Research Article

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Formulation and evaluation of chaturjat churna: Containing 4 ingredients

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Abstract

Despite the growing interest in the safety of herbal medicines, national surveillance systems to monitor and evaluate adverse reactions associated with herbal medicines are rare. Moreover, there is a lack of effective communication on this subject at all levels, from international to local. A recent WHO survey showed that around 90 countries, less than half of WHO's Member States, currently regulate herbal medicines and an even smaller proportion has systems in place for the regulation/qualification of providers of herbal medicines. Moreover, there are disparities in regulation between countries and this has serious implications for international access to and distribution of such products. In this paper attempt has been made to evaluate Chaturjat churna, a polyherbal formulation consisting of 4 ingredients with specific morphological parts. The ingredients were collected from market and churna was prepared and evaluated as per WHO guidelines.

Key-Words: Evaluation, Charutjat churna, Microbial contamination, Physicochemical Evaluation and HPTLC Profile.

Introduction

Herbal medicine is the oldest form of healthcare known to mankind. Herbs had been used by all cultures throughout history. It was an integral part of the development of modern civilization. Primitive man observed and appreciated the great diversity of plants available to him. The plants provided food, clothing, shelter, and medicine. Much of the medicinal use of plants seems to have been developed through observations of wild animals, and by trial and error. As time went on, each tribe added the medicinal power of herbs in their area to its knowledgebase. They methodically collected information on herbs and developed well-defined herbal pharmacopoeias. Standardization of herbal drugs is not an easy task as numerous factors influence the bio efficacy and reproducible therapeutic effect. In order to obtain quality oriented herbal products, care should be taken right from the proper identification of plants, season and area of collection and their extraction and purification process and rationalizing the combination in case of polyherbal drugs.

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The paper present developmentof methods for the evaluation of Chaturjat churna containing *Cinnamomum zeylanicum, Elettaria cardamomum, Cinnamomum tamala* and *Tribu*lues terrestris as the main ingredient. It consists of fine powders of all ingredients. All the four ingredients of Pharmacopoeia quality were washed, dried and powdered individually & passed through sieve 85 meshes separately. Each Ingredient was weighed separately, mixed together in specified ratio & passed through sieve no 44 to obtain a homogenous Blend. [1-4]

Material and Methods

Method of Preparation of churna

All the four ingredients of Pharmacopoeia quality were washed, dried and powdered individually & passed through sieve 85 meshes separately. Each Ingredient was weighed separately, mixed together in specified ratio & passed through sieve no 44 to obtain a homogenous Blend. Finally packed in tightly closed containers to protect from light & moisture.[5-6]

Microscopy of Chaturjat Churna

About 2 g of churna taken and washed thoroughly with water, poured out the water without loss of material and mounted in glycerin ; warmed a few with chloral hydrate solution , washed and mounted in glycerin ; treated a few with iodine in potassium iodide solution and mounted in glycerin.[7]

Determination of total ash

About 2 to 3g of churna was accurately weighed in a previously ignited and treated crucible (platinum or silica dish) and incinerated at a temperature not exceeding 450^0 C until it was free from carbon, cooled and weighed.

Determination of acid insoluble ash

The total ash obtained as mentioned in the above procedure was boiled with 25ml hydrochloric acid for 5minutes, the insoluble matter was collected in an ash less filter paper, washed with hot water, ignites, cooled in desiccators and weighed. The percentage of acid insoluble ash was calculated with reference to the air-dried churna.

Determination of water soluble ash

The total ash obtained as mentioned in the above procedure was boiled with 25 ml of water for 5 minutes and filtered through an ash less filter paper no-41. It was followed by washing with hot water. The filter paper was ignited in the silica crucible, cooled and the water insoluble matter was weighted. The water soluble ash was calculated by subtracting the water insoluble matter from the total ash.

Determination of alcohol soluble extractive value

About 5g of the air dried material was macerated with 100ml alcohol of specified strength in a closed flask for 24hours, shaking frequently during the first 6hours was done and then allowed to stand for 18hours. It was then evaporated 25ml dried in tarred flat bottom shallow dish at 105^0C and residue was weighed. The percentage of alcohol soluble extractive was calculated with reference to the air dried churna.

Determination of water soluble extractive value

About 5g of the air dried material was macerated with 100ml water of specified strength in a closed flask for 24hours, shaking frequently during the first 6hours was done and then allowed to stand for 18hours. It was then evaporated 25ml dried in tarred flat bottom shallow dish at 105^0C and residue was weighed. The percentage of water soluble extractive was calculated with reference to the air dried churna.

Determination of loss on drying

About 1-2 gm of the powdered material was accurately weighed in a glass stoppered weighing bottle which is previously dried for 30 minutes in the drier. Then, the sample was gently shaken side wise for even distribution and dried in an oven at 100^0 C to 105^0 C by removing the stopper. It was cooled in a dessicator and again weighed. The loss on drying was calculated with the reference to the amount of air dried powder taken.[8-13]

HPTLC Profile

The crude drug sample extracted in Methanol (150 ml x 5) through Soxhlet apparatus was filtered & concentrated to 5-10 ml. High Performance Thin Layer Chromatography was carried out by applying 6 µl of the sample on TLC Silica gel plate 60 F_{254} (from Merck India Ltd, Germany) and developed the plate to a distance of 10 cm using Toluene : Ethyl acetate (9 : 1) as mobile phase. After derivatization the Plates were allowed to dry in Room Temperature & examined under Ultra Violet Light at 254 nm; under 366 nm; after derivatization with 5% methanollic sulphuric acid solution.[14-15]

Test For Microbial Contamination

PDA, Mac-conkey broth, XLD, Plate count agar, Citrimide agar and Staphylococcus aureus enriched agar media were used for the isolation of the yeast & mould, *E.coli*, *Salmonella sp*, TBC, *Staphylococcus sp*. & *Pseudomonas sp*. To perform this test different proportion of different media were dissolved in different flasks containing distilled water. All the media were prepared & autoclaved at 15 lb/in² pressure at 121ºc for 15-20 minutes. After autoclaving media were poured into Petri plates & allowed to solidify. Plates were prepared & marked for different isolates. Culture suspension was prepared in 1gm of test sample in 100 ml. distilled water. 0.1 ml. suspension was spreaded on the plates with the help of spreader. All plates were prepared, inoculated & incubated at 26-28 ºc for 3-4 days. After incubation period, the observations were taken for presence or absence of the colonies. The concentration of different microorganisms and media used were given.[13,16]

MPN method for *E. coli*

Broth was separately weighed into the flask & dispends it into the screw cap tube with Durham's tube inverted inside them & autoclaved. After autoclaving 1 ml. test sample was inoculated in these tubes. All tubes were incubated at 37ºc for 24 hours for gas production. After incubation observation was taken.[13&16]

Results and Conclusion

Powder microscopy of churna reveled the following cells

1. Pollen grains
2. Rosette crystals
3. Endospern fiiled with starch grains.
4. Trichomes
5. Prismatic & Rhomboidal crystals of Calcium oxalate
6. Starch Granules.
7. Oil Globules
8. Cork cells
9. Groups of stone cells
10. Paracytic stomata
11. Spiral & Scalariform thicking
12. Thick-walled parenchyma cell

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13. Fibres
14. Parenchyamatic cells filled with starch grains
15. Tannin cells
16. Elongated cells

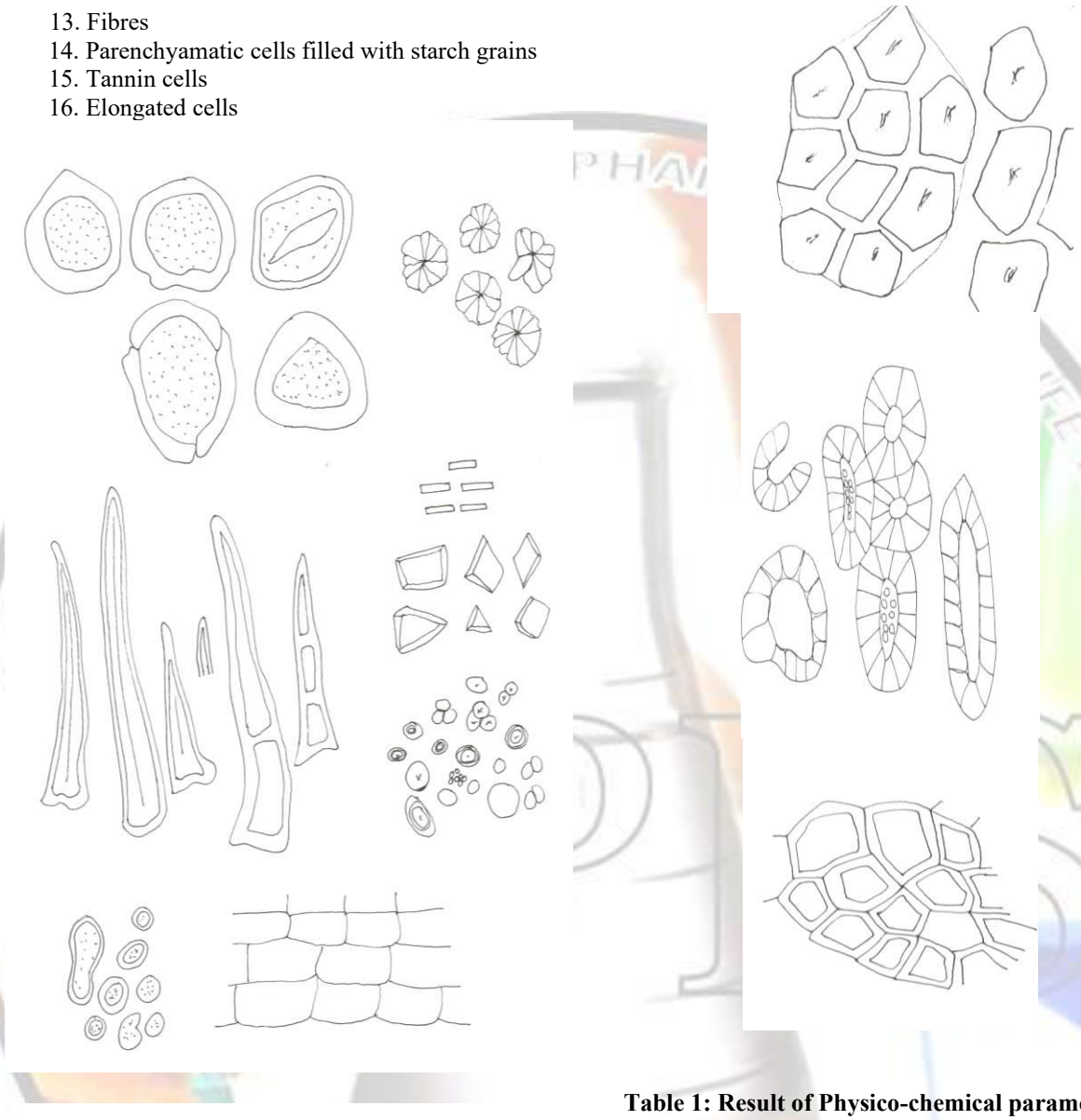

Fig. 1: Powder Microscopy of Chaturjata churna

Table 1: Result of Physico-chemical parameters in two batches of Chaturjata Churna

Parameters	Chaturjata Churna		Average value
	Batch A	Batch B	-
LOD at 105°	8..02%	8.25%	8.13
Total Ash	5.65	5.66	5.65
Acid-insoluble ash	0.29	0.31	0.3
Water-soluble extract	9.25%	9.73%	9.49
Alcohol-soluble extract	8.05%	7.95%	8.0

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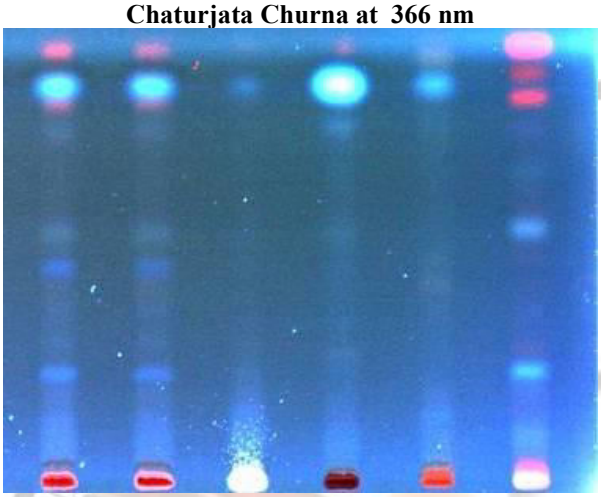
Fig. 2: TLC Finger prints of test solution of Chaturjata Churna at 366 nm



A: Batch A, **B:** Batch B,
1: *Elettaria cardamomum,* **2:** *Tribu*lues terrestris,
3: *Cinnamomum zeylanicum,* **4:** *Cinnamomum tamala*

Table 2: Different Rf values in TLC finger prints of Chaturjat Churna at 366 nm

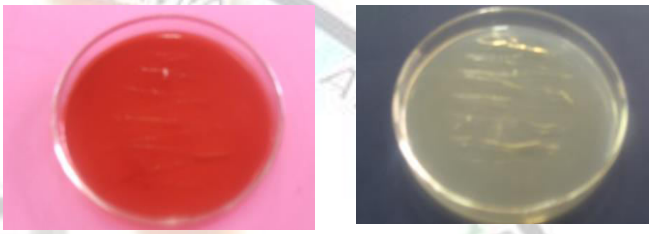
Rf	Chaturjata Churna			Single ingredients		
	A	B	1	2	3	4
R_f 1	0.24	0.24	NA*	0.24	NA*	0.24
R_f 2	0.47	0.47	NA*	NA*	0.47	
R_f 3	0.54	0.54	NA*	0.54	NA*	0.54
R_f 4	0.76	0.76	NA*	0.76	0.76	
R_f 5	0.80	0.80	NA*	NA*	NA*	0.80
R_f 6	0.84	0.84	0.84	0.84	0.84	0.84
R_f 7	0.92	0.92	NA*	0.92	0.92	0.92

*NA- Not Appeared

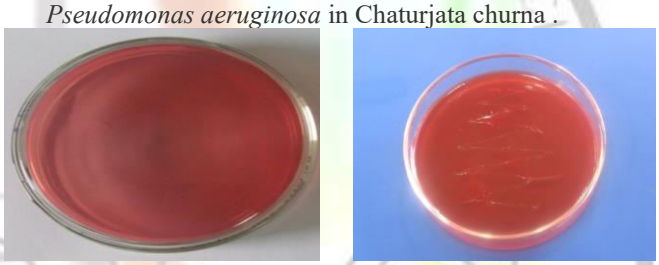
Table 3 : Determination of Microbial Load in 05 batches of Chaturjata Churna

Parameters	Chaturjata Churna		Permissible Limits API part II Append 2.4
	Batch 002A	Batch 002B	
Staphylococcus aureus /g	Absent	Absent	Absent
Salmonella spp. /g	Absent	Absent	Absent
Pseudomonas aeruginosa /g	Absent	Absent	Absent
E.coli	Absent	Absent	Absent
Total Aerobic microbial count. (AMC)	195 cfu/g	178 cfu/g	10^5 /gm
Total Yeast & mould.	18 cfu/g	14 cfu/g	10^3 /gm

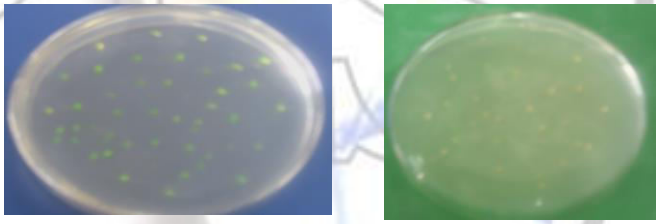
Fig. 3: Determination of Microbial Load in Chaturjata Churna



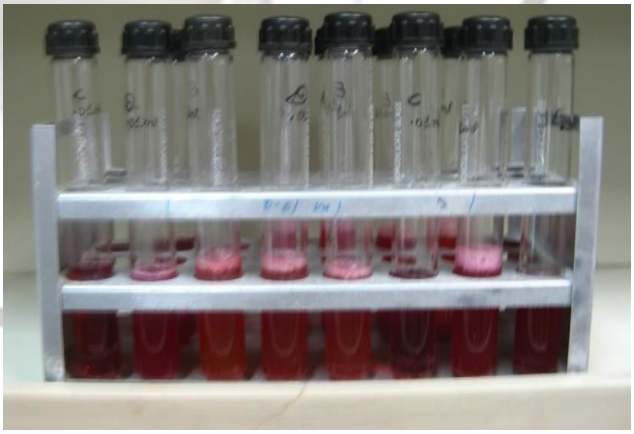
Plates- 1: Plate showing negative results for *Staphylococcus aureus* in Chaturjata churna
Plates- 2: Plate showing negative results for *Pseudomonas aeruginosa* in Chaturjata churna .



Plates- 3: Plate showing negative results for *E.coli* in
Plates- 4: Plate showing negative results for *Salmonella* in Chaturjata Chaturjata churna



Plates- 5: Plate showing Total Bacterial Counts in Chaturjata churna
Plates- 6: Plate showing yeast & Moulds in Chaturjata churna



The present study was undertaken for the appraisal of chaturjat churna as a versatile ayurvedic formulation. It has already formulated on the basis of ayurvedic formulary of India but now we evaluated its various evaluation parameter including powder microscopy, HPTLC profile and its microbial load test. Thus, from current study the importance of ayurvedic formulation is clear and doesn't have any side effect, as well as to increase the market potential of ayurvedic preparations.

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