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Preparation and quality control profiling of triphala churna

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ABSTRACT

Ayurvedic Formulation is prepared from the raw materials derived from Plant origin, mineral origin, metal, animal or a marine origin. Most of the traditional system of medicine is effective but they required standardization. Standardization of traditional medicine is essential in order to assess their quality, purity, safety & efficacy. It is majorly used in the treatment of eye disorders, oral ulcers, liver disorders, edema and inflammation. In the present study preparation of *Triphala Churna* was carried out and then it was subjected to various quality control parameters. The formulation was prepared as per the guidelines mentioned in pharmacopeia & various tests performed were physical properties (such as moisture content), biochemical test, ash value, HPLC, IR spectroscopy. The above parameters can be used as preliminary standardization for quality control of *Triphala Churna*.

Keywords: Standardization, herbal formulation, Triphala Churna, Physicochemical properties.

INTRODUCTION

Every science and scientific system has its pros and cons, but generally we are unaware about the negative aspect of certain systems and these systems are always projected as the best, which is not even near the truth.Many ayurvedic drugs have been found far superior to their allopathic counterparts. Ayurveda is completely natural and it believes that to follow the nature is the only way to achieve the complete wellness.Allopathic system, though faster, has lots of side effect and disease is not completely eliminated. It is only used to treat the symptoms not the cause. There are many cases where disease comes back after some duration of time. Ayurveda is slow process but it cures the cause and symptoms ultimately are eliminated. The recent emerging trends have made people more inclined towards natural way of living and holistic approaches to maintain the health [1-5].

Standardization of drugs means confirmation of its identity, determination of its quality, purity and detection of nature of adulterant by various parameters like morphological, microscopical, physical, chemical and biological evaluations.The propagation and acceptance of ASU (Ayurveda, Siddha & Unani) particularly Ayurveda, is increasing.*Triphala Churna* is a herbal formulation used extensively as treatment of It is used in the treatment of eye disorders with inflammation, wherein it is used to wash the eyes.It is used to treat oral ulcers, wherein it is used for gargling.Oral intake is indicated in liver disorders, edema and inflammation.It is used along with cow urine cow urine to treat testicular disorders.It Balances vatta and kapha.Churna can be defined as a dried powdered mixture of one or multiple herbs.*Triphala Churna* is prepared from three herbs Amlaki (*Embilica officinalis*), Haritaki (*Terminalia chebula*) and Bibhitaki (*Terminalia bellerica*).

The Pharmacopeial standards in Ayurvedic, Siddha and Unani are not adequate enough to ensure the quality of formulations. Analysis of marker compounds is necessary to maintain the quality and identity of the formulations. In order to assess the quality of inhouse formulation, it was prepared at laboratory scale as per pharmacopoeial standards and it was subjected to various quality control tests [6-10].

MATERIALS AND METHODS

Raw Materials, Chemicals and Reagents

Plant Raw materials used for the preparation of *Triphala Churna* were procured Ayurvedic Proprietory Medicines Shop (Mumbai) with the knowledge of Ayurvedic physician. The materials were dried in an oven preset at 45°C, powdered, sieved through an 85-mesh (BSS) sieve and stored in air tight containers.

The Gallic Acid standard was procured from Himedia and Assigned purity: 98%.

Preparation of Triphala Churna

Raw materials complying the pharmacopoeial quality and quantity were subjected to the preparation of Triphala Churna as per the composition [Table 1]. All the prepared powders amlaki , haritaki and bibhitaki were mixed thoroughly as per the standard protocol and stored in air tight container.

| | Table 1. Formulation composition | | | | | | |
|--------|----------------------------------|-------------------------|--------------------|--|--|--|--|
| S. No. | Ayurvedic name | Botanical /English name | lish name Quantity | | | | |
| 1 | Amlaki | Embilica officinalis | 1 parts | | | | |
| 2 | Haritaki | Terminalia chebula | 1 parts | | | | |
| 3 | Bibhitaki | Terminalia bellerica | 1 parts | | | | |

Table 1: Formulation composition

Quality Evaluation of Triphala Churna

Organoleptic evaluation

The formulation was studied for its preliminary characters like colour,texture,odour and taste.

Preliminary Phytochemical and Biochemical Evaluation

Phytochemical screening of some major secondary metabolites (Flavonoids, Tannins, Alkaloides, Glycosides, Terpenoids, Steroids, Phlobatannin, Phenolic Compounds and Saponins) and Biochemical for Carbohydrates ,Proteins and Fats in Triphala Churna was carried out by performing preliminary colour based tests.

Physicochemical Evaluation

The prepared formulation was subjected for physical studies like Bulk density, Tap Density, Compressibility Index, Housner Ratio and Ash Value.

Chromatographic Evaluation

Preparation of Standard

Gallic Acid standard was prepared in methanol with initial concentration of 1000 ppm.Further dilution of 100 ppm was prepared using mobile phases.

Preparation of Sample

All the raw materials and prepared formulation powders were dissolved in Methanol and kept overnight.Next day al the solutions were filtered through whattman filter paper to obtain clear extracts.

HighPerformanceThinLayerChromatography (HPTLC)Fingerprinting

10 μ l of the filtered solution of formulation extract and standard was applied on the HPTLC plate as per conditions mentioned in table 1a followed by development, derivatizing with vanillin sulphuric acid agent and scanning at 513 nm.

| Stationary Phase | HPTLC plates silica gel 60 F 254 | | | |
|---------------------|--|--|--|--|
| Plate size | 10.0x10.0 cm | | | |
| Mobile Phase | Ethyl Acetate : Methanol : water (40.48 : 5.46 : 4.04) | | | |
| Saturation Time | 20 min. | | | |
| Standard Used | 100 ppm Gallic Acid | | | |
| Spot Volume | 10 µl | | | |
| Band Length | 8.0mm | | | |
| Solvent Front | 80mm | | | |
| Wavelength and Lamp | 366nm & Mercury lamp | | | |
| Sample Applicator | CAMAG Linomat 5 | | | |
| Sample Detection | CAMAG Visualizer : 200480 | | | |
| Number of Tracks | 5 | | | |

| Table 1a :Chromatographic Conditions for | HPTLC |
|--|-------|
|--|-------|

High Performance Liquid Chromatography (HPLC) evaluation

HPLC was also performed to find out the gallic acid content in prepared formulation as per conditions mentioned in table 1b.

| Table 1b :Chromatographic Conditions for HPLC | | | | | |
|---|--|--|--|--|--|
| Mobile phase | Acetonitrile : water (20:80) [pH 3 by ortho phosphoric acid] | | | | |
| Stationary Phase | $C_{18} (4.6 \times 250 \text{ mm}, 5 \mu\text{m}).$ | | | | |
| Flow rate | 1 ml/min | | | | |
| Injection volume | 20 µl | | | | |
| Detection | UV at 272nm | | | | |

RESULTS AND DISCUSSION

Triphala churna was prepared in the laboratory as given in standard Ayurvedic literature. The observed results clearly indicates good quality of Triphala churna. The organoleptic characters (table help to indentify the formulation from its 2) external appearance. Studies on physicochemical constants (table 3) can provide valuable source of information and suitable standards to determine the quality of the formulation. Phytochemical evaluation helped to understand the presence of various therapeutically active constituents in Triphala Churna. It found that was tannins, Steroides, saponins and phenolic compounds were present (table 4). The presence and absence of these phytoconstituents in particular formulation depends upon raw materials present into it and the procedure used for ots preparation.Biochemical tests were also performed to check presence of nutritives like Carbohydrates ,proteins ,Fats and Starch.The tests were positive

for all except Fats(table 5). These Phytochemical and Biochemical tests are important to obtain preliminary information on the quality. According to Mohan et al. different chemical compounds detected in whole plant extracts could make the plant useful for treating different ailments as having a potential of providing useful drugs of human use.

The prepared formulation was then assessed for its quality by checking the presence of marker compound Gallic acid by hyphenated techniques like HPTLC and HPLC. HPTLC fingerprinting and HPLC both are very useful techniques to check the presence or to confirm raw materials in formulations. For monitoring quality ,one can visualize the presence of various plant chemical constituents in raw materials as well as formulation , out of these a marker compound can serve as a characteristic fingerprint for that formulation (Fig 1 and 2).

| | Table 2 :Organoleptic Characters | | | | |
|-------------|----------------------------------|------------------|--|--|--|
| Sr. | No. Characters | Triphala Churna | | | |
| 1 | Colour | Burdywood | | | |
| 2 | Taste | No Specific | | | |
| 3 | Texture | powder | | | |
| 4 | Odour | No Specific | | | |
|] | Table 3 : Physicoche | mical evaluation | | | |
| Sr. No. | Parameters | Triphala Churna | | | |
| 1 | Bulk Density | 0.476gm/ml | | | |
| 2 | Tap Density | 0.644gm/ml | | | |
| 3 | Hausner Ratio | 1.352 gm/ml | | | |
| 4 | Compressibility In | dex 16.8 % | | | |
| 5 Total Ash | | 0.049 | | | |

| Table 4 : Phytochemical Evaluation | | | | | |
|------------------------------------|--|-------------------------|---------|--|--|
| SR NO. | TESTS | OBSERVATION | RESULTS | | |
| 1 | Tannin: | Brownish green or Blue | + | | |
| | 1ml Aq. Extract + 0.1% FeCl ₃ dropwise | black colour | | | |
| 2 | Alkaloids: | Yellow ppt | - | | |
| | 1ml Alc. Extract + 1ml conc. HCl + Hager's | | | | |
| | Reagent | | | | |
| 3 | Glycosides: | Brown Ring | - | | |
| | 1ml extract + 0.5ml Glacial Acetic acid + few drops of | | | | |
| | Dil. FeCl ₃ till colourless + 1ml Dil. H ₂ SO ₄ | | | | |
| 4 | Flavonoids: | Yellow colour disappear | - | | |
| | 1ml extract+ 1ml Dil. ammonia solution + | | | | |
| | Conc. H ₂ SO ₄ | | | | |
| 5 | Steroids: | | + | | |
| | 1 ml extract + 1 ml chloroform + Conc H ₂ SO ₄ | Red colour after stand | | | |
| 6. | Phlobatannin: | Ppt present | - | | |
| | 0.5ml aq. Extract+ Boil with 1ml 1% HCl | | | | |
| 7. | Phenolic Compounds: | Violet colourppt | + | | |
| | $1 \text{ ml extract} + \text{dropwise} \text{FeCl}_3$ | | | | |
| 8. | Saponin: | Froth | + | | |
| | 1ml extract + Few drops of olive oil+ Shake | | | | |
| | vigorously | | | | |
| 9. | Terpenoids: | Yellow colour | - | | |

Key : + positive, - Negative

1ml extract +0.5ml CHCl₃+ 1ml Conc. H₂SO₄

| | Tests Observation Carbohydrate: Blue Colour + | | | | | |
|-----|--|--------------------------|---------|--|--|--|
| Sr | Tests | Observation | | | | |
| no. | | | Results | | | |
| 1. | Carbohydrate: | Blue Colour | + | | | |
| | 1ml extract + 1ml Fehling A + 1ml Fehling B | | | | | |
| 2. | Proteins: | Violet or pink colour | + | | | |
| | 1ml extract + 1ml 4% NaOH + few drops 1% CuSO ₄ | | | | | |
| 3. | Fats and Fixed oils: | Formation of froth and | | | | |
| | 1ml extract + 1ml KOH + 2drops of phenolphthalein + | neutralisation of alkali | - | | | |
| | heat for 15mins on water bath | | | | | |

Table 5 : Biochemical Evaluation

4 Starch: 1ml extract + iodine

Blue colour

+



Solvent front : 6.5 cm

| Track No. | Sample Name |
|-----------|-------------|
| 1 | Haritaki |
| 2 | Bibhitaki |
| 3 | Amalaki |
| 4 | Formulation |
| 5 | Gallic Acid |



| # | Peak Name | CH | tR [min] | Area [µV·sec] | Height [µV] | Area% | Height% | Quantity | NTP | Resolution |
|-----|-------------|----|----------|---------------|-------------|--------|---------|----------|-------|------------|
| 1 | Unknown | 1 | 2.742 | 1658266 | 197285 | 59.781 | 53.498 | N/A | 5918 | N/A |
| 2 | Unknown | 1 | 3.333 | 89907 | 7637 | 3.241 | 2.071 | N/A | N/A | N/A |
| 3 | gallic acid | 1 | 3.758 | 707948 | 135636 | 25.522 | 36.780 | N/A | 13821 | 2.973 |
| - 4 | Unknown | 1 | 4.242 | 128676 | 15938 | 4.639 | 4.322 | N/A | 7329 | 1.973 |
| - 5 | Unknown | 1 | 4.800 | 24790 | 2013 | 0.894 | 0.546 | N/A | 2705 | 3.917 |
| 6 | Unknown | 1 | 6.400 | 110416 | 6003 | 3.981 | 1.628 | N/A | 3237 | 3.561 |
| 7 | Unknown | 1 | 7.675 | 30248 | 3043 | 1.090 | 0.825 | N/A | 13124 | 1.403 |
| - 8 | Unknown | 1 | 8.300 | 23637 | 1218 | 0.852 | 0.330 | N/A | 2816 | N/A |

CONCLUSION

Quality control parameters are of key importance if traditional medicines are to be given credibility as modern medicine has. In order to have consisitancy and uniformity in the production of these medicines on large scale, there is a need to set a standard protocol for preparation and for assesment of quality, efficacy. Ayurvedic formulation Triphala Churna was prepared as

described in classical texts and it has been assessed for its quality by intervention of modern scientific quality control measures in the traditional preparation. The quality profile obtained from the present study for the formulation could be employed for evaluating its identity and can be used for routine analysis. It can be concluded that this profile might be helpful in establishing standardization of the formulation.

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