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MICROBIOLOGICAL ASSESSMENT OF NATURAL THERAPEUTIC HERBAL DRUGS

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ABSTRACT

Worldwide demand for therapeutic herbal and nutraceutical preparations has increased greatly in past few years, so there is a need to put strict regulations over the microbial quality of such preparations since they are consumed internally and safety is of prime concern. This investigation was designed to focus on the microbial status of herbal drugs. Herbal drugs of different pharmacies were collected from market. The samples were examined for their microbial profile. All samples were serially diluted and plated on SDA and NAM and counted cfu/ml. The most prominent fungal genera encountered were *Aspergillus, Mucor, Cladosporium, Penicillium*, etc and in case of bacteria *E.coli* and *Bacillus cereus* were prominent which was identified by 16SrRNA sequencing. These fungi are reported to have ability to produce mycotoxin like aflatoxins etc. It assessed the incidence of microflora in herbal drugs samples in market which used for direct human and pharmaceutical purpose and can be able to cause serious consequences.

Keywords: Therapeutic, Nutraceutical, Herbal drugs, Incidence, Pharmaceutical, Fungal contaminants.

INTRODUCTION

Herbal drug is a preparation of stem, bark, root, rhizome, leaves, flowers, fruits and seeds of medicinal plants, used to prevent and treat diseases of humankind. Over 8000 plant species have been reported to prepare some 25,000 formulations, to treat various ailments (6). Herbal drugs are preferred to cure diseases because of better cultural acceptability, compatibility with the human body with lesser side effects (11). Although the World Health Organization (WHO) has advocated for the integration of herbal medicinal products into the primary health

care system of developing countries (18,19), safety issues related to herbal drugs continue to be ignored by the herbalist whose methods of concocting herbal preparations for the public are usually unhygienic with the attendant microbiological hazards (17). The unscientific methods of harvesting, collection, storage of raw materials, processing and poor storage of herbal drugs, retailed in market openly in unhygienic conditions, are the main causes considered to make both, raw materials as well as herbal drugs prone to microbial infections (8). The contaminates has been reported to affect the chemical composition of the raw materials and thereby, decreases the medicinal potency of the herbal drugs (16), whereas effects of these contaminants causes several ailments of liver, kidney, nervous system, muscular, skin, respiratory organs, digestive tract, genital organs, etc. (13,14). Present study is an attempt to identify the microorganism present in herbal drugs which can be toxic to human health.

MATERIALS AND METHODS

Collection of samples

Total thirty six samples were used in this study, out of thirty six samples thirty one were of cough syrup and liver tonic and four were crude sample of dry plant i.e *Ocimum sanctum, Tinospora cordifolia, Glycyrrhiza glabra, Piper longum, Triphala*. These samples were purchased from local market of Haridwar and its adjoining places. These products were chosen according to their commercial availability and uses. Crude samples were chosen according to their presence in syrups and tonics.

pH measurement

For pH measurement, a 1:10 sample/distilled water suspension of each sample was prepared and stirred for 24 h in 200 ml beaker. The pH of the suspension was measured using an electronic pH meter (12).

Microbiological analysis

Serial dilution method was used for isolation of fungi and bacteria from herbal drugs. 1ml of each sample were transferred into test tube containing 9 ml of sterile distilled water and was then mechanically homogenized at constant speed for 15 min on electronic shaker. The sample-water suspension was allowed to stand for 10 min with intermittent shaking before being plated. Appropriate tenfold serial dilutions (1:10) were prepared and 1 ml aliquot of each dilution was aseptically surface plated and distributed uniformly on culture medium with the help of sterilized L-shaped glass spreader. For microbiota analysis, freshly prepared

Sabouraud Dextrose Agar (SDA) medium for fungi and nutrient agar for bacteria were used; three replicates were used for each sample. The plates were incubated at 28±2°C for 7 days for fungi and 35±2 for 24 Hrs for bacteria and examined daily. The counts were recorded only after 4–5 days for fungi. (3). After incubation, the plates were examined visually and under a microscope. Identification of bacteria was done by 16SrRNA sequencing and fungal species was done by culture and morphological characteristics with the help of Barnett (4).

The percent relative density of different fungi on the samples was calculated (1)

$$\textbf{Relative density \%} = \frac{\textbf{No: of colony of the fungus}}{\textbf{Total no. of colonies of all fungal species}} \times 100$$

Percent frequency of occurrence of mycobiota on individual raw materials of herbal drug samples was determined following (12).

$$\textbf{Occurrence frequency} = \frac{\textbf{No: of fungal isolates on a drug sample}}{\textbf{Total no. of fungal isolates on all drug samples}} \times 100$$

RESULTS

Out of thirty six samples, twenty five were contaminated with fungi and twenty were contaminated with bacteria **(Table.1**). Incase of Bacteria ten samples were contaminated with *Bacillus cereus (*27.7%), seven samples were contaminated with *E.coli* (19.4%), and six were contaminated with *Staphylococcus aureus* (16.6%). *Bacillus cereus* was found to be most dominant bacteria followed by *E.coli.* (**Fig2**)

Maximum number of isolates was recorded of *A. flavus* followed by *Mucor* followed by *Penicillium.* The genus *Aspergillus* was found to be the most dominant genus encountered, with three species viz. *A. flavus, A. niger*, and *A. oracheus.* Relative density value (%) was estimated to determine the abundance of fungal isolates of given species among all samples. The highest percentage relative density was shown by *A.* flavus (22%), followed by *Mucor* (18.4%). The lowest relative density was recorded of *Trichoderma* sp. (.6%). (**Fig.1**) The hydrogen ion concentration (pH) of samples falls in the magnitude of acidic condition. In all the samples the pH ranged from 3.5- 6.8 pH.

Table 1: Microbial contaminants in herbal drugs

Sample type	Sample code	Bacteria isolated	Fungi isolated
COUGH SYRUP	A1	Nil	*A.flavus,* *Cladosporium*
	A2	Nil	*A.sp,* *Penicillium,* *Mucor*
	A3	Nil	*Mucor,* *Helminthosporium,* *Penicillium*
	A4	Nil	*Actinomycetes*
	A5	*a)Bacillus*	*Penicillium,* *Mucor,* *Fusarium,* *Trichoderma*
	A6	Nil	*Mucor*
	A7	*Bacillus*	*A.sp,* *Penicillium*
	A8	*b) E.coli*	*a) A.sp,* *b) Penicillium,* *c)Mucor,* *d)Fusarium*
	A9	*a) E.coli*	*a) Gliocladium,* *b)A.flavus,* *c) Penicillium*
	A10	*Staphylococcus aureus*	Nil
	A11	Nil	Nil
	A12	*Staphylococcus aureus*	Nil
	A13	*Klebsiella pneumoniae*	Nil
	A14	NIL	Sterile mycelium
	A15	*a) E.coli*	*a) Mucor,* *b) Helminthosporium,* *c)Penicillium,*

			d)Fusarium
	A16	Nil	*a)Trichoderma* *b)Cladosporium*
	A17	*E.coli,* *Bacillus cereus*	*a) Actinomycetes* *b)A.flavus,* *c)A.niger*
	A18	*E.coli,* *Bacillus subtilis*	Nil
	A19	Nil	Nil
LIVER TONIC	B1	Nil	*a)Penicillium,* *b)Fusarium,* *c)Cladosporium*
	B2	*a) E.coli*	*a)Actinomycetes*
	B3	*a) Bacillus cereus*	*a)A.flavus,* *b)A.niger,* *c)A.orachaeus*
	B4	*Staphlococcus aureus*	*A.flavus,* *A.niger,* *A.fumigatus,* *Penicillium*
	B5	*Bacillus*	*Mucor,* *A.fumigatus,* *Cladosporium,* *Fusarium*
	B6	*Bacillus*	*A.flavus,* *A.fumigatus,* *A.orachaeus,* *Helminthosporium*
	B7	*a) S.aureus*	*a) Actinomycetes*
	B8	*S.aureus,* *Bacillus*	Nil
	B9	*Bacillus*	Nil
	B10	Nil	Nil

	B11	Nil	*A.flavus*
	B12	*E. coil*	Nil
CRUDE SAMPLE	C1	*Bacillus cereus*	*Mucor,* *A.flavus*
	C3	*a) E.coli,* *b) Staphylococcus aureus*	*a) Mucor,* *b) Penicilluim*
	C3	*Bacillus cereus*	*Mucor*
	C4	*Bacillus cereus*	*Penicillium,* *A.flavus*
	C5	*a) E.coli*	*a) Mucor,* *b) Penicilluim, ,* *c) A.flavus*

- *A > Aspergillus*

S > Staphylococcus

E > Escherichia

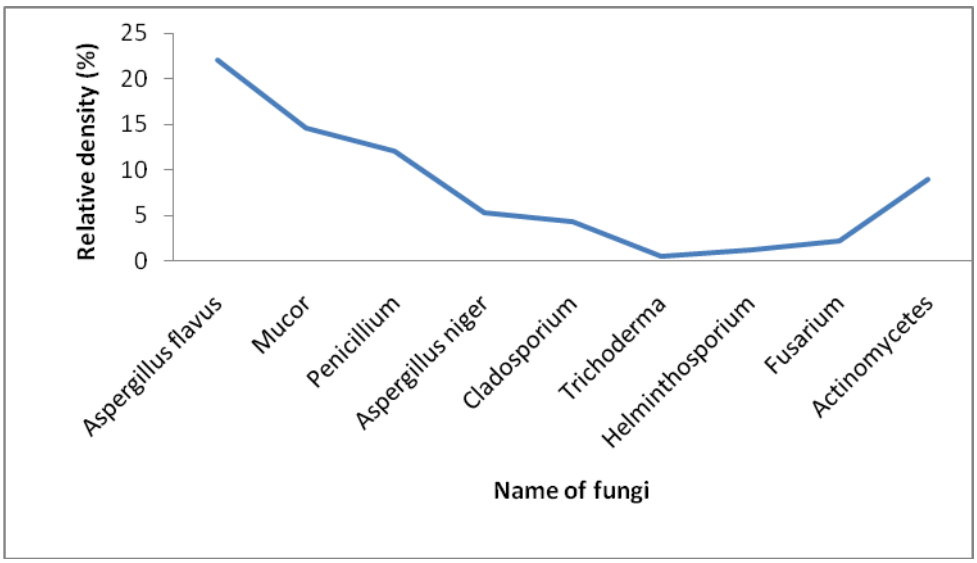

Fig 1: **Presence of Fungi in herbal drugs**

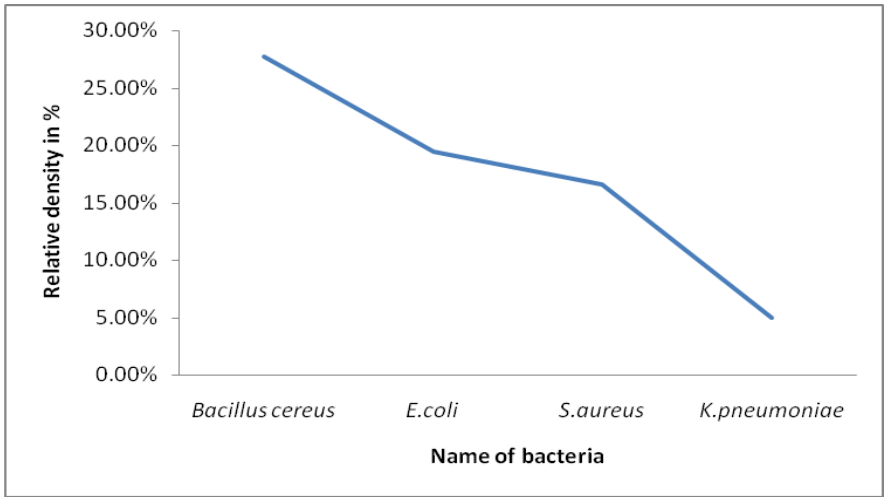

Fig 2: Presence of Bacteria in herbal drugs

DISCUSSION

The presence of mycotoxin producing fungal species of *Aspergillus, Penicillium, Mucor, Helminthosporium,* in herbal drugs revealed that these herbal syrups and tonic are not acceptable for human consumption. Species of *Aspergillu*s and *Penicillium* dominate the mycoflora of collected powdered samples of *E. officinalis, T.bellirica and T. Chebula*, were already reported as dominating mycobiota of stored herbal drugs (3, 4, 10). Presence of Aflatoxins is also a matter of great concern because once the drugs are contaminated with aflatoxin they are not fit for use. Because, even routine boiling will also not be able to detoxify them since aflatoxins have been reported to be heat stable up to,269°C (9). The presence of *A. fumigatus* was also found in the powdered herbal drugs analysed in present study. Hence the presence of wide range of fungi in these medicinally important herbal drugs showed that there is a potential risk for mycotoxins contamination, especially during prolonged storage in poor conditions without temperature and moisture control (5, 7, 16). Isolation of *E.coli* and *Bacillus cereus* in these products indicates public health risk and adverse effect on health and spoilage potential of these contaminants highlighted the need to reduce the degree of contamination of such products by establishing official guidelines such as GMP.

CONCLUSION

High no. of these bacteria and fungi in these samples suggest that these organisms become resistant to preservatives added. So we should add those compounds which have antifungal and antibacterial activity it may be a plant extract which have both quality. There is a need for constant monitoring and control of quality of the herbal medicine product manufactured,

sold, advertised and used. There should be an adequate standard and specification for HMPs being sold to public. The persons who are involved in harvesting, storage, processing and post processed storage of these herbal drugs are required to take precautions. Hawkers of herbal medicinal products also contribute immensely to the poor quality of the products, due to their poor and hygienic practices. Hawking of herbal medicines should be dissuaded and only thoroughly monitored herbal products (i.e. those whose microbiological, phytochemical, and physiochemical qualities are government or NAFDAC approved should be allowed for sale to the public (1083).

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REFERENCES

1. Agrawal G.P., Thakur M.K., Awasthi S. Studies on the wheat grain storage on Madhya Pradesh "fungi associated with different varieties of freshly harvested wheat grains". Nat Acad Sci Lett, 1980; 3:195–197
2. Aryes, G.I., Mund, T.I., Sondin, E.W.. Microbiology of food spices and condiments. A series of books in food and nutrition Edn. Schmeigert, 1980, pp- 249.
3. Aziz NH, Youssef YA, EL-Fouly MZ, Moussa LA Contamination of some common medicinal plant samples and species by fungi and their mycotoxins. Bot Bull Acad Sinica, 1998; 39:279–285.
4. Barnet, H. L. & Hunter, Barry B. Illustrated Genera of Imperfect Fungi. 4th Edition, Macmillan Publishing coy, New York, Collier Macmillan Publishers, London, 1987.
5. Bugno, A., Adriana, A.B.A., Tatiana, C.P., Terezinha, A.P.,Myrna, S. Occurence of toxigenic fungi in herbal drugs. Brazillian Journal of Microbiology, 2006; 37: 47-51.
6. Dubey, N. K., Kumar R. and Tripathi, P. Global promotion of herbal medicine: India's opportunity. Current Science, 2004; 86: 37- 41.
7. Efuntoye, M.O. Fungi associated with herbal drug plants during storage. Mycopathologia, 2004; 136: 115-118.
8. Essono, G., Ayodele, M., Akoa, A., Foko, J., Olembo, S. and Gock, J.. Aspergillus species on cassava chips in storage in rural areas of southern. Cameroon: their relationship with storage duration, moisture content and processing methods. African Journal of Microbiology, 2007: 001-008.
9. Frazier, W.C. and Westhoff, D.C. Food Microbiology. 4th ed. International edition, McGraw-Hill, New York, NY,1988.

10. Hitokoto,H., Morozumi, S., Wauke, T., Saka, S., Kurata, H..Fungal contamination and mycotoxin detection of powdered herbal drugs. Applied and Environmental Microbiology, 1978; 36:252-256.
11. Kamboj, V.P.. Herbal medicines. Current Science, 2000; 78(1): 35-39.
12. Mandeel, Q.A. Fungal contamination of some imported species. Mycopathologia, 2005; 159:291–298
13. Muntanola, M. General mycology. Beograd: NIRO. Knjez evne novine, 1987; 257–269.
14. Butler, W.H. Aflatoxin. In Purchase, I.F.H., ed. Mycotoxins. Amsterdam. Elsevier scientific publishing company, 1974; 1–28.
15. Roy, A.K. Mycological problems of crude herbal drugs-Overview and Challenges. Indian Phytopathology, 2003; 56: 1–13.
16. Singh, P., Srivastav, B., Kumar, A., Dubey,N.K. Fungal contamination of raw material of some herbal drugs and recommendation of Cinnamomum camphora oil as herbal fungitoxicant. Microbial Ecology, 2008; DOI 10.1007 /s00248-008-9375-x
17. Tella A Traditional medicine: Safety and Efficacy. Niger. J. Pharm, 1977; 8: 89-90.
18. WHO ,WHO technical report series no.622,WHO Geneva,1978.
19. WHO Document No. WHO/TRM 19-1 Geneva, 1989, pp.7-9.