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Original Research Paper

ASSESSING OF MARKETED BENZOIN SAMPLES FOR DIFFERENT QUALITATIVE AND QUANTITATIVE ATTRIBUTES

Kawade Vikram S[1]*, Jadhav Sharmila S[2] and Killedar Suresh G[2]

[1]Department of Pharmaceutical Chemistry, Bharati Vidyapeeth College of Pharmacy, Near Chitranagari, Kolhapur, 416 013, Maharashtra, India

[2]Department of Pharmaceutics, Bharati Vidyapeeth College of Pharmacy, Near Chitranagari, Kolhapur, 416 013, Maharashtra, India

ABSTRACT

Balsamic resin obtained from certain tropical Asian trees of the genus *Styrax* and used in perfumery and medicine. It has been observed that several malpractices exist in market during sale of benzoin, which is typically known as "Dhoop" in the folks. Market is flooded with the adulterated benzoin. Considering the above observations, objective of our experiment is to check and detect the purity of the products under consideration in experiment. Thus, though malpractices are occurring in various forms, this experiment helps in identifying a part of adulterations taking place under the name of pure benzoin or pure dhoop in the market. In this experiment we have selected various samples A, B, C and D from market. For identification and purity of samples we performed various tests like morphological test, chemical quantitative test, standardization and estimation of total balsamic acid. By performing different quantitative and qualitative analysis it was found that all samples under consideration were adulterated. Though this experiment it was confirmed that malpractices exist in market. The samples collected from local market were found in different size, shape, color, solubility and some proximate values.

Keywords: Benzoin, Standardization, Estimation, Malpractice.

INTRODUCTION

Generally herbal formulation involves the use of fresh or dried plant parts. Correct knowledge of crude drug is very important aspect in preparation, safety and efficacy of the herbal product (Ahmad, 2013). Standardization is a process of prescribing a set of standards or inherent characteristics, constant parameter, definitive, qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility. It is a system to ensure that every packet of medicine that is sold has the correct amount and will induce its therapeutic effect. Specific standards are worked out by experimentation and observation, which would lead to the process of prescribing a set of characteristics exhibited by particular crude product. Hence it is a tool in quality control process (Kunle, 2012). Benzoin is a balsamic resin obtained from *Styrax benzoin* Dry or *Styrax paralleloneurus Perkins* and other species of Styrax known in the market as Sumatra benzoin or it may also contain the balsamic resin from *Styrax tonkinesis* and other species commercially known as Siam benzoin. It is an organic compound with the formula PhCH(OH)C(O)Ph. It is a hydroxy ketone attached to two phenyl groups. Sumatra benzoin has an aromatic and balsamic odor. Whenheated it does not emit a

pinaceous odor. Its taste is aromatic and slightly acrid. Siam benzoin has an agreeable,balsamic, vanilla-like odor. Its taste is aromatic and slightly acrid (USP, 2009). Benzoin resin is obtained from the bark of several species of trees in the genus Styrax. The chemical constituent present in the benzoin are Cinnamic, benzoic and sumaresinolic acid esters, benzoic acid, cinnamic acid, sumaresinolic acid, benzaldehyde and vanillin. Sumatra benzoin contains free balsamic acid (benzoic acid and cinnamic acid) and esters derived from them. Triterpenoids such as summaresinolic and siarsinolic acids are also present. The major constituent of Siam benzoin is an ester coniferyl benzoate. Benzoin has therapeutic actions like stimulant, expectorant, astringent, antispasmodic, antiseptic, carminative, diuretic. It may be applied topically to wounds and ulcers to protect and disinfect the skin. It is also useful in respiratory difficulties, providing useful in cases of bronchits and asthmait in form of tinctures. It acts as a carminative when taken internally and is rapidly absorbed. In this article the description of the results of standardization of benzoin which is available in the market. For the first time we have come with the comparative report of morphological tests, identification tests, standardization and estimation of different samples. In addition since adulteration of other substances like benzoin to be a cumulative process in malpractices of benzoin, it stands to reason that alteration in colours, odor of benzoin may be an early event in the development of malpractices of marketed samples of benzoin. It is our contention that a better understanding of the chemical composition of benzoin may facilitate the discovery of improved strategies for the prevention, detection of malpractices of marketed samples of benzoin.

MATERIALS AND METHODS

Materials

Ethanol (Jiang Huaxi International Trade Co. Ltd., China), Potassium permagnate (Loba Chemie), Toulene (Molychem), Ethyl acetate (Loba Chemie), Chloroform (Molychem), Ether (Loba Chemie), Light petroleum (Loba Chemie), Anisaldehydesulphuric acid reagent (E-Merk), Sodium hydroxide (Merk Specialities Pvt. Ltd.), Phosphorus pentoxide (Molychem), 0.5 N alcoholic potassium hydroxide (Molychem), Magnesium sulphate (HiMedia Laboritories), Hydrochloric acid (Molychem), Sodium bicarbonate (Molychem), Phenol red indicator (Molychem).

Electronic balance (Seico India), Muffle furnace (Meta Lab), Soxhlet Extractor (J-SIL), Steam Bath (Meta Lab), Hot Air Oven (Meta Lab).

Methods

Identification Test for Benzoin

I. Alcoholic solution of benzoin with water gave milky white solution.
II. Small quantity of benzoin in a test tube was heated and covered with a glass plate. Contents of the test tube were cooled and the glass plate was examined under the microscope. The crystals of cinnamic acid were observed.
III. About 2.5g of benzoin was added to 10 mL of ether, to this solution 3 drops of sulphuric acid was added. Sumatra benzoin showed the deep brown color and purple color was observed for siam benzoin.
IV. Exactly 4 mL of potassium permanganate was added to 1 g of benzoin. Odor of Benzaldehyde confirms the sample as Sumatra benzoin (IP, 2007).

Standardization of Benzoin

I. Acid insoluble ash

Standard: Not more than 1% in Sumatra benzoin and not more than 0.5% in siam benzoin, determined on 2 g (IP, 2007).

Procedure

Total Ash: Exactly weighed 2 g of air dried drug was taken in silica crucible and incinerated at temperature 450^{o}C and the charred mass was wash with the hot water and the residue was collected on the ashless filter paper (Whatman-41), carbon free white ash was obtained after evaporation. Percent of ash was calculated on the dry drug basis.

Acid Insoluble Ash: Ash obtained from the total ash was boiled with 25 mL of 2 M hydrochloric acid for 5 min. Insoluble matter was collected on ashless filter paper, washed with hot water and then kept in vacuum desiccators. Percent of acid insoluble ash was calculated on the dry drug basis (Ahmad, 2013).

II. Foreign organic matter

Standard: Not more than 1% (IP, 2007).

Procedure

100 g of original sample was weighed and spread out in a thin layer on white glossy paper. The sample was inspected with unaided eye and foreign organic matters were separated manually. After the complete removal of foreign matter from each sample, the separated foreign matter was weighed and percent of foreign organic matter was calculated (Kunle, 2012).

III. Ethanol soluble extractive

Standard: Not less than 75% in Sumatra benzoin and not less than 90% in siam benzoin (IP, 2007).

Procedure

2 g of accurately weighed coarse powder was taken in trade extraction thimble and subjected for soxhelation. 0.1 g of NaOH was placed in the receiving flask. Sample was extracted with ethanol (95%) for 5 h. After the complete extraction, thimble was dried to constant weight at 105°C. Ethanol soluble extractive was calculated from the increase in weight of the thimble (Ahmad, 2013).

IV. Loss on drying

Standard: Not more than 10% (IP, 2007).

Procedure

2 g of coarse powdered sample was dried over phosphorus pentaoxide at pressure 2.7 kPa for 4h (Gautam, 2010).

Estimation of Balsamic Acid

Procedure

2 g of benzoin was boiled with 25 mL of 0.5 N alcoholic potassium hydroxide solution under the reflux condenser for 1 h. The alcohol was removed and the residue was digested with 50 mL hot water. The liquid was cooled and 150 mL of water was added to it. 2.5 g of magnesium sulphate was added to 50 mL of water and allowed it to stand for 10 min. Further filter under vacuum. The residue was again washed with 20 mL of water. The liquid was acidified with HCl and extracted with successive quantities of 50, 40, 30, 30, 30 mL of solvent ether. The combined ethereal extract was shaken with the successive quantities of 20, 20, 10, 10 mL of sodium bicarbonate solution. Mixed aqueous extract was acidified with HCl. Acidified aqueous extract was shaked with successive quantities of 30, 20, 10, 10 mL of chloroform. Chloroform layer was separated, evaporated and the residue was dissolved by warming with 10 mL of alcohol. Solution was titrated against 0.1 N NaOH using phenol red as indicator. Total balsamic acid was calculated as:

Each mL of 0.1 N sodium hydroxide is equivalent to 0.01482 g of total balsamic acids calculated as cinnamic acid (Kokate, 1994).

RESULTS AND DISCUSSIONS

From above experiment following results were found.

From the practical values it has been observed that all results complies with official standards expect sample D for foreign organic matter. The above result shows that sample A, B and C though complies as per official standard with respect to physical constants they differ individually. Ethanolic extractive values were found to be 82.51% in sample B which deemed as necessary required limits as per standards for Sumatra benzoin. Loss on drying values were observed for samples A, B, C and D as 0.82%, 1.36%, 0.91% and 2.15% respectively indicates excess moisture content in sample D. Acid insoluble ash value in sample D was found 0.92% indicate more silicate as an impurity compare to other samples. Also sample D showed loss on drying (2.15%) highest amongst samples tested indicates adulteration of essence. From the results

it is confirmed that commercial samples tested shows close resemblance to Sumatra benzoin except sample D. Overall results shows sample D was found to be more impure, more fragrant and sticky in nature.

CONCLUSION

From our experimentation it was concluded that, the marketed products of benzoin are adulterated with other substances and lots of variation among them with respect to physical constants. The values also confirm that sample A, B and C are Sumatra benzoin with respect to ethanol soluble extractive value. Sample D failed to show most of the specific physico-chemical tests for benzoin and hence it is not the benzoin but adulterated mass. Thus, it was confirmed that the malpractices are occurring in various form in market under the name of benzoin. Further studies are on with some more samples from different regions of our country and estimation of balsamic acid using specific spectral analysis.

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Figure 1 (A, B, C & D): Marketed samples of benzoin

Table 1: Organoleptic characters

Sample	Color	Odor	Taste
A	Yellowish-brown	Aromatic	Sweet & Acrid
B	Greyish-brown	Agreeable and vanilla like	Sweet & Acrid
C	Yellowish brown	Aromatic and agreeable	Sweet & Acrid
D	Yellowish brown	Very aromatic	Sweet & Acrid

Table 2: Comparative identification test of sample

Test	Observation	Sample A	Sample B	Sample C	Sample D
0.5g Benzoin + 5 mL $KMnO_4$	Odor of Benzaldehyde	Slight	Strong	Moderate	No odor
Benzoin+ Ethanol (95%) + Water	Milky Acidic	Seen	Seen	Seen	Not seen
0.5g Benzoin+ Heat (to melt)	White Fumes	Seen	Seen	Seen	Not seen
2.5 g of Benzoin + 10 mL Ether + 2-3 mL of Suphuric acid	Deep Brown or Deep Purplish Red	Deep Brown	Deep Purplish Red	Upper layer deep brown and lower layer purplish red	No change

Table 3: Standardization of benzoin

Sample	Acid insoluble ash*	Foreign organic matter*	Ethanol soluble extractive*	Loss on drying*	Total balsamic acid*
A	0.41±0.02%	0.62±0.05%	64.83±1.20%	0.82±0.06%	2.37±0.06%
B	0.23±0.03%	0.34±0.01%	82.51±1.50%	1.36±0.60%	3.25±0.09%
C	0.48±0.05%	0.48±0.06%	73.64±1.68%	0.91±0.09%	1.78±0.01%
D	0.92±0.08%	1.32±0.05%	35.24±0.50%	2.15±0.50%	0.59±0.01%

* Mean ± SD

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Correspondence Author:

Kawade Vikram S

1Department of Pharmaceutical Chemistry, Bharati Vidyapeeth College of Pharmacy, Near Chitranagari, Kolhapur, 416 013, Maharashtra, India

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