



Siddiquee M.R.A. et al.; International Journal of Pharmamedix India, 2014, 2(1), 669-78.

“Scientific Review of Os Sepia (*Jhaag-e-Darya/Samudra Phena*) with Respect to Indian Medicine and its Characterization on Physicochemical Parameters.”

Siddiquee M.R.A.^{1*}, Sultana A², Siddiquee A³

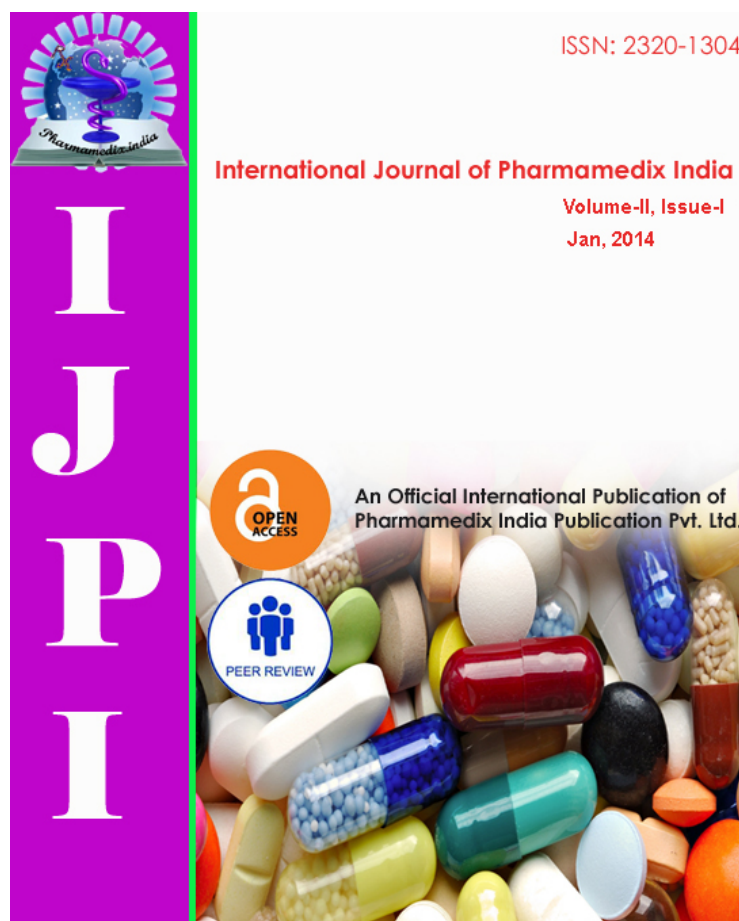
***Author for correspondence**

M R A Siddiquee^{*1},
Lecturer, Jamia Tibbiya Deoband
Phone No. 01336-222379, 222468
mdrizwans@yahoo.com

²Associate professor, Ajmal Khan Tibbia
College, Aligarh, U.P.

³Associate Professor, Faculty of Unani
Medicine, Jamia Hamdard, New Delhi.

Institutional Address: Faculty of Unani
Medicine, Jamia Hamdard, New Delhi .Pin
110062 Phone no. 26059688 Ext. 5713



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Abstract:

Cuttlebone also called *Os sepia* had drawn considerable attention of researchers now a day as an important marine product due to its pharmacological and non-pharmacological uses. However it had been long used in traditional system of medicine in India and China due to its various pharmacological effects. The work was carried out to firstly, update the knowledge on cuttlebone as a drug including its structure, identification, pharmacological effects and medicinal uses. The second part of paper includes original research work on characterization of this drug on physiochemical laboratory parameters.

Keyword: Cuttlebone, aragonite, chitin, ash value, density, successive extraction.

Introduction

Cuttlefish bone, as the name suggest is a hard, brittle internal structure found in all members of the family Sepiidae, commonly known as cuttlefish. Being light, it floats in water, and during monsoon, these bones are drifted ashore in such great numbers that they have been named as sea-foam. (Kotpal, n.a.) Cuttlebone signifies a special class of ultra-lightweight cellular natural material possessing unique chemical, mechanical and structural properties, which have drawn considerable attention in the literature. (Cadmana, Zhou, Li, & Chen, 2012) However pharmacological effects and uses of cuttlebone as drug in Ayurveda, Unani and

Chinese traditional system of medicine are elaborately mentioned in classical literatures with the name of *Samudra phena*, *Jhag-e-darya* and *Hai piao xiao* respectively. Now a day, researches to proof efficacy mentioned in classical literature and also to find out new medicinal uses are being carried out. Cuttlebone has also got a number of non-pharmacological uses, thus attracting number of traders of marine products. (Sathiadhas & Aswathy, 2004)

Indian pharmacopeia of Unani drug as well as Ayurvedic drug have not yet published its monographs in their recent editions and therefore updated knowledge about the drug

and its physiochemical properties is lacking in medicinal context. This original research paper tries to fill few of these loopholes. Characterization was done on the parameters of microscopic view, moisture content, ash value, density, pH and successive extraction.

Literature review:

Crystal structure of the cuttlebone is an aragonite form of CaCO_3 , and density of aragonite is 2.70 g/cm^3 . (Sirilux, Ikeda, Kokubo, & Shiono, 2008) The microscopic structure of cuttlebone consists of narrow layers connected by numerous upright pillars. (Rexfort & Mutterlose, 2006).

It is tasteless, sometime sour and does not have any odour (Abdul-Hakeem). It inhabits the Atlantic Ocean and the Mediterranean Sea too (Lucius & Sayre, 1917) It is also found in Indian coast and commercially utilized.

Variants of Cuttlebone

The bone from all species of cuttlefish is of medicinal value and considered as *Jhag-e-Darya*. Thus source of cuttlebone are *Sepia officinalis* Linnaeus (CSIR, 1962), *Sepia rugosa* (Bowdich, 1822), *Sepia vicellius* (Gray, 1849), *Sepia zebrine* (Risso, 1854), *Sepia filliouxii* (Lafont, 1869), *Sepia officinalis mediterranea* (Ninni, 1884; Gofas & Rosenberg, 2010), Hai Piao Xiao (YYH Staff, 2013; MPA Media Publication)

Pharmacological Actions

Detergent (Kabeeruddin, n.a.) ,Antacid (Muzaffer-Hussain, n.a.; Radhika Gopal,

2008), Astringent (Muzaffer-Hussain, n.a.; CSIR, 1962; Radhika Gopal, 2008) ,Sedative (CSIR, 1962; Radhika Gopal, 2008) ,Metal adsorbent (You-Zhi, et al., 2010) , Emmenagogue (Kabeeruddin, n.a.), Diuretic (Abdul-Hakeem) ,Improves Vision (Kabeeruddin, n.a.), Antiemetic (Abdul-Hakeem), Digestive (Abdul-Hakeem), Anti-inflammatory (Abdul-Hakeem)

Uses

Cuttlebone had found its way in verity of uses. For convenient here it is summarized into i) Non pharmacological uses and ii) Pharmacological uses

Non pharmacological Uses

It is used as Calcium-rich dietary supplements of caged birds, chinchillas, hermit crabs, snails, and turtles. (Norman & Reid, 2000). It have its importance in mold-making material for small metal casting for the creation of jewelry and small sculptural objects and also as polishing agent. It can be used as metal adsorbent of low cost. (You-Zhi, et al., 2010). Cuttlebone was proved to be a biomass for new reinforcing filler for natural rubber. (Sirilux et al., 2008)

Pharmacological uses

Cosmetology: Chitin, major constituent of the exoskeleton material of cuttlefish is used in preparation of natural cosmetic soap, preparation of slimmer capsules, preparation of hair dyes (Sathiadhas & Aswathy, 2004)

Dermatology: Its paste along with lemon juice is usefully applied in itches and other skin diseases like melasma, freckles.

(Kabeeruddin, n.a.; Khan, 1940; Nadkarni, 1979; Abdul-Hakeem ; Md Rizwan, Sultana, Khanna, Tanveer, & Shiffa, 2012) In prickly heat cuttlefish powder is applied on body along with rose water. (Nadkarni, 1979; Radhika Gopal, 2008) Topically, cuttlefish bone can be used as a poultice to treat skin rashes, ulcers and lesions.(MPA Media Publication)

ENT: Used as dusting powder to relieve the pain of ear ache or otorrhoea (Nadkarni, 1979; Radhika Gopal, 2008). Medicated oil prepared by boiling fine scraping of the bone in sweet or sesame oil is also used as drops for ear pain and otorrhoea (Radhika Gopal, 2008). It is used as an ingredient of tooth powder. (Kabeeruddin, n.a.; Radhika Gopal, 2008)

Gastrology: It helps in neutralizing excess of gastrointestinal acidity as it harmonizes the stomach - epigastric pain, acid reflux.(YYH Staff, 2013) Some patients with stomach problems may take it to combat acid reflux disease and some intestinal disorders (MPA Media Publication) Powder is also given internally to control spure and dysentery (Radhika Gopal, 2008), diarrhea (YYH Staff, 2013; Lucius & Sayre, 1917). It find its uses in cases of Ascites. (Abdul-Hakeem) Cuttlebone-fritillary bulb pair is used on stomach pain (Fan Gang, 1993)

Gynecology: It stops uterine bleeding with vaginal discharge and also helps treat leucorrhea (YYH Staff, 2013) , Internally, it is taken to help stop bleeding of the uterus

(MPA Media Publication) It is also used in amenorrhea. (MPA Media Publication)

Ophthalmology: Paste made of Cuttlefish bone and rock-salt (Sodium chloride impura) in rose water base is usually applied to the eyes in conjunctivitis. (Nadkarni, 1979; Radhika Gopal, 2008). It is also used to improve vision (Abdul-Hakeem).

Orthopedics: It is used as rich source of calcium (Sathiadhas & Aswathy, 2004) It enhance osteogenesis and thus osteoconduction. (Lucius & Sayre, 1917). Osteogenic capacity and bone repairing suggest that cuttlefish backbone could be considered an alternative xenograft material in orthopedic applications. (Zafer & Vildirim, 2005). Hydroxyapatite structures for tissue engineering applications have been produced by hydrothermal (HT) treatment of aragonite in the form of cuttlefish bone at 200°C (Ivankovic, Ferrer, Gallego, Tkalcec, Orlic, & Ivankovic, 2009)

Surgery: It is used externally to cease bleeding. (Lucius & Sayre, 1917). It cures wound (Gupta, Silori, Mistry, & Dixit, 2003). It resolves dampness and promotes healing of ulcer and rashes. (Lucius & Sayre, 1917; YYH Staff, 2013) Cuttlebone has been used for making surgical sutures (Sathiadhas & Aswathy, 2004)

Urogenital-System: It helps restoration of kidney functions (Lucius & Sayre, 1917). Also expel kidney stones (Abdul-Hakeem), prevent enuresis and cures retention of urine.(MPA Media Publication) It is helpful

in curing nocturnal emission and premature ejaculation. (MPA Media Publication).

In traditional Chinese medicine, cuttlefish bone has salty and warm properties, and is associated with the kidney, liver and stomach meridians. Its functions are to stop bleeding, harmonize the stomach and improve kidney deficiency.

Characterization of *Jhag-e-Darya* (Os Sepia)

After Identification of this drug on following description, drug was characterized on parameters of microscopic view, loss of weight on drying, density, pH, successive extraction and ash values like total ash, water insoluble ash, acid insoluble ash, sulphated ash.

Identification

Jhag-e-Darya, cuttlefish bone is 1 to 3 inches in width and 5 to 10 inches in length. The skeleton is an oblong, elliptical or oval substance, of whitish colour. (Nadkarni, 1979) Identification is very easy due to its characteristic shape. Cuttlebone is flat, broad and oval in shape, represented by phragmocone with a broader and rounded oral end, called pro-ostracum, and a narrow, pointed aboral end, called rostrum. The shell is entirely dead and composed of calcareous rather than horny matter. (Kotpal, n.a.). Difference may lie due to difference in the species of cuttlefish. But in Unani medicine, *Kaff -e-darya* represents all range of cuttlefish bone from different species from the family Sepiidae as seen in Fig. 1.



Figure No 1: It shows a specimen of *Os sepia*.

Methods, Observations and Results

Microscopic view

A part of transverse section of bulk part of the drug was viewed under compound microscope and it was seen that crystalline form is like multistoried structure on evenly distributed pillars. (Figure no. 2)

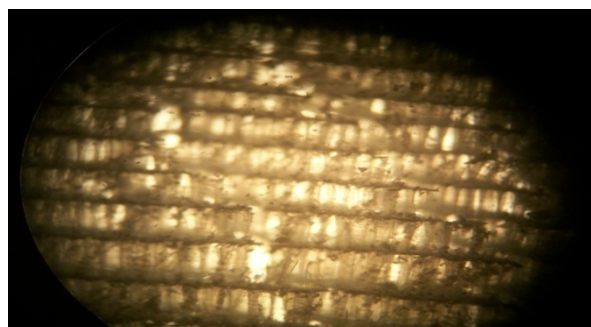


Figure No. 2 : It shows microscopic view of T.S. of part of Cuttlebone

Loss on drying / Moisture content

The drug was powdered and a sample 5g was placed on a dry Petri dish. The Petri dish along with drug was dried at 105°C for 2 hrs. in oven and weighed. The drying was continued until two successive readings matched with each other. It was found that weight of the dried drug was 4.92 g. Thus loss of weight was calculated i.e. 0.08g which represent 1.6% of crude weight of drug.

Ash Value

Controlled incineration of crude drug results in ash residue consisting of non volatile inorganic material. This value varies within fairly wide limits and is, therefore, an important parameter for the purpose of evaluation of crude drugs. The ash value determined here is the total ash, the acid insoluble ash, the water-soluble ash and the sulphated ash.

Total Ash: Crucible is weighed (C1) and then 5g of dry drug is kept in it and weighed again (C1 + D). Crucible with drug was kept in Muffle furnace at not more than 450 degree centigrade for 4 hrs. Crucible with resulting ash was cooled and weighed. Now, again crucible with ash was kept in Muffle furnace for two hours at same temperature and weighed again. The weight was found constant (C1 + A). The process was repeated for tree times. Thus, Average Total ash (%) $(92.6+96.4+92.6)/3 = 93.87\%$

Water Soluble Ash: Total Ash was calculated by the above mentioned method and 20 ml of water was put in the crucible and heated over waterbath. The resultant was filtered through ash-less filter paper and again the crucible was kept in Muffle furnace for 2 hours at 450°C. Crucible was cooled and weighed again. Total ash was 4.63g and total insoluble ash was found to be 4.61g. Thus water soluble ash is 0.02g which is 0.4% of drug taken.

Acid Insoluble Ash Total ash was calculated as mentioned. The total ash was boiled with 25 ml of 2N HCl for 5 min. The insoluble matter was collected on an ash less filter paper after filtering. The crucible with ash less filter paper and insoluble matters was again kept in Muffle furnace for 2 hrs at same temperature. The percentage of acid insoluble ash with reference to the air-dried drug was calculated. By this process it was found that in a sample of 5g drug, average of total ash was 4.63g and acid insoluble ash was found to be 0.48g which is 9.6% of drug weight taken.

Sulphated Ash To the sample of 5g drug, 10ml of 25% Sulphuric acid is added in a weighed crucible. Muffle furnace was set at 600°C. and the crucible was kept in it for 5 hours. Crucible was cooled and weighed. It was again kept in furnace for 2hr and constant weight was found. It was observed that in a drug of 5g weight sulphated ash was 6.23 which are calculated to be 124.6% of the drug.

Successive Extraction

The dried and coarsely powdered material (50 gm) was subjected to successive extraction in a Soxhlet apparatus with different solvents like petroleum ether, chloroform, methanol and water. The extracts were evaporated to dryness and their constant weights were recorded. (See table no.2)

Table No. 1: It shows values obtained in successive extraction of Cuttlebone

Wt. in Gram→ Solvent↓	Wt. of Beaker(B)	Wt. of beaker + extract	Wt. of extract	Percentage
Petroleum ether	91.82	91.83	0.01	0.02%
Chloroform	100.46	100.47	0.01	0.02%
Methanol	95.72	95.99	0.27	0.54%
Water	96.98	98.16	1.18	2.36%

Density

Density is evaluated on the formula, density = mass/ volume.

Since, the drug is porous as well as lighter than water, density calculation by immersing in water give altered result. So, drug was cut in a form of three cuboids with known dimension, thus having a definite volume and then weighed. The density in various sample was found 0.240, 0.225, 0.259 g/cm². The average of the three is taken. Thus, density of the drug is 0.241 g/cm³.

pH value

10% aq. solution was made with the help of sonicator and pH reading was taken by pH meter. Result: pH value of 10% aq. solution of cuttlefish bone is 8.6.

Discussion:

The paper constitutes distinctly two parts: Review and laboratory works. Review works meant to provide updated knowledge of cuttlebone for its medicinal uses which includes Indian traditional knowledge and recent works for its medicinal uses. Characterization of the drug was done to

further improve the knowledge about its physiochemical characteristics.

After classical literature of this drug in ancient texts, the information gets copied in literature with few changes. The changes were not of research work. However new sources of this drug in the form of different species of cuttlefish were identified by scholars in 19th century.(Gofas & Rosenberg, 2010) In second decade of 20th century, Lucius and Sayre in Sayre's-Materia-Medica: A Manual of Organic Materia Medica and Pharmacognosy updated the previous knowledge of cuttlebone with its medicinal efficacy. (Lucius & Sayre, 1917) Wealth of India and Indian Meteria Medica in sixties and seventies of previous century mentioned the drug which was authentic in Indian context (CSIR, 1962; Nadkarni, 1979). This drug gained importance due to its non-pharmacological uses which affected its marketing all over the world. (Norman & Reid, 2000; Sirilux, Ikeda, Kokubo, & Shiono, 2008; You-Zhi, et al., 2010) Modern medical sciences researches found its importance as xenograft material to be used in orthopedics (Zafer & Vildirim,

2005; Lucius & Sayre, 1917), in tissue engineering and its utility in cosmetics and surgical sutures (Sathiadhas & Aswathy, 2004). Also, scientific proving of efficacy mentioned in traditional medicine gave a new dimension in research work. (MdRizwan, Sultana, Khanna, Tanveer, & Shiffa, 2012). Thus in 21st century, cuttlebone gained its importance as a drug and further more research are expected in near future (Radhika Gopal, 2008; Cadmana et al., 2012; Rexfort & Mutterlose, 2006).

Characterization parameters were chosen in accordance with the standardization of drugs in Unani system of medicine published by Indian government authority, CCRUM. However the nature of this drug is more mineral type than inorganic type except the thin layer dried animal matter on one surface of the cuttlebone containing chitin, chitosan, amino acids etc. Moisture content of the drug had shown very minor loss in drying i.e. 1.6% as drugs available in market are already dried. Also, main content of the bone, aragonite form of CaCO_3 is not water retaining. Ash value was found to be 93.87% and value seems fairly high with respect to other drug of plant and animal origin and it is because of its high mineral content. At temperature less than 450°C , some of the CaCO_3 is get converted into Calcium Oxide and Calcium dioxide and remain in equilibrium with CaCO_3 . (Anonymous, Calcium Carbonate, 2013) Thus some weight is lost due to difference in molecular weight of calcium carbonate and

calcium oxide. Water soluble ash was found to be 0.4% as solubility of calcium oxide and calcium carbonate is very low at room temperature i.e. 0.119 gm/L (25°C) and 0.0013 g/100 ml respectively. (Anonymous, Calcium Carbonate, 2013) Acid insoluble ash was found to be 9.6% which mean ash is very soluble in acid and this result confer physio-chemical activities of both calcium carbonate and calcium oxide (Anonymous, Calcium Carbonate, 2013). Results of sulphated ash were astonishing at first as the ash value was 124.6%. But reactions of these two compounds in sulphuric acid easily define the increase in weight due to attachment of sulphate anion with calcium.

Results of successive extraction of the drug with chloroform, petroleum ether, methanol and water show low values i.e. 0.02%, 0.02%, 0.54%, and 2.36%. It is owing to less organic part in cuttlebone.

Density calculated had shown vast difference with that of shown above in literature review. This is due to difference in type of aragonite. Also I prefer to mention my work as correct in spite of some working loopholes because the drug floats in water showing its density is less than water. The pH of 10% aqueous solution was found with the help of pH meter is 8.6 which is near to standard Acidity (pKa value) 9.0 (Anonymous, Calcium Carbonate, 2013).

Conclusion

Cuttlebone, earlier used in traditional medicine in India loose importance in 20th

century but now lots of research work are being carried out to establish its pharmacological effects and non-pharmacological uses. Classical literature and recent advancements had established its application in GIT disorders, kidney disorders, gynecological disorder, dermatological manifestations, and wound healing processes as well as in orthopedic cases. Results of characterization showed more of its value relating its mineral content as calcium carbonate rather than showing characteristics of typical drug of animal origin.

Summary

Cuttlebone, called *Jhaag-e-darya* in Unani system of Medicine had long been used traditionally as a drug in Ayurveda as well as Unani systems. Important pharmacological properties mentioned with this drug are detergent, antacid, sedative, metal absorbent, diuretic, antiemetic, digestive and anti-inflammatory. It had its effects in multiple system of the body and therefore it had found its uses in dermatological, gastrointestinal, gynecological, surgical, ophthalmological and urogenital systems of the body. Recent researches had proved their efficacy of that mentioned in the classical literature of Indian medicine as well as new therapeutic roles especially in orthopedics.

Laboratory characterization of this drug was done. It was found to lose 1.6% of its weight on drying. Total ash, water soluble ash, acid

insoluble ash, sulphated ash were found 93.87%, 0.4%, 9.6%, 124% respectively. Successive extraction was also done in petroleum ether, chloroform, Methanol and water and extraction values were 0.02%, 0.02%, 0.56%, and 2.36% respectively. Density was measured experimentally and was found to be 0.241 g/cm³. pH of the 10% aqueous solution of its powder with the help of pH-meter was 8.6

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