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Simple modus operandi to bring down microbial load of herbal drugs to Pharmacopoeial limit - A study on ingredients of *Hutabhugadi curna*

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Abstract

Limit for microbial load in raw drugs intended for preparation of medicines for internal or external use has been prescribed by World Health Organization (WHO). In present study, *Hutabugadi curna* as well as six raw drugs of *Hutabugadi curna*, viz. Hutabhuga (*Plumbago zeylanica* Linn.), Ajamoda (*Apium leptophyllum* (Pers.) F.V.M.ex Benth), Saindhava Lavana (Rock salt), Magadha (*Piper longum* Linn.), Marica (*Piper nigrum* Linn.), and Pathya (*Terminalia chebula* Retz.), were analysed for microbial load (total bacterial count - TBC & total yeast and mould count -TFC) as per Ayurvedic Pharmacopoeia of India (API). The total bacterial and fungal count of the raw sample as such was found to be above the limits. Ingredients when washed with either water at 60 ºC or 50% ethanol, the microbial limit was found to be in compliance with API. The study gave an experimental evidence to show that a simple surface wash of ingredients would be sufficient to bring the high content of microbial load to the prescribed limit.

Keywords: Herbal formulations, *Hutabugadi curna*, Microbial limit, Surface swabbing.

Introduction

Nowadays herbal medicines are preferred over the modern medicines since there are number of advantages of herbal drugs over pharmaceutical products such as lower cost, availability, immunity, lesser side effects. The medicinal plants and their parts such as root, stem, seed, fruit etc are used as such on commercial scale for drug formulation are called crude drugs or raw material. The herbal drugs are commonly used as single drug or as ingredient of herbal formulations.

Apart from the great beneficial aspects of herbal drugs they will effect when contaminated with pathogenic microorganism. Medicinal plants are associated with a broad variety of microbial contaminants mainly bacteria and fungi. Also, broad diversity of bacterial, fungal cells and viruses can be found either in or on the plant material. Among micro-organisms, occurrence of pathogens particularly limits the use of these plants.[1] The practices of harvesting, handling, production and storage cause additional contamination and microbial growth. Aflatoxin, even in very small amounts, released into the plant materials by fungal growth can cause health hazards. Various conventional methods of sterilization and reduction of microbial loads are used; the

major methods are fumigation with gaseous ethylene oxide, or propylene oxide, and application of steam.[2] These methods are, however, recognized as less safe, and are now prohibited or being increasingly restricted in most countries. Therefore, microbial load should be determined after using a suitable cleaning procedure. In the present investigation standard procedure is determined to establish experimental evidence for methods to abolish microbial load in herbal drugs and their formulations. The study was done on six ingredients of *Hutabhugadi curna* (Table 1) mentioned in Ayurvedic Formulary of India. The formulation is used in the treatment of Agnimandya (digestive impairment), pandu (anemia), sopha (oedema) and arsa (piles).[3] The effect of washing on five of six ingridients (except Saindhava lavana) of the formulation using either water at 60 ºC or 50% ethanol on microbial load was documented in comparison to no washing.

Table 1: Details of raw materials used

S. No.	Commercial Name	Botanical / Chemical Name	Part taken for study
1.	Citraka	*Plumbago zeylanica* Linn.	Root
2.	Ajamoda	*Apium leptophyllum* (Pers.) F.V.M.ex Benth	Fruit
3.	Saindhava lavana	Rock salt	-
4.	Pippali	*Piper longum* Linn.	Fruit
5.	Marica	*Piper nigrum* Linn.	Fruit
6.	Haritaki	*Terminalia chebula* Retz.	Fruit (pericarp)

Materials and methods

Collection and identification of plant samples

Authenticated samples required for the study were collected from the raw drug section of SDM Ayurveda pharmacy; Udupi .The samples were stored in clean and dry zip lock polythene covers until the initiation of the study. Voucher specimens of the plants (No. SDM/UGC-MRP/HC/01-06) have been deposited in the crude drug museum of Pharmacognosy department of SDMCRAAS, Udupi.

Sample preparation

Three sets of raw drugs were taken; the first set of samples were powdered directly, surface of the second and third set of raw drugs were washed by dipping in 60 ºC warm water and 50% ethanol for one minute respectively. Adhering soils and other unwanted materials were removed by rubbing the surface during the dipping. Washed raw drugs, other than rock salt, were dried and ground into fine particles under aseptic conditions and were stored in sterile containers until analysis. *Hutabhugadi curna* was prepared as per standard operating procedure for curnas.[4]

Determination of microbial contamination

For the evaluation of microbial load pour plate technique was employed. This is performed to quantitatively estimate the viable microorganisms present in the sample. Bacteria and Fungi present in the sample are determined using two different media. The sample as such and the sample after washing the external surface with water at 60 ºC and 50% ethanol were evaluated for the quantity of total bacteria and fungi.[5]

Media use

Soya bean digest agar (SBA) was used for isolation of bacteria, Sabouraud dextrose agar (SDA) was used for isolation of fungi and Buffered Sodium chloride – peptone solution (SPS) was used for serial dilution.

1 g of the three sets of samples was mixed with 10 ml of sterile SPS to make dilution 10^{-1}. 1 ml from 10^{-1} dilution is transferred to 9 ml of sterile SPS to make a dilution of 10^{-2}. Similarly dilutions till 10-4 are carried out. A petri dish of measurement of 10 cm in diameter is taken and 1 ml inoculam of each dilution was introduced into sterile petri dishes. Molten media cooled to 45ºC is poured into petri dishes containing the specified amount of sample. Following the addition of molten and cooled agar, the cover is replaced and the plate is gently rotated in a circular motion to achieve uniform distribution of the sample. This is left undisturbed till the media solidified. The petri dishes were transferred to the BOD incubator. Plates with SBA were incubated at 30˚C for 5 days for bacterial count and plates with SDA were incubated at 25˚C for 5 days for fungal count. The developed microbial colonies were counted using digital colony counter and

computed as colony forming units per gram (CFU/g) of plant material.

Results and discussion

The total fungal and bacterial load was counted for the *Hutabugadi curna* as well as the raw materials of *Hutabugadi curna*. The total bacterial count of the raw sample as such was found to be above the limits. Surfacial washing of the ingredients using water at 60 ºC and 50% ethanol was found to bring the microbial load of all the ingredients to the limit prescribed in API. Wash with water at 60 ºC was found to bring down / nullify the total bacterial count (TBC) in Citraka, Ajamoda and Haritaki. However, Saindhava lavana showed the TBC within the limit without any washing operations. The TBC was high in Marica, hence the analysis was done at 10-5 dilution and the microbial load after the treatment was within the limit. The total fungal count (TFC) was found to be within the limit for all the ingredients without any washing operations except for Citraka. The TFC in Citraka was found to reduce to API limit either by washing with water at 60 ºC or 50% ethanol (Table 2).

The ingredients which had the microbial limit in compliance with API were chosen for preparation of *Hutabhugadi curna*. The final formulation of *Hutabhugadi curna* prepared from the ingredients showed total bacterial count within the limit and fungal count was found to be nil.

Table 2: Microbial limit in ingredients of *Hutabhugadi curna*

Sl. No.	Name of the sample	Dilution	Total bacterial count			API limit (CFU/g)	Total fungal count			API limit (CFU/g)
			Direct	60 ºC warm water	50%Ethanol		Direct	60 ºC warm water	50%Ethanol	
1.	Citraka	1/10000 (10^{-4})	INC[1] INC[2]	01[1] 02[2]	INC[1] INC[2]	-	INC[1] INC[2]	0[1] 0[2]	0[1] 0[2]	10^5
2.	Ajamoda	1/10000 (10^{-4})	INC[1] INC[2]	0[1] 0[2]	2[1] 1[1]	-	0[1] 0[2]	0[1] 0[2]	0[1] 0[2]	10^5
3.	Saindhava lavana	1/10000 (10^{-4})	01[1] 02[2]	-	-	-	0[1] 0[2]	-	-	10^5
4.	Pippali	1/10000 (10^{-4})	03[1] 02[2]	2[1] 2[2]	INC[1] INC[2]	-	0[1] 0[2]	0[1] 0[2]	0[1] 0[2]	10^5
5.	Marica	1/10000 (10^{-4})	9[1] 9[2]	0[1] 0[2]	0[1] 0[2]	-	0[1] 0[2]	0[1] 0[2]	0[1] 0[2]	10^5
6.	Haritaki	1/10000 (10^{-4})	01[1] 03[2]	0[1] 0[2]	01[1] 05[2]	-	0[1] 0[2]	0[1] 0[2]	0[1] 0[2]	10^5
7.	Hutabhugadi curna	1/10000 (10^{-4})	01 01	-	-	$<10^5$	-	-	-	-
		1/10000 (10^{-3})	-	-	-	-	0[1] 0[2]	-	-	10^3

Conclusion

Microbial contamination in herbal drugs can cause health hazards. Also they yield the low quality of the drugs as a result of the activities of different microorganisms. So, bacterial and fungal contamination can be reduced by decontamination of raw drugs using proper methods prior to formulation. The present study, by taking *Hutabhugadi curna* as a test sample, revealed that medicinal plants with high microbial load when washed with either water at 60 ºC or 50% ethanol can bring down the load to Pharmacopoeial limit.

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