

**PHARMACOGNOSTICAL AND PHYTOCHEMICAL ANALYSIS OF
ANUBHUTA RASAYAN YOGA-AN AYURVEDIC
POLYHERBOMINERAL FORMULATION FOR DIABETIC
RETINOPATHY**

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ABSTRACT

Diabetic retinopathy is a micro vascular complication of Diabetes mellitus. Anubhuta Rasayan Yoga is an Ayurvedic Polyherbomineral formulation for Diabetic retinopathy. The quality assessment of herbal formulation is of vital importance in order to justify their acceptability in the modern circumstances. Standardization and quality control strategies are more required to provide effective and safe drugs. Thus Anubhuta Rasayan Yoga was evaluated by Pharmacognostical, Preliminary Physicochemical and Phytochemical studies. The powder microscopical study showed the presence of pitted vessels and sclerides, annular and scalariform vessels, dark brown coloured

content, yellow colouring matter, silica deposits, stone cells, cork cells, collenchyma cells and rhomboidal crystals. Preliminary physicochemical parameters showed that water soluble extractive value is more than alcohol soluble extractive value. Qualitative analysis of methanolic and water extract showed the presence of tannin, phenolic compounds, alkaloid, glycoside and steroid.

KEYWORDS: Anubhuta Rasayan Yoga, Diabetic retinopathy, Pharmacognosy, Physico-chemical analysis.

INTRODUCTION

Diabetic retinopathy (DR), the leading cause of visual disability in diabetics, is an important complication of diabetes mellitus (DM).^[1-5] The conventional treatment for DR is LASER Photocoagulation and intravitreal pharmacotherapies, which has got many side effects and also high cost. Hence it is high time to address the issue of diabetes, prevention of its complications and management with all seriousness and find affordable medical care in alternative system of medicine. Anubhuta Rasayan Yoga is an Ayurvedic Polyherbomineral formulation for Diabetic retinopathy. It acts at the level of Rasayani Daurbalya (weakness of structural and functional integrity of nutritive channels) which is seen in the pathogenesis of Diabetic retinopathy. For a quality assured herbal product standardization is always required. Standardization should be based on microscopical, physical, chemical and phyto-chemical parameters. The detailed pharmacognostical and phytochemical evaluation of an herb or formulation provides a means of standardization which is useful for future reference. In the present paper an attempt has been done to standardize Anubhuta Rasayan Yoga based on microscopical, physical, physico-chemical and phytochemical characteristics. The yoga consists of Haritaki (*Terminalia chebula* Retz.), Amalaki (*Embilica officinalis* Gaertn), Vibhitaki (*Terminalia bellerica* Roxb.), Haridra (*Curcuma Longa* Linn.), Guduchi (*Tinospora cordifolia* [Thunb] Miers), Musta (*Cyperus rotundus* Linn), Yastimadhu (*Glycyrrhiza glabra* Linn.), Vasa (*Adathoda vasica* Nees.) and Swarna maksika bhasma.

MATERIALS & METHODS

Collection of drugs

Individual powder microscopy was done at Pharmacognosy unit, IPGT&RA, Jamnagar to prove the authenticity of the drug.

Preparation of powder

All the drugs except Swarna maksika bhasma were powdered separately and the powder was sieved through mesh size #85. All the eight drugs except Swarna maksika bhasma were taken in equal quantity and mixed together. Then Swarna maksika bhasma was added to make the formulation.

Preparation of extracts

About 5g of the test drug (formulation) was macerated with methanol (100ml) in a closed flask for 24 hours with initial shaking frequently during first 6hrs and kept it for 18 hrs. After 24 hours it was filtered and alcoholic extracts were collected in semisolid form. The same procedure was followed to obtain aqueous extracts of the test drugs.^[6]

Organoleptic characters

Organoleptic characters of test drug such as odour, taste, texture and colour were observed and recorded.^[7]

Powder microscopy

For examining characters of the test powder, pinch of powder was taken on glass slide and observed as such to see their cell contents and then stained with phloroglucinol and hydrochloric acid to observe the lignifications of the cell wall.^[8] The sample was observed under compound microscope and photographs were taken.

Physicochemical parameters

Physico-chemical Parameters like Loss on drying, alcohol soluble extractive and water-soluble extractive values and pH were determined as per the API guidelines for the test sample.^[9]

Phytochemical parameters

Preliminary phytochemical studies of methanolic and aqueous extract of the test drug was carried out. Presence of various phyto-constituents viz., alkaloids, starch, proteins, amino acids, glycosides, and phenolic compound and amino acids were evaluated.^[10,11]

OBSERVATIONS AND RESULTS**Organoleptic characters**

Colour: Greenish yellow.

Odour: Aromatic.

Taste: Astringent ends with sweet.

Texture: Fine.

Powder Microscopy

The powder microscopy of the test drug revealed the presence of silica deposits of *Musta*, sclerides of *Yashtimadhu*, stone cells of *Yashtimadhu*, unicellular trichomes of *Vibhitaki*,

yellow colouring matter of Haridra, border pitted vessels of Guduchi, stone cells of Haritaki, dark brown tannin content of Haritaki, sclerides of Vibhitaki, sclerides of Amalaki, cork cells of Guduchi in surface view, rhomboidal crystals of Yashtimadhu, collenchyma cells of Guduchi, crystal fibres of Yashtimadhu, annular and scalariform vessels of Vasa, scalariform vessels of Haridra and epidermal cells of Vasa.(Plate 1).

Physicochemical parameters

Physicochemical characters like Loss on Drying, Total Ash Value, pH are scientifically studied and results are depicted in the table no 1.

Phytochemical parameters

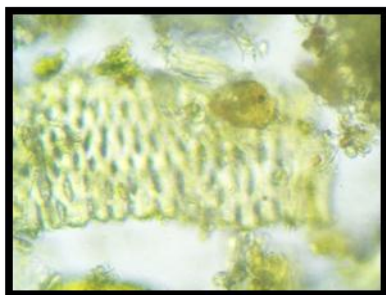
Qualitative analysis was carried out by using methanolic and aqueous extracts of the test sample. The test sample was evaluated for carbohydrate, amino acids, proteins, starch, alkaloid, tannin, steroid, flavonoids etc. Their results are as quoted in table no 2.

Table 1: Physico chemical parameters of Anubhuta Rasayan yoga

| No | Parameters | Results |
|----|-----------------------------------|---------|
| 1 | Loss on Drying (%) w/w | 0.0622 |
| 2 | Total Ash Value (%)w/w | 4.0081 |
| 3 | Water soluble extractive(%) w/w | 34.45 |
| 4 | Alcohol soluble extractive(%) w/w | 31.11 |
| 5 | pH | 3.5 |

Table 2: Phytochemical parameters of Anubhuta Rasayan yoga

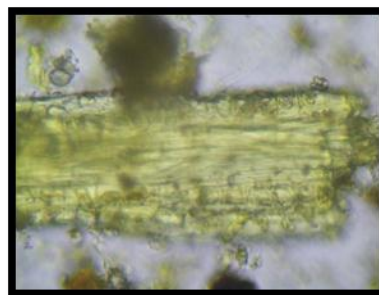
| No. | Phytoconstituents | Test performed | Results obtained | |
|-----|--------------------|----------------------|------------------|-----|
| | | | M.E | W.E |
| 1 | Carbohydrate | Molish's test | - | - |
| 2 | Reducing Sugar | Fehling's test | - | - |
| 3 | Amino acids | Ninhydrin test | - | - |
| 4 | Alkaloid | Dragondorff's test | + | + |
| | | Wagner's test | + | + |
| 5 | Protein | Biuret's test | - | - |
| 6 | Tannin | Lead acetate test | + | + |
| 7 | Steroid | Salkowaski test | + | + |
| 8 | Flavonoids | Lead acetate | - | - |
| 9 | Glycosides | Keller-Killiani Test | + | + |
| 10 | Saponin | Foam Test | - | - |
| | | Lead acetate test | - | - |
| 11 | Phenolic compounds | Lead acetate | + | + |



A) Border pitted vessels of Guduchi



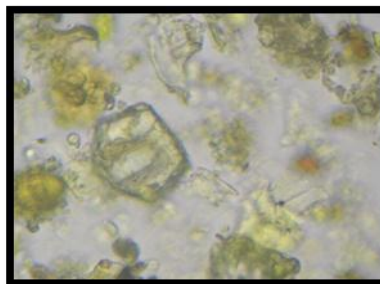
B) Annular and scalariform vessels of Vasa



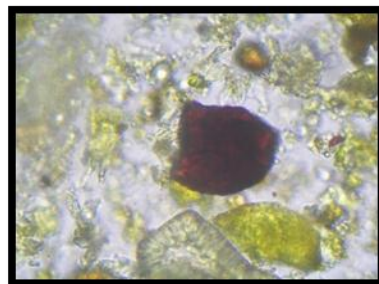
C) Crystal fibres of Yashtimadhu



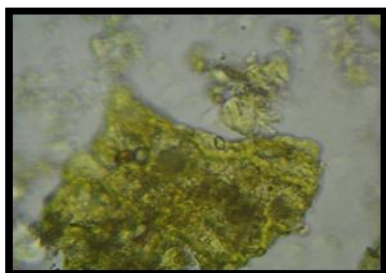
D) Cork cells of Guduchi in surface view



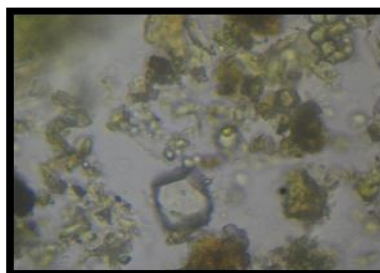
E) Collenchyma cells of Guduchi



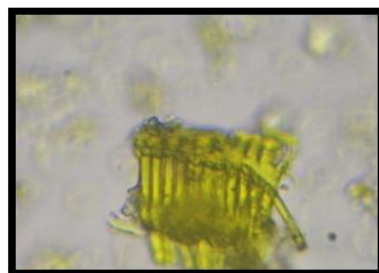
F) Dark brown tannin content of Haritaki



G) Epidermal cells of Vasa



H) Rhomboidal crystals of Yashtimadhu



I) Scalariform vessel of Haridra



J) Sclerides of Amalaki



K) Sclerides of Vibhitaki



L) Sclerides of Yashtimadhu



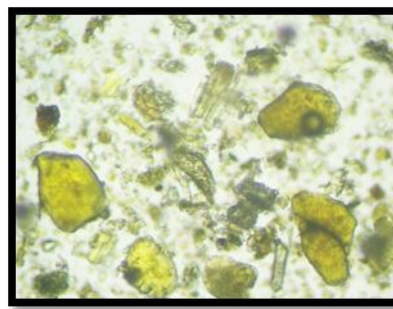
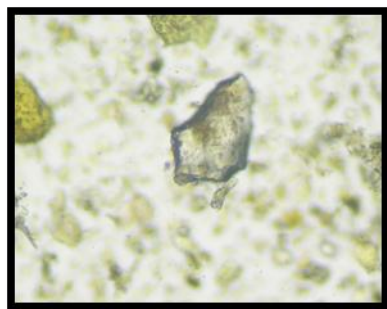
M) Stone cells of Haritaki



N) Stone cells of Yashtimadhu



O) Unicellular trichome of Vibhitaki



P) Silica deposits of Amalaki

Q) Yellow colouring matter of Haridra

Plate: No.1 Microscopic characteristics of Anubhuta Rasayan yoga.

DISCUSSION

Microscopical analysis of the formulation revealed the presence of silica deposits, sclerides, stone cells, unicellular trichomes, yellow colouring matter, border pitted vessels, dark brown tannin content, cork cells, rhomboidal crystals, collenchymas cells, crystal fibres, annular and scalariform vessels and epidermal cells. Rhomboidal crystals were present in Yashtimadhu. Sclerides of Yashtimadhu, Vibhitaki and Amalaki were present. It shows that the finished product contains all ingredients which were used. The physical constant evaluation of a drug is an important parameter in detecting adulteration or improper handling of drugs. The total ash is particularly important in the evaluation of purity of drugs i.e, the presence or absence of foreign inorganic matter. The moisture content of the drug is very low thus it could discourage the multiplication of bacteria, fungi and yeast. Preliminary physicochemical parameters showed that water soluble extractive value is more than alcohol soluble extractive value, which indicates the presence of more water soluble contents in the formulation. pH of the drug determines acidity or alkalinity of drug. The test drug has pH 3.5 indicating its acidic nature. Qualitative analysis of methanolic and water extract showed the presence of steroid, glycoside, tannin and phenolic compounds and alkaloid. The test drug showed negative results for proteins, aminoacids, flavonoid, saponins, carbohydrate and reducing sugars.

CONCLUSION

The ability to provide timely, accurate and reliable data is an essential component for the discovery, development and manufacture of Pharmaceuticals. The Pharmacognostical, Physico-chemical characters and phytochemical parameters of Anubhuta Rasayan yoga may be useful to generate standards to assess the quality and purity of the formulation in further research works.

REFERENCES

1. Danaei G, Finucane MM, Lu Y, Singh GM, Cowan MJ, Paciorek CJ, et al. National, regional, and global trends in fasting plasma glucose and diabetes prevalence since, 1980: Systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2.7 million participants. *Lancet*, 2011; 378: 31-40.
2. World Health Organization. *Global Health Risks. Mortality and Burden of Disease Attributable to Selected Major Risks*. Geneva: World Health Organization, 2009.
3. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Med.*, 2006; 3: e442.
4. World Health Organization. *Global Status Report on Non Communicable Diseases 2010*. Geneva: World Health Organization, 2011.
5. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Res Clin Pract*, 2010; 87: 4-14.
6. Anonymous, *The Ayurvedic Pharmacopoeia of India, Part I, Appendix 2*, 1st edition, New Delhi, Govt. of India publication, Ministry of Health & FW, Dept. of ISM and H, 1999; 1: 207.
7. Trease and Evans, *Pharmacognosy*, 15th Ed., W.B. Saunders Company Ltd, 1996; 569: 570.
8. Khandelwal KR. *Practical Pharmacognosy – Techniques and Experiments*. 9th ed: Nirali Prakashan, Pune, 2002; 24-29: 149-153.
9. Anonymous, *The Ayurvedic Pharmacopoeia of India, Part-I*, New Delhi, Govt. of India, Ministry of Health & FW, Dept. of ISM and H, 1999; 1-4: 213-14.
10. Shukla VJ, Bhatt UB. *Methods of Qualitative Testing of some Ayurvedic Formulations*. Jamnagar, Gujarat Ayurved University, 2001; 5-10.
11. Khandelwal KR. *Practical Pharmacognosy – Techniques and Experiments*. 9th ed.: Nirali Prakashan, Pune, 2002; 149-153.