A Modern Approach for the Analytical Determination of Natural Berry Fruit Juice Freeze Dry Powders for Cosmeceutical Applications

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Abstract

Naturally obtained berry fruits are useful for treatment of various skin disorders, inflammations, for scurvy etc., as they possess potential antibacterial and antioxidant activities. These properties were investigated according to the current pharmacopoeia guidelines in the current study. Physicochemical properties, phytochemical analysis, fluorescence study and HPTLC fingerprint were evaluated to establish reliable supporting data. Phytochemical screening indicated the existence of various phytochemicals like polyphenols, flavonoids, tannins, total polysaccharides and the HPTLC fingerprint developed for the separation of phytochemicals was reported. HPTLC fingerprint has been developed for the natural berry fruit juice freeze dry powder, which can be used in any quality control setup as identification and chromatographical chemical fingerprinting technique which in turn can provide referential information for exact identification and accurate standardization of Natural berry fruit freeze dry powder.

Keywords: Pomegranate; Mango; Lemon; Jamun; Acai berry; Macaque berry; Black berry; Goji berry; Fingerprint HPTLC; Spectrophotometry

Introduction

Plants and herbal preparations have been used for medicinal and cosmeceutical applications since ages and the traditional systems of medicine have become a topic of global importance especially in the developed countries. Majority of the population in most of the developing countries still relies heavily on traditional practitioners and medicinal plants to meet primary health care needs. Even the population of the developed countries has begun to turn to alternative or complementary therapies, including medicinal herbs. Some of the most significant plants were considered for the current study.

Commonly known as Lemon tree, belongs to family Rutaceae can be used for the production of natural Lemon oil which is an important component of aromatherapy [1]. Lemon oil aroma does not influence the human immune system [2] but may contribute to relaxation. It is also called as Mosambi in Hindi. Drinking mosambi juice regularly reduces weakness and fatigue, and in fact boosts your energy. The composition of vitamins and minerals in mosambi juice makes it a great food for gorgeous healthy skin. Mosambi juice detoxifies the body and anything that detoxifies the body is great for our skin. It helps cure pimples and problematic skin. It is rich in antioxidant Vitamin C, a vital component for healthy glowing skin [3]. Due to its mild bleaching and cleansing properties, Mosambi juice can help get rid of spots, blemishes and even pigmentation. Mosambi juice has great anti-ageing properties and helps reduce wrinkling of the skin. It promotes the development of healthy collagen, provides firmness and prevents sagging [4].

Mango tree belongs to family Anacardiaceae. Some members of this family produce a viscous or adhesive fluid which can be used as a varnish or for reversing skin tanning and even as a mordant for red dyes [5]. Medicinally the edible nuts from this family are very good for the overall brain health. In a Korean study conducted in 2013, mango extracts were found to act against the UVB-induced skin aging in mice [6].

Mangoes are very rich in beta-carotene and vitamin A and as per a German study, these carotenoids can help enrich skin and promote healthy skin [7-10]. Beta-carotene being a natural photoprotective agent protects skin from harmful UV radiations by quenching the photochemical reactions in the epidermis. According to a Chinese study, the polyphenols in mangoes exhibit anticancer activity and hence can prevent skin cancer [11].

Jamun tree belongs to family Myrtaceae. The seeds of the Jamun fruit are used in various alternative therapies like Ayurveda, Unani and traditional Chinese medicine. Wine and vinegar are also made from the fruit [12-15]. Jamun fruit is rich in vitamin A and vitamin C which has been known to have an amazing effect on skin. It has been proven to brighten and lighten skin. The antioxidant attributes of the Jamun can be used as an anti-aging agent as it helps skin in fighting free radicals, producing collagen, aids in skin regeneration and improving skin texture. Scars and blemishes can be faded by applying Jamun seed powder on to the affected areas of skin.

Pomegranate plant belongs to family Lythraceae. Regular consumption of pomegranate juice can slow down the process of ageing by reducing wrinkles and fine lines caused by constant exposure to sun. It also helps in the regeneration of skin cells and prevents hyper pigmentation and occurrence of dark spots [16]. Pomegranate juice works wonders for dry skin. It also protects the skin from sun burns and heals the damage caused to the skin due to constant exposure to the sun. Pomegranates are also known to extend the life of fibroblasts which are responsible for the production of collagen and elastin that tighten the skin and prevent the formation of fine lines and wrinkles. Collagen and elastin gives skin the strength and support thus pomegranate acts as an astringent. Anthocyanins and hydrolysable tannins present in pomegranate possess strong anti-oxidant and anti-
tumor properties. When applied directly on the skin, ellagic acid, a polyphenol antioxidant found in pomegranates inhibits skin cancer [17].

Acai berry plant belongs to family Arecaceae [18]. The fruit is processed into pulp for supply to food product manufacturers or retailers, sold as frozen pulp, juice, or an ingredient in various products from beverages, including grain alcohol, smoothies, foods, cosmetics and supplements [19].

Acai berries are a abundant of many important nutrients with antioxidant properties which can fight the free radicals and delay aging. These properties make them a perfect ingredient for anti-aging face and body creams. Their daily consumption can keep the skin glowing and healthy. Due to the anti-inflammatory properties, Acai berry can be used in treating hyper pigmentation, controlling acne and breakouts, repair the damage caused by exposure to sun, pollutants etc., and can maintain the natural moisturizing power of the skin [20].

Goji berry plant belongs to family Nightshade [21]. This claim apparently originated in a 2003 booklet by Earl Mindell, who had stated that Goji berry has anti-cancer properties [22]. The anti-cancer properties can be attributed to the anti-inflammatory and anti-oxidant properties of the berries. They can be used in conditions of skin inflammation and they can reduce and prevent acne breakouts. Direct consumption of the juice can treat inflammation and acne breakouts from inside. Additionally, one can apply Goji berry juice or extract to the face and wash with cold water after 15 minutes. The berries are rich in Vitamin C which can help in firming up your skin and reduce wrinkles too. This basically means you can also use them for their anti-aging benefits [23].

Blackberry fruit belongs to family Rosaceae. Blackberry was found to be one of the most beneficial ingredients to treat hyperpigmentation [24].

Blackberries are rich in Vitamins A, C and K which are excellent for skin rejuvenation. Seeds are rich in omega-3 and omega-6 fatty acids and there fatty acids are vital for healthy skin. Blackberries are found to contain the anti-oxidants namely Anthocyanocides and polyphenols which can help in fighting free radicals. Vitamin C and Vitamin A present in blackberries act as antioxidants and also build collagen, making the skin stronger. Regular consumption of blackberries protects the skin from the damaging UVA and UVB rays and aids in skin cell renewal [25].

Maqui berry fruit belongs to family Elaeocarpaceae. There are some publications suggesting the usage of Maquiberry for supporting the weight loss regime, slowing down the aging process, help with dry eye, improving skin health and the immune system [26].

Free radicals can damage the DNA, can break the collagen forming wrinkles and fine lines on the skin, all of which are the signs of aging. Anti-oxidant rich Maqui berry can help slow down the premature aging by fighting these free radicals. Maqui berry slows down aging and is also able to nourish the skin cells, providing a radiant and youthful skin [27].

The present investigation of evaluation of berry fruit juice freeze dry powder and establishing quality control parameters was taken up and the complete process adheres to the guidelines of Indian Pharmacopoeia and WHO. The evaluated quality control parameters helps in identification as well as in standardization of the berry fruit juice freeze dry powder [28,29]. As per WHO guidelines, fingerprint chromatography of medicinal herbs can be used as an identification and quality control technique, since 1991 [30]. Fingerprints can be a unique identification utility for berry fruits and their different species [31,32]. Therefore, HPTLC fingerprint, tannins, polyphenols, Flavonoids content has been also developed for berry fruit juice freeze dry powders.

Freeze drying, also known as Lyophilization is a process of drying in which the material is first frozen completely before drying by direct sublimation of the ice under reduced pressure [33]. To carry out a successful freeze-drying operation, the pressure in the drying chamber must be maintained at an absolute pressure of at least 620 Pa [34]. Freeze drying is considered as the best method for producing high quality dried products [35]. Limitations of freeze drying are high production costs, high energy consumptions, and low throughputs [35-37].

Studies were reported that compared the influence of freeze-drying methods on various quality attributes of berry fruits, including the color of dehydrated Mango, jamun, pomegranate, sweet lemon, acai berry, goji berry, black berry and Macqui berry. However, no studies have been conducted to evaluate the effect of drying methods on freeze dried powders in terms of color, Loss on drying, pH, total ash, acid insoluble ash, sieve, total polysaccharides, water and alcohol soluble extractive, total polyphenols, flavonoids and tannins. Thus, the objective of this work is to establish a detailed report about understanding the influence of freeze-drying methods, the physical properties of resulting freeze dry powders, selection of freeze dry fruit powders for the manufacture of high-quality fruit powders and cosmetics etc.

Materials and Methods

Freeze drying was carried out in a laboratory freeze dryer (Cosmeinnova Specialty Ingredient India Mumbai-400037). Berry fruits were juiced, and the juice was poured into a stainless pan to form a layer of about 15 mm. These samples were placed at -25°C for 24 Hrs and then transferred to the freeze dryer. The vacuum pressure of the dryer was set at 20 Pa, the plate temperature was 20°C, and the condenser was at -60°C. The residence time needed to dry the berries fruit juice to below 0.05 kg water/kg dry solids was determined when the vacuum pressure had dropped to 30 mTorr (4 Pa). Indian pharmacopoeia methods were used for the determination of physicochemical properties such as the percentage of total ash, acid insoluble ash, water soluble ash; water soluble and alcohol soluble extractive values, sieve, total polysaccharides, total carbohydrates, total nitrogen and protein content, microbial and elemental analysis [38]. Fluorescence analysis was conducted using methods of Kokoski [39] and Chase and Pratt [40]. Thin layer chromatography (TLC) described by Wagnar and Bladt method was used for phytochemical analysis. The said method is a qualitative densitometric HPTLC analysis performed with methanolic extract for the development of characteristic fingerprint profile. This method may be used for evaluation of quality and standardization of the drug. 10 µl of the methanolic extract was spotted on pre-coated silica gel G60 F254HPTLC plates (Merck) with the help of CAMAG Linomat V applicator. The plate was then developed in glass twin trough chamber (20 cm*10 cm) pre-saturated with mobile phase (Toluene: Ethyl acetate: Formic acid: Methanol in the ratio 5:3:1:1). The plate after elution was derivatized using vanillin sulphuric acid reagent and scanned using TLC Scanner 3. (CAMAG).
Results

Physico-chemical analysis

Ash and loss on drying of any organic material is composed of their non-volatile inorganic components. Controlled incineration of crude drugs results in an ash residue consisting of an inorganic material (metallic salts and silica). These values usually vary within certain fairly wide limits owing to various parameters. Hence these parameters are important in the evaluation of crude drugs [32]. Therefore, percentage of total ash, acid insoluble ash, water soluble ash, total polysaccharides, total nitrogen and protein content were determined for the evaluation. The extraction of any crude drug with a particular solvent yields an extract containing different phytoconstituents at different proportions. Extractive value is also useful for evaluation of crude drug, which gives an idea about the nature of the chemical constituents present in a crude drug and is useful for the estimation of specific constituents, soluble in that particular solvent used for extraction [33]. Loss on drying is the loss of mass expressed as percent w/w [34]. Results are tabulated in Table 1.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Test parameters</th>
<th>Observed values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Name of fruit juice freeze dry powder</td>
<td>Mango</td>
</tr>
<tr>
<td>1</td>
<td>Description</td>
<td>Free flow orange color powder</td>
</tr>
<tr>
<td>2</td>
<td>pH (1% solution in water)</td>
<td>4.30</td>
</tr>
<tr>
<td>3</td>
<td>Loss on drying at 105 ± 5°C by IR Moisturizer</td>
<td>6.33%</td>
</tr>
<tr>
<td>4</td>
<td>Total ash content at 625±25°C</td>
<td>1.84%</td>
</tr>
<tr>
<td>5</td>
<td>Total polysaccharides content</td>
<td>% Acemannan (methanol precipitation)</td>
</tr>
<tr>
<td></td>
<td>% Acetone precipitation</td>
<td>17.18%</td>
</tr>
<tr>
<td>6</td>
<td>Water soluble extractive</td>
<td>92.60%</td>
</tr>
<tr>
<td>7</td>
<td>Alcohol soluble extractive</td>
<td>24.48%</td>
</tr>
<tr>
<td>8</td>
<td>Sieve 80# (Residue retained on)</td>
<td>Nil</td>
</tr>
<tr>
<td>9</td>
<td>Total nitrogen content</td>
<td>0.57%</td>
</tr>
<tr>
<td>10</td>
<td>Total protein content</td>
<td>3.56%</td>
</tr>
</tbody>
</table>

Table 1: Physicochemical Analysis of Fruit Juices.

Phytochemical analysis

The preliminary phytochemical screening of fruit powder was carried out using various solvents viz., Ethanol, methanol and water. These extracts were subjected to various qualitative chemical analysis which shows the presence of total polyphenols, tannins, flavonoids and total carbohydrates. The results of phytochemical analysis are tabulated in Table 2.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Test parameters</th>
<th>Observed values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Name of fruit juice freeze dry powder</td>
<td>Mango</td>
</tr>
<tr>
<td>1</td>
<td>% of Tannins</td>
<td>Nil</td>
</tr>
<tr>
<td>2</td>
<td>% of Flavonoids</td>
<td>Nil</td>
</tr>
</tbody>
</table>
Table 2: The results of phytochemical analysis.

**HPTLC fingerprint**

HPTLC fingerprint patterns of fruit juice freeze powder are useful for the quantification of active ingredients and maximum band separation [40]. HPTLC fingerprinting studies of methanolic fruit juice extract showed distinct band pattern before and after spraying with derivatizing reagent (methanolic vanillin sulphuric acid) and at different wavelengths. HPTLC fingerprints before and after derivatization are tabulated in Table 3 (Figure 1-(254 nm); Figure 2-(366 nm); Figure 3 (white light)).

Table 3: HPTLC fingerprints before and after derivatization.

**Microbial analysis**

The preliminary Microbial screening of fruit juice freeze dry powder was carried out using Pharmacopoeia method. These extracts were subjected to qualitative analysis to determine the presence of bacteria, fungi, and pathogens. The results of microbial analysis are tabulated in Table 4.

Table 4: Microbial analysis of fruit juice freeze dry powder.

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therapeutic values. According to World Health Organization (WHO),
been emphasis on standardization of fruit juice freeze dry powders of
powder was carried out using Pharmacopoeia method.
the quality of berry fruit freeze dry powder also. Recently there has
fruit juice freeze dry powder is an integral part of establishing its
Elemental analysis

<table>
<thead>
<tr>
<th>S. No</th>
<th>Test parameters</th>
<th>Name of fruit juice freeze dry powder</th>
<th>Observed values</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Escherichia coli/gm</td>
<td>-Ve</td>
<td>-Ve</td>
</tr>
<tr>
<td>4</td>
<td>Staphylococcus aureus/gm</td>
<td>-Ve</td>
<td>-Ve</td>
</tr>
<tr>
<td>5</td>
<td>Pseudomonas aeruginosa/gm</td>
<td>-Ve</td>
<td>-Ve</td>
</tr>
<tr>
<td>6</td>
<td>Salmonella/gm</td>
<td>-Ve</td>
<td>-Ve</td>
</tr>
<tr>
<td>7</td>
<td>Burkholderia cepacia/gm</td>
<td>-Ve</td>
<td>-Ve</td>
</tr>
<tr>
<td>8</td>
<td>Coliforms/gm</td>
<td>-Ve</td>
<td>-Ve</td>
</tr>
<tr>
<td>9</td>
<td>Candida albicans/gm</td>
<td>-Ve</td>
<td>-Ve</td>
</tr>
<tr>
<td>10</td>
<td>Bile-Tolerant gram-negative bacteria/gm</td>
<td>-Ve</td>
<td>-Ve</td>
</tr>
</tbody>
</table>

Table 4: The results of microbial analysis.

Elemental analysis

The preliminary Elemental screening of fruit juice freeze dry powder was carried out using Pharmacopoeia method. These extracts were subjected to qualitative analysis to determine the presence of heavy metals and elements like Lead, cadmium, mercury, Arsenic Iron etc. The results of microbial analysis are tabulated in Table 5.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Test parameters</th>
<th>Name of fruit juice freeze dry powder</th>
<th>Observed values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Arsenic</td>
<td>0.013 ppm</td>
<td>0.016 ppm</td>
</tr>
<tr>
<td>2</td>
<td>Cadmium</td>
<td>BDL (0.005 ppm)</td>
<td>BDL (0.007 ppm)</td>
</tr>
<tr>
<td>3</td>
<td>Mercury</td>
<td>BDL (0.005 ppm)</td>
<td>BDL (0.005 ppm)</td>
</tr>
<tr>
<td>4</td>
<td>Lead</td>
<td>0.316 ppm</td>
<td>0.514 ppm</td>
</tr>
<tr>
<td>6</td>
<td>Antimony</td>
<td>0.009 ppm</td>
<td>0.005 ppm</td>
</tr>
<tr>
<td>7</td>
<td>Iron</td>
<td>47.925 ppm</td>
<td>50.742 ppm</td>
</tr>
<tr>
<td>8</td>
<td>Nickel</td>
<td>0.123 ppm</td>
<td>0.301 ppm</td>
</tr>
<tr>
<td>9</td>
<td>Cobalt</td>
<td>0.082 ppm</td>
<td>0.094 ppm</td>
</tr>
</tbody>
</table>

Table 5: Elemental screening of fruit juice freeze dry powder.

Discussion

Proper control and sourcing of the starting materials is of utmost importance for any final product to be consistent. This stands true for the quality of berry fruit freeze dry powder also. Recently there has been emphasis on standardization of fruit juice freeze dry powders of therapeutic values. According to World Health Organization (WHO), establishing the identity and purity of plant-based products should be performed first before any further tests [36]. The standardization of fruit juice freeze dry powder is an integral part of establishing its correct identity for inclusion of crude drug in Pharmacopoeia. The results obtained from the present investigation could, therefore, serve as a basis for proper identification, collection and investigation of the fruits and their extracts [37].

The present study is focused on the evaluation of features and physicochemical parameters of fruits juice freeze dry powder. The physicochemical standards, such as loss on drying, ash values, extractive values, polysaccharides, pH, sieve, microbial, total nitrogen and protein content, total carbohydrates and elemental analysis will be useful to identify the authenticity of the drug even from the crushed or
powdered fruit juice freeze dry materials and these can be referred to as a standard data for the quality control of the preparations containing this fruit juice freeze dry powder. The fruits can be differentiated from other related species by the usage of the data generated here [38].

Preliminary phytochemical and HPTLC analysis showed the presence of various phytochemical compounds such as, tannins, Polyphenols, flavonoids, which could make the fruits useful for treating different skin conditions and ailments. These preliminary screening tests may be useful in the determination of the bioactive principles and drug discovery and development [39].

HPTLC technique is useful in confirming the identity and purity of fruit juice freeze dry powder and for detecting adulteration and substitution. HPTLC fingerprint profile can be generated and compared with the recorded reference standard for further research and confirmation of the fruit juice freeze dry powder [39].

**Conclusion**

Berry fruit juice freeze dry powders are being used in many developed and developing countries as traditional home remedies, over-the-counter drug products and raw materials for the pharmaceutical industry and represent a substantial proportion of the global herbal drug market. Therefore, it is essential to ensure reproducible quality of herbal products. Thus in recent years there has been an emphasis on standardization of fruit juice freeze dry powder of therapeutic values. Despite the modern techniques, identification and evaluation of fruit juice freeze dry powder by pharmacognostical studies is still more reliable, accurate and inexpensive means. Since all fruit juice freeze dry powder are known for its various medicinal and cosmecutical properties, the present study could be useful to supplement information with respect to its identification, authentication and standardization. The information generated can also be used for preparation of a monograph of the fruit juice freeze dry powder, for the Indian Herbal Pharmacopoeia.

**References**

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