S1	S2	S3	S4	S5	S6
Lead	<0.1 mg/kg	ND	ND	ND	0.3	0.08	0.4
Cadmium	<0.1 mg/kg	ND	ND	ND	ND	0.06	ND
Arsenic	<0.1 mg/kg	ND	ND	ND	ND	ND	ND
Mercury	<0.1 mg/kg	ND	ND	ND	0.06	ND	0.05

Table 6. Microbial limit tests for six herbal pharmaceutical products

	Product					
Microbial type (Limit)	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**
TNAM (<10 CFU/g)	ND	ND	ND	12	ND	10
TYM (<10 CFU/g)	ND	ND	ND	ND	ND	ND
BTGN <10 PN/g)	ND	ND	ND	ND	ND	ND
Salmonella species (in 10 g)	ND	ND	ND	ND	ND	3×10^3
E. coli (in 1 g)	ND	ND	ND	1.4×10^3	ND	4.5×10^3
Staphylococcus aureus (in 1 g)	ND	ND	ND	ND	ND	ND
Pseudomonas aeruginosa (in 1 g)	ND	ND	ND	ND	ND	ND

*(TNAM): Total number of aerobic microorganisms; (TYM): Total yeasts and molds; (BTGN): Bile-tolerant Gram-negative bacteria; and ND: not detected.

Commonly, NPPs are contaminated with bacteria and molds from contaminated water, soil, and atmosphere. The presence of high levels of water content and poor manufacturing practice are the main reasons for microbial growth. Of concern is the contamination of products by organisms that are considered pathogenic, such as *Salmonella* spp. and *E. coli*.

The findings of this study show that NPPs sold in Iraqi markets and consumed by people, unfortunately, do not have an acceptable pharmaceutical and/or microbial quality. Therefore, there is a crucial need for more rigorous monitoring and quality assessment of herbal products, which are manufactured, imported, sold, and used in Iraq. The quality requirements for synthetic medical preparations made by Drug Regulatory Authority are strict whereas such stringent standards are not enforced for NPPs. A real concern about the safety of such products has risen. Drug Regulatory Authorities in Iraq are required to establish laws and regulations to ensure the safety and validity of NPPs and protect people from the toxic and adverse effects of such non-safe products. Additionally, health professionals are required to educate people about the health consequences of consuming low-quality natural products and change their belief that "natural" is the synonym for "safe".

Authors' Contribution

Study concept and design: H. J. M. A.
Acquisition of data: H. J. M. A.
Analysis and interpretation of data: H. J. M. A.
Drafting of the manuscript: H. J. M. A.
Critical revision of the manuscript for important intellectual content: H. J. M. A.
Statistical analysis: H. J. M. A.
Administrative, technical, and material support: H. J. M. A.

Conflict of Interest

The authors declare that they have no conflict of interest.

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References

1. Atanasov AG, Waltenberger B, Pferschy-Wenzig EM, Linder T, Wawrosch C, Uhrin P, et al. Discovery and resupply of pharmacologically active plant-derived natural products: A review. Biotechnol Adv. 2015;33(8):1582-614.
2. Abdel-Aziz SM, Aeron A, Kahil TA. Health Benefits and Possible Risks of Herbal Medicine. In: Garg N, Abdel-Aziz SM, Aeron A, editors. Microbes in Food and Health. Cham: Springer International Publishing; 2016. p. 97-116.
3. Monera-Penduka TG, Jani ZT, Maponga CC, Mudzengi J, Morse GD, Nhachi CF. Quality and labeling information of Moringa oleifera products marketed for HIV-infected people in Zimbabwe. J Public Health Afr. 2016;7(2):84-8.
4. Kulhari A, Sheorayan A, Bajar S, Sarkar S, Chaudhury A, Kalia RK. Investigation of heavy metals in frequently utilized medicinal plants collected from environmentally diverse locations of north western India. Springerplus. 2013;2:676.
5. Gaston TE, Mendrick DL, Paine MF, Roe AL, Yeung CK. "Natural" is not synonymous with "Safe": Toxicity of natural products alone and in combination with pharmaceutical agents. Regul Toxicol Pharmacol. 2020;113:104642.
6. World Health Organization. Quality control methods for herbal materials. World Health Organization; 2011.
7. USP -N. Articles of Botanical Origin and Loss on drying. 2020.
8. Indrayanto G. Recent Development of Quality Control Methods for Herbal Derived Drug Preparations. Nat Prod Commun. 2018;13.
9. Li G, Schoneker D, Ulman KL, Sturm JJ, Thackery LM, Kauffman JF. Elemental Impurities in Pharmaceutical Excipients. J Pharm Sci. 2015;104(12):4197-206.
10. Wacoo AP, Wendiro D, Vuzi PC, Hawumba JF. Methods for Detection of Aflatoxins in Agricultural Food

Crops. J Appl Chem. 2014;2014:706291.

11. Bosiljkov T, Levaj B. The Effect of Extraction Solvents, Temperature and Time on the Composition and Mass Fraction of Polyphenols in Dalmatian Wild Sage (Salvia officinalis L.) Extracts. Food Technol Biotech. 2013;51(1):84-91.
12. Mahdi H, Khan N, Roziahanim M, Asmawi M, Vikneswaran A. LC/MS, GC/MS screening and in vivo anti-inflammatory activity of Malaysian Moringa oleifera Lam leaf extracts and fractions against carrageenan-induced paw oedema in rats. J Innov Pharmac Biol Sci. 2017;4(3):48-54.
13. Jan AT, Azam M, Siddiqui K, Ali A, Choi I, Haq QM. Heavy Metals and Human Health: Mechanistic Insight into Toxicity and Counter Defense System of Antioxidants. Int J Mol Sci. 2015;16(12):29592-630.
14. Kumar P, Mahato DK, Kamle M, Mohanta TK, Kang SG. Aflatoxins: A Global Concern for Food Safety, Human Health and Their Management. Front Microbiol. 2016;7:2170.