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PHARMACOGNOSTICAL AND PHYTO-CHEMICAL STANDARDIZATION OF PATHADI CHOORNA- A POLYHERBAL FORMULATION FOR POLYCYSTIC OVARIAN SYNDROME

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ABSTRACT

Introduction: Ayurveda has become significantly more popular all over the world because of the effective and curative nature for chronic disease with less toxicity. Most of the Ayurvedic drugs are very effective but lack standardisation. Standardization of drugs means confirmation of its identity and determination of its quality and purity. *Pathadi choorna* was described by Acharya Susruta in the context of *Granthi artavadushti* as a kwatha formulation. Polycystic Ovarian Syndrome (PCOS) can be compared with *granthi artavadushti* due to the *grathila* (polycystic) appearance of ovaries along with abnormal uterine bleeding in the form of Oligomenorrhoea or a phase of amenorrhoea followed by severe bleeding with clots. The Polycystic Ovarian Syndrome (PCOS) is a syndrome of ovarian dysfunction along with the cardinal features of Hyperandrogenism and polycystic ovarian morphology. *Pathadi choorna* was selected as the trial drug for polycystic ovarian Syndrome. **Aim:** To develop the pharmacognostical and phytochemical profile of *Pathadi choorna.* **Material and Methods:** *Pathadi choorna* was prepared as per classical methods and analytical findings were systematically recorded. The samples were subjected to organoleptic analysis,

physicochemical analysis and HPTLC examination by optimizing the solvent systems. **Results and Conclusions:** Pharmacognostical profile of *Pathadi choorna* was established. The presence of prismatic crystals, spool cells, fibres, stone cells, black debris, were the characteristic features observed in the microscopy of the finished product. Physico-chemical analysis showed Loss on drying 9.24% w/w, ash value 5.54% w/w, and pH-6.5. HPTLC fingerprinting profile of *Pathadi choorna* revealed 5 spots at 254 nm and 6 spots at 366 nm.

KEYWORDS: Organoleptic, Pharmacognosy, PCOS, *Pathadi choorna*, Physio-chemical

INTRODUCTION

Ayurveda, a part of cultural heritage of India, is widely respected for its uniqueness and global acceptance as it offers natural ways to treat diseases and promote healthcare.[1] Most of the Ayurvedic drugs can be effectively used for curing different chronic diseases with fewer side effects. Standardisation is an essential part of herbal medicine to prove its quality, purity and safety. Most of the herbal medicines available in the market lack this standardisation. Standardization minimizes batch to batch variation; assure safety, efficacy, quality and acceptability of the polyherbal formulation.[2]

Polycystic Ovarian Syndrome (PCOS) is extremely prevalent and probably constitutes the most frequently encountered endocrinopathy in women of reproductive age. It is reported to affect between 5-10% of women of reproductive age.[3] PCOS is a syndrome of ovarian dysfunction along with the cardinal features of Hyperandrogenism and polycystic ovarian morphology.[4] It increases the risk of insulin resistance (IR), Type 2 Diabetes, Obesity and Cardiovascular disease. The aetiology of the disease remains unclear, however there has been evidence that shows both environmental as well as genetic factors play a role in the aetiology.[5] Stress, lack of exercise, depression, abnormal food habits have all contributed to the increase in prevalence of the disease. The indefinite diagnostic criteria in addition to its immense complexity make PCOS a challenging area of research.

Ayurveda explained mainly four etiological factors like unhealthy lifestyle (*Mithyachara)*, menstrual disorders (*Artava dushti*), genetic defects (*Beeja dosha*) and certain unknown factors (*Daivata*) are responsible for the development of female genital disorders[6] and it is seems that all these aetiologies contribute to the development of PCOS as a whole. *Mithyachara* along with existing *Artava dushti* plays an important role in the pathogenesis of PCOS. As the ultimate effect of *Artava dushti* being *abeejatva* (anovulation), diagnosis and

treatment of *artava dushti* is of most important as it hinders the main function of female genital tract. *Granthibhoota artavadushti* being said to be *krichrasadhya/asa*dhya in prognosis [7,8] and some of clinical symptoms of PCOS may simulate *granthibhoota artava dushti* and if not treated early lead to the full manifestations and complications of PCOS. *Pathadi yoga* mentioned by Acharyas in the context of *granthibhoota artavadushti chikitsa* was taken for the clinical trial in the form of powder. Standardization is needed to establish quality control parameters for each traditional drug before it is released for use without the fear of toxicity and contamination.[9] So the present work comprises of Pharmacognostical, physiochemical and HPTLC fingerprinting profile for *Pathadi choorna*.

MATERIALS AND METHODS

Collection of raw materials

Raw drugs of *Pathadi choorna* were procured from the local market and Pharmacy of Gujarat Ayurved University, Jamnagar. The ingredients were identified and authenticated at Pharmacognosy laboratory Institute for Postgraduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar.

Pharmacognostical evaluation

Raw drugs were identified and authenticated by the Pharmacognosy Lab. The identification was carried out based on the organoleptic features and powder microscopy of the individual drugs. Later, Pharmacognostical evaluation of *Pathadi choorna* was carried out. *Pathadi choorna* (fine powder) dissolved in small quantity of distilled water, was studied under the Carl zeiss trinocular microscope attached with camera, with stain (Phloroglucine and concentrated HCl) and without stain. The microphotographs were also taken under the microscope.[10]

Preparation of Pathadi Choorna

Pathadi choorna was prepared as per classical method. Reference of *Pathadi choorna* is available in *Susruta Samhita.* All ingredients were taken in prescribed ratio equal in quantity (Table 1). Powdered and stored in airtight glass jars under hygienic conditions.

Analytical study

Raw materials of *Pathadi choorna* and prepared final product in choorna (fine powder) form were analyzed by employing various analytical parameters. Oraganoleptic characteristics colour, odour, taste and touch were analyzed. Physicochemical analysis such as loss on

drying at 1100C, pH value, Ash value, Water soluble extract, Alcohol soluble extract tests were carried out.[11]

HPTLC Profile: Instrument used was CAMAG make HPTLC with WINCATS 1.4.3 software and Linomat 5 sample applicator. The stationary phase used was HPTLC plate's silica gel 60 F254 and mobile phase was Toluene: Ethyl Acetate (8:2). The sample was prepared in methanol, and 2µl sample was applied as 8 mm band for each spot. The plate was visualized under short and long ultraviolet (UV) radiations and density of the separated spots was recorded using scanner III. The plate was sprayed with vanillin-sulphuric acid reagent and observed in daylight. The Rf values were recorded.[12]

RESULTS AND DISCUSSION

Pharmacognostical evaluation

Organoleptic characters: Organoleptic parameters like Taste, Colour, odour and touch were scientifically studied and results are as per depicted in Table 2.

Table 1: Composition of *Pathadi Choorna*.

S. No.	Drugs	Botanical Name	Officinal part	Quantity
1	*Patha*	*Cissampelos pareira*.Linn	Dried Root	1 part
2	*Pippali*	*Piper longum* Linn	Dry Fruit	1part
3	*Sunthi*	*Zingiber officinale* Roxb.	Dry Rhizome	1 part
4	*Marica*	*Piper nigrum* Linn.	Dry Fruit	1 part
5	*Vrikshaka*	*Holarrhena antidysentrica* Linn.	Dried Seed	1 part

Table 2: Organoleptic characters of *Pathadi Choorna*.

Serial No.	Characters	Results
1	Colour	Greenish
2	Odour	Bitter
3	Taste	Bitter, pungent
4	Touch	Smooth

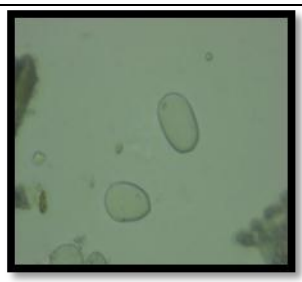
Table 3: Physico-chemical assay of *Pathadi Choorna*.

Serial No.	Parameter	Value
1	Loss on Drying (% w/w)	9.24
2	Ash value (% w/w)	5.54
3	Water soluble extract (% w/w)	15.2
4	Alcohol soluble extract(% w/w)	15.64
5	pH (5% aqueous solution)	6.5

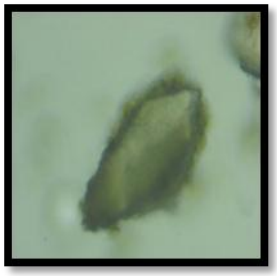
Table 4: HPTLC Profile of *Pathadi Choorna*.

Under 254 nm		Under 366 nm	
No of Peaks(Spots)	**Rf Values**	**No of Peaks(Spots)**	**Rf Values**
5	0.01, 0.48, 0.62, 0.75, 0.87	6	0.00, 0.34, 0.66, 0.75, 0.86, 0.95

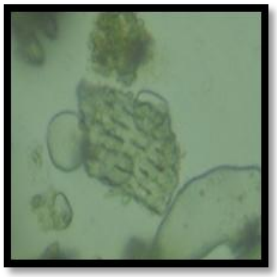
Plate 1: Microphotographs of *Pathadi Choorna.*



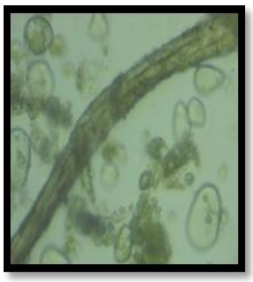
1.Starch grains of *Sunti*



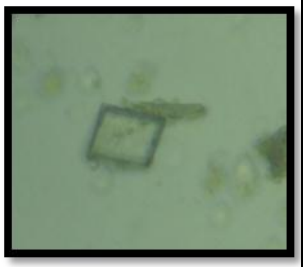
2.Black debris of *Marich*



3.Pitted vessel of *Patha*

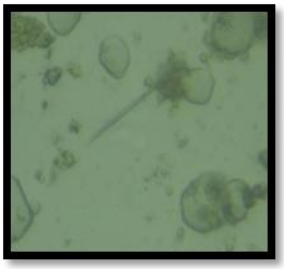


4.Fibres of Kutaja



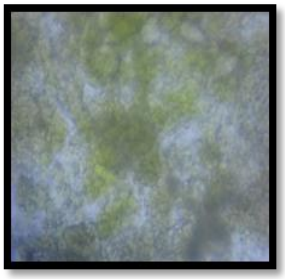
5. Prismatic crystals of *Kutaj*

6.Stone cells of *Pippali*

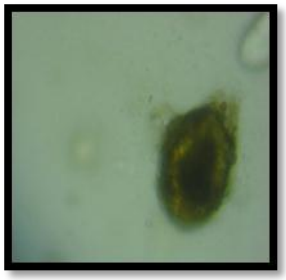


7. Acicular crystal of *Patha*

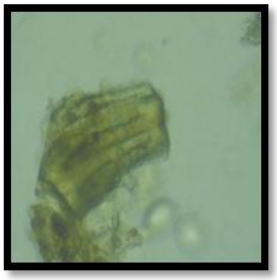
8.Spool cells of *Kutaj*



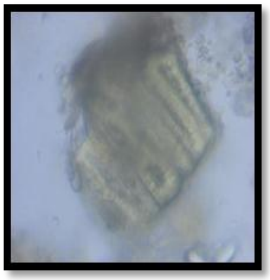
9. Epidermal cells of *Patha*



10. Stone cells of *Marich*

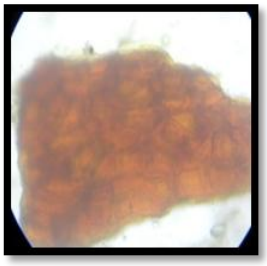


11. Bottleneck stone cells of *Pippali*

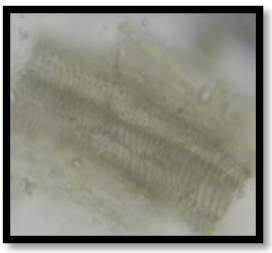


12. Group of Stone cells of *Pippali*

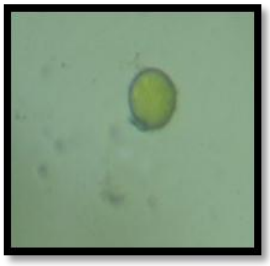
13. Septate fibres of *Patha*



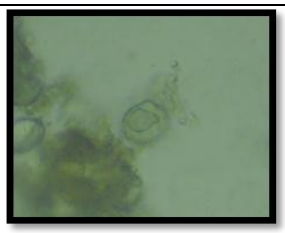
14.Epicarp cells of *Kutaj*



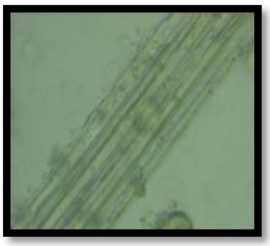
15.Scalariform vessel of *Sunti*



16. Oil globule of *Kutaj*



17. Aluron grain of *Kutaj*.



18. Fibres of *Sunti*.

19. Group of stone cells of *Pippali*.

Plate 2: HPTLC of *Pathadi Choorna*.

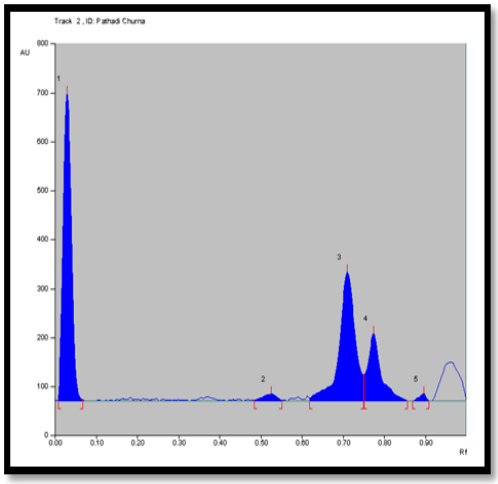


Fig: 20. HPTLC at 254nm

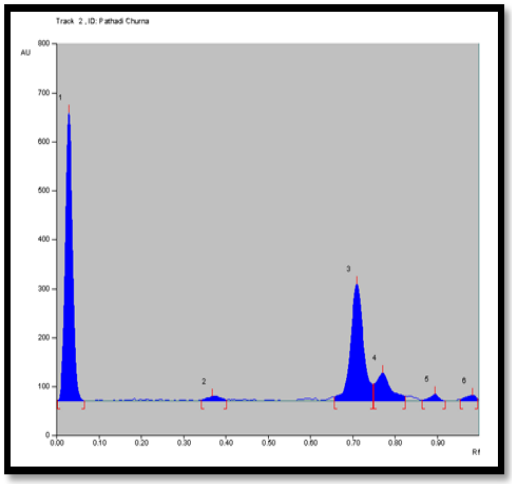
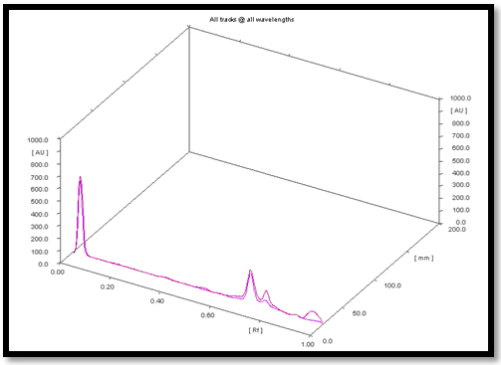


Fig: 21. HPTLC at 366nm

Plate: 3. 3-D Diagram of HPTLC of *Pathadi Choorna*.



Microscopic characters

Diagnostic characters were observed under the microscope were pitted vessel, epidermal cells, septate fibres and Acicular crystals from *Patha*. Starch grains, fibres and scalariform vessel of *Sunti*, Black debris and stone cells of *Marich*, bottle neck stone cells and a group of stone cells of *Pippali*. Prismatic crystals, spool cells, epicarp cells, aleurone grain and oil globules of *Kutaj*. Details of which are depicted in plate 1.

Physicochemical analysis

Physicochemical analysis of *Pathadi choorna* ie Ash value, loss on drying, water soluble extract, methanol soluble extract and pH were scientifically studied and the results were detailed in Table 3.

HPTLC Study

Chromatographic study (HPTLC) was carried out under 254 and 366 nm UV to establish fingerprinting profile and the results were depicted in Table 4. It showed 5 of spots at 254 nm and 6 spots at 366 nm. Phyto-components with 0.75 Rf values were recorded in both long and short UV, they showed that one spot merging which may be responsible for expression of its pharmacological and clinical actions. This shows common characters in both the wavelength and it reflects presence of steroidal components in product.

DISCUSSION

Pharmacognostical evaluation showed that the *Yoga* contains all the ingredients which were observed in the microscopical characters, this shows that the purity and quality of the product. Physicochemical analysis showed that material gains moisture during storage, which eventually may affect the quality of product. Here, average value of Loss on drying (LOD) was found within normal limits (9.24% w/w), which indicates prompt care taken during packaging and storage of product. The obtained values of these tests were found within normal limits in *Pathadi choorna*, which indicate good quality of product. The pH value of the compound was found to be 6.5 and Ash value was 5.54% w/w, the water soluble extract was found to be 15.2%w/w, the alcohol soluble extract was 15.64% w/w. HPTLC results showed that the 5 spots at 254 nm and 6 spots at 366 nm, where one common spot observed at both spectrum.

CONCLUSION

Pharmacognostical and physico-chemical evaluation of *Pathadi choorna* illustrated the specific characters of ingredients which were used in the preparation. Physico-chemical profile is an essential parameter for quality assurance; in present work the obtained results were found within prescribed limits. On the basis of observations and experimental results, this study may be used as reference standard in the further quality control researches.

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