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Pharmacognostical and Phytochemical Standardization of *Nimbadi Yonivarti*- an Ayurvedic Poly herbo-mineral Formulation

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ABSTRACT

Ayurveda, being as ancient system of medicine, which comprises hundreds of *Kalpanas* of pharmaceutical preparations but only few of these *Kalpanas* are in practice. In this modern era many good preparations have been extinguished in the passage of time among these, *Varti Kalpana* is main one. Now-a-days, when human life is very fast, we should be ready to make necessary changes in our science according to the need of hour. In present study *Nimbadi Yonivarti* is selected for the management of *Swetapradara* which has been mentioned as *Anubhuta Yoga* by Vd. Devisharan Garga Ayurveda Upadhyaya in "*Nari Roganak*". The present study was aimed at setting up a standard profile of *Nimbadi Yonivarti* which was prepared using pharmacognostically authenticated raw drugs followed by subjecting it to detailed pharmacognostical, physicochemical and phytochemical (including Thin Layer Chromatography) analysis as per standard protocol. The observations were systematically recorded. Pharmacognostical findings (crystals, epicarp cells, trichom, etc.) confirm the ingredients present in the finished product. Identified phytochemical components (Carbohydrates, tannins, fanoic compound, glycosides, steroids, flavonoids.etc.) support the intended action of the formulation in vaginal discharge.

Keywords: HPTLC, Pharmacognosy, Phytochemistry, *Nimbadi Yonivarti*, *Shwetapradara*.

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INTRODUCTION

In this present era of materialism, women are surrounded with various kinds of disease in which vaginal discharge is most common. Vaginal discharge very much depends upon woman's own perception, power of observation and tolerance etc. or has great individual variation. Vaginal discharge is indicative of some white creamy, yellowish, greenish discharge from vagina as abnormal or pathological process in the genital tract. It usually shows symptoms in association with other illness and commonly caused by fungal, parasitic, bacterial and sexually transmitted disease like trichomonas vaginalis. In Ayurvedic texts, though various conditions are described in which *Swetasrava* occurs but the *Swetapradara* as a special disease is not mentioned anywhere in Brihatrayee and Laghutrayee. For bleeding per vagina *Raktapradara*, *Pradara* and *Asrigdara* and for white discharge *Swetasrava* or *Yoni Srava*, *Slesmala Yoni* words have been used. The signs and symptoms of *Slesmala Yoni Vyapada* can be correlated with the term *Swetapradara* which is commonly seen in practice¹. Commentator Cakrapani, Sharngadhara Samhita, Bhavaprakasa and Yogaratnakara have used the word *Swetapradara* for white vaginal discharge. Commentator Cakrapani (11thcentaury) has well narrated this term². In *Acharana Yoni* there is no direct discription of vaginal discharge in classics, but due to uncleanliness and presence of microbes vaginal discharge along with severe itching is seen³. According to WHO -every year 333 million new cases of curable vulvovaginitis infections are registered. 10% to 41% of women have had abnormal vaginal discharge at least once in a life. In the last 20 years – incidence is doubled. So it is great scope of research to find out safe, potent, cost effective remedy from Ayurveda for the management of *Swetapradara*. Thus *Nimbadi Yonivarti* has been selected for the trial which has been mentioned as *AnubhutaYoga* by Vd. Devisharan Garga Ayurveda Upadhyaya in “*Nari Roganak*”⁴. The drugs which are used in it are *Tridoshashamaka*, *Krimighna*, *Stambhana*, *Shoshana*, *Samgrahni*, *Kledaghna*, *Kaphaghna*, *Putihara*, *Kandughna*, Antimicrobial, Antifungal, Antibacterial properties. Easily available in sufficient quality noncontroversial and also economical⁵. Dose maintenance is to be perfect in *Varti* form and it does not need any special precautionary measures during its administration. Even patient can administer this form of drug herself without the aid of anyone. In condition of abnormal vaginal discharge local route is to be better as compare to oral because the high concentrations are attained at the desire site without exposing the rest of body⁶. The systemic side effect or toxicity is consequently absent or minimal. Oral antiprotozoal like (Metronidazole) and antifungal (Ketoconazole, Fluconazole) causes many

side effects like nausea, burning sensation, headache, rashes and gastric discomfort etc⁷. So, use of topical route for the treatment of abnormal vaginal discharge is safe and efficacious.

MATERIALS AND METHOD

Collection of Raw Drugs

Most of the raw drugs of *Nimbadi Yonivarti* were collected from Pharmacy of Gujarat Ayurved University, Jamnagar. Fresh *Nimbapatra* had been collected from Botanical garden, Gujarat Ayurved University, Jamnagar. Ingredients and the part used are given in Table 1.

Table 1: Showing contents of *Nimbadi Yonivarti*

| No. | Drug | Latin Name | Family | Part used |
|-----|------------------|------------------------------------|----------------------|-----------|
| 1. | <i>Nimba</i> | <i>Azadirachta indica</i> A. Juss. | Meliaceae | Leaf |
| 2. | <i>Amalaki</i> | <i>Embllica officinalis</i> Linn. | Euphorbiaceae | Fruit |
| 3. | <i>Haritaki</i> | <i>Terminalia chebula</i> Retz. | Combretaceae | Fruit |
| 4. | <i>Bibhitaki</i> | <i>Terminalia bellirica</i> Roxb. | Combretaceae | Fruit |
| 5. | <i>Madhu</i> | <i>Apiscerana</i> Fabr. (source) | Apidae ¹⁵ | - |
| 6. | <i>Sphatika</i> | - | - | - |

Preparation of *Nimbadi Yonivarti*

Nimbadi Yonivarti was prepared in the Rasa Shastra Dept. I.P.G.T. & R.A., GAU, Jamnagar.

Method of preparation

Nimbadi (*Nimba Patra: Triphala Yavakuta-3:1*) *Kwatha* had been prepared by *Kwatha Vidhi* (1/8 reduction of water) then filtered it & evaporated by mild heating to made it semi solid. Then Gelatine powder had added in *Kwatha*, stirring by help of spatula & heated till melt into a homogeneous mixture. Then *Su. Sphatika, Madhu* & preservative added to it and the whole mixture became poured into lubricated mould and allowed to set in refrigerator. Finally the *Varti* had packed in Aluminium foil.

Weight of each *Varti*:

About 3 gm.

Shape:

Oviform shape.

Size:

About 1.5" x 0.5" cm

Storage:

Nimbadi Yonivarti should be kept in well closed polythene bags and stored in refrigerator. Ingredients and their required quantity for each *Nimbadi Yonivarti* of 3g are given in Table 2.

Table 2: Ingredients for individual *Nimbadi Yonivarti*

| Ingredients | For 1 tab. of 3 gm. |
|--|----------------------------|
| 1. <i>Kwatha</i> of <i>Nimba</i> and <i>Triphala</i> | 3.33ml |
| 2. <i>Madhu</i> | 0.16ml |
| 3. <i>Su. Sphatika</i> | 0.11gm |
| 4. Gelatine powder | 1.3gm |
| 5. Methyl-P-Hydroxy Benzoate | 0.01gm |
| 6. Paraffin Liquid | as lubricant |

Pharmacognostical Evaluation^{8,9}

As per API drugs which are used in the finished product of *Nimbadi Yonivarti* were identified and authenticated by the Pharmacognosy department. The identification was carried out based on the morphological, Organoleptic features and microscopy of the finished product.

Pharmaceutical Evaluation

Physico-chemical Parameters¹⁰

Following parameters were analyzed for different physico-chemical parameters by today's routine methods at the pharmaceutical chemistry lab, IPGT& RA.

➤ Physico-chemical Parameters for *Nimbadi Yonivarti*

- a. Uniformity of Weight
- b. Loss on drying
- c. Total ash
- d. Water soluble extract
- e. Methanol soluble extract
- f. PH

a. Determination of uniformity test of *Nimbadi Yonivarti*:

Weight of individual 20 *Yonivarti* were noted. After that average weight of each *Yonivarti* were calculated by dividing weight of total 20 *Yonivarti* by 20. After that variation of each *Yonivarti* with the average were noted, these were within the allowed variation.

b. Loss on drying of *Nimbadi Yonivarti*

The loss on drying of the samples was determined to find out the moisture content in it by using the following method. Accurately weighed (2.5 gm) samples were taken in a Petridish and kept in the oven at 105°C for 1 hour. From the weight lost the percentage of loss on drying or moisture content was calculated and expressed as % w/w.

c. Total Ash of *Nimbadi Yonivarti*

The ash value of the sample was determined according to IP'85. About 2 gm of drug was accurately weighed and taken in a silica crucible incinerated in a muffle furnace at a temperature not exceeding 450°C for about 4 hours, cooled and weighed.

d. Determination of water soluble extract of *Nimbadi Yonivarti*

About 2.5gm, accurately weighed, *Nimbadi Yonivarti* sample was taken in a conical flask, 50ml of distilled water added to it, shaken and was kept overnight. Next day it was filtered, 20 ml of filtrate was taken in a previously dried and weighed, porcelain evaporating dish and evaporated on a hot water bath. They were dried to constant weight in an oven and weighed. From the weight of the residue obtained the water soluble extractive percentage was calculated on the basis of air dried sample.

e. Determination of methanol soluble extract of *Nimbadi Yonivarti*

About 2.5gm, accurately weighed, *Nimbadi Yonivarti* sample were taken in a conical flask, 50ml of methanol added to it, shaken and was kept overnight. Next day it was filtered, 50ml of filtrate was taken in a previously dried and weighed, porcelain evaporating dish and evaporated on a hot water bath. They were dried to constant weight in an oven and weighed. From the weight of the residue obtained the water soluble extractive percentage was calculated on the basis of air dried sample.

f. Determination of pH of sample of *Nimbadi Yonivarti*

The pH conventionally represents the acidity or alkalinity of an aqueous solution. The pH value of an aqueous liquid may be defined as the common logarithm of the reciprocal of the hydrogen ion concentration. pH value of sample was determined by Potentiometer or pH meter. The apparatus was calibrated using standard buffer solutions and then the electrodes were immersed in the syrup to be examined and pH was measured at the same temperature as for the standard solutions.

Qualitative test for various functional groups^{11,12}

The techniques employed to isolate active substance are termed as extraction method. Crude extracts obtained from such processes can be qualitatively tested to ascertain the presence of different types of components. Qualitative tests are used to detect the presence of functional groups, which plays very important role in the expression of biological activity.

High Performance Thin Layer Chromatography¹³

The sample was evaporated to dryness, the residue was extracted with methanol by maceration and the sample for spotting was prepared. H.P.T.L.C. study of the samples was carried out.

RESULTS AND DISCUSSION

Pharmacognostical study

The initial purpose of the study was to confirm the authenticity of the drugs used in the preparation of *Nimbadi Yonivarti*. For that Finished product microscopy of the drugs was studied microscopically and microscopic characters of them were drowned. Results matched with the API and thus confirmed the genuineness of all the drugs used in the finished product.

Organoleptic findings

Nimbadi Yonivarti shows that dark green in colour, taste *Kashaya*, characteristic aromatic odour and semisolid touch (Jelly like).

Finished product microscopy

Microscopic evaluation finished product of *Nimbadi Yonivarti* was conducted, Characters were noted down and microphotographs were taken. Diagnostic characters of *Nimbadi Yonivarti* showed that brown content of *Bibhitaki*, simple starch grain without hilum of *Bibhitaki*, simple starch grain with hilum of *Haritaki*, starch grain with concentric line *Nimba*, prismatic crystals of *Nimba*, epicarp cells of *Haritaki*, simple fibre of *Amalaki*, oil globule of *Nimba*, calcium oxalate prismatic crystals of *Nimba*, parenchyma cells of *Nimba*, simple trichome of *Bibhitaki*, aluerone grains of *Nimba*, mesocarp cells of *Amalaki*, tannin content of *Haritaki*, crushed stone cells of *Haritaki*, rosette crystals of *Bibhitaki*, scleroids of *Haritaki*, silica deposition of *Amalaki*. Microphotographs are placed in (Plate-1).

Plate 1: Microphotographs of *Nimbadi Yonivarti*

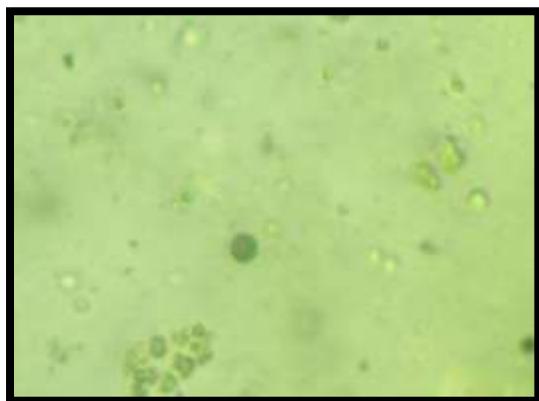


Figure 1: Alueron grains of Nimba

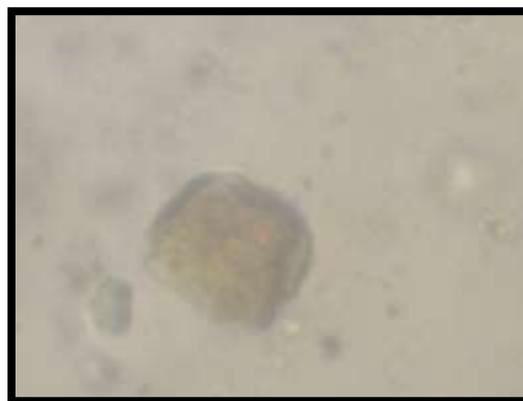


Figure 2: Brown content of Bibhitaki

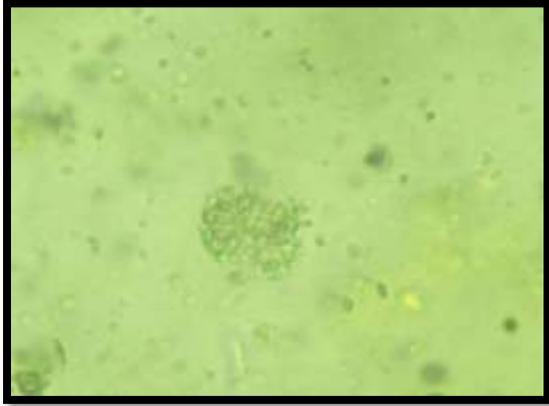


Figure 3: Epicarp cells of Haritaki

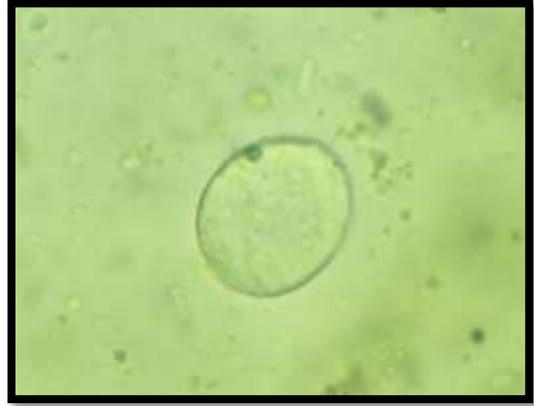


Figure 4: Oil globule of Nimba

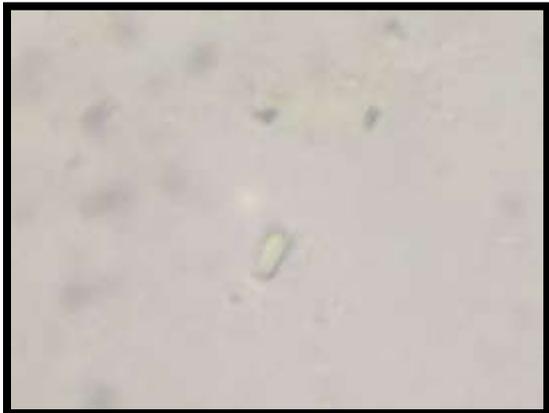


Figure 5: Prismatic crystal of Nimba

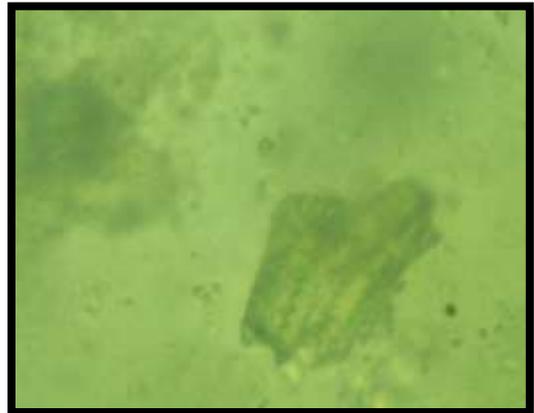


Figure 6: Scleroid of Haritaki



Figure 7: Silica deposition of Amalaki



Figure 8: Simple trichom of Bibhitaki

Pharmaceutical Evaluation

Physico-Chemical parameters of *Nimbadi Yonivarti* like pH, Loss on drying, water soluble extract, and methanol soluble extract all were found to be within the normal range. Details are given in Table 3. Qualitative test for various functional groups reveals the presence of Carbohydrates, tannins, fanoic compound, glycosides, steroids and flavonoids in drug. Details are given in Table-

4. HPTLC profile of methanolic extract of *Nimbadi Yonivarti* was done and details of number of spots and Rf value are given in Table 5 (Plate 2).

Table 3: Showing Physico-Chemical parameters of *Nimbadi Yonivarti*:

| Parameters | <i>Nimbadi Yonivarti</i> |
|-------------------------------|--------------------------|
| 1 Uniformity of weight (gm) | |
| Maximum weight | 3.170 gm |
| Minimum weight | 3.028 gm |
| Average weight | 3.044 gm |
| 2 Loss on drying (%) | 46.71 |
| 3 Ash value (%) | 1.174 |
| 4 Water soluble extract % w/w | 18.72 |
| 5 Methanol soluble extract % | 13.81 |
| 6 pH Value (By pH meter) | 5.5 |

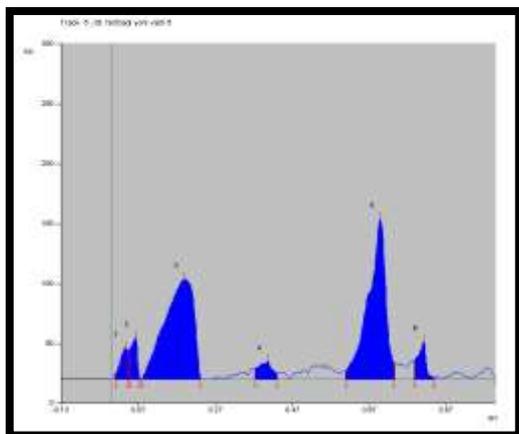
Table 4: Showing qualitative test for various functional group of *Nimbadi Yonivarti*

| Sr. No | Component | Test | Result |
|--------|-------------------------------|---------------------|--------|
| 1. | Carbohydrates | Molish Test | +ve |
| 2. | Sugar | Fehling's test | - ve |
| 3. | Proteins | T.S.+4% NaOH+CuSo4 | - ve |
| 4. | Aminoacid | Ninhydrin | - ve |
| 5. | Steroids | Salkowski | +ve |
| 6. | Glycosides | Keeler-Kiliani Test | +ve |
| 7. | Saponin | Foam test | - ve |
| 8. | Flavonoids | Lead acetate | + ve |
| 9 | Alkaloids | Dragendroff's test | - ve |
| 10. | Tannins and Phenolic Compound | Lead acetate | +ve |

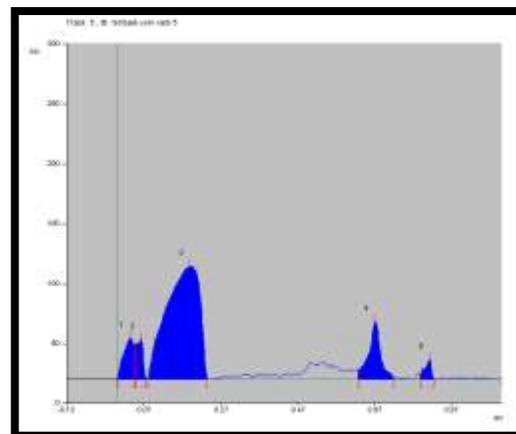
Table 5: HPTLC profile of *Nimbadi Yonivarti*

| Ultra Violet rays | Number of spot | Rf value |
|-------------------|----------------|------------------------------------|
| 254nm | 6 | 0.04, 0.06, 0.19, 0.41, 0.70, 0.81 |
| 366 nm | 5 | 0.04, 0.06, 0.19, 0.67, 0.81 |

Plate 2: Showing HPTLC profile of *Nimbadi Yonivarti*



Peak display 254 nm



Peak display 366nm

In the present study a pharmaceutical preparation of *Nimbadi Yonivarti* was tried. Its pharmaceutical properties had to be studied; hence the formulation was subjected to minimum Pharmacognostical and Pharmaceutical analysis. Pharmacognostical evaluation of *Nimbadi Yonivarti* showed the specific characters of *Azadirachta indica* A. Juss., *Emblica officinalis* Linn., *Terminaliachebula* Retz., *Terminaliabellicrica* Roxb., etc. present in the preparation. Features found in microscopy such as brown content, simple starch grain without hylum, simple starch grain with hylum, starch grain with concentric line, prismatic crystals, epicarp cells, simple fibre, oil globule confirm the same and showed all the ingredients features i.e. genuinity of the finished product. Considering the physicochemical parameters, pH of prepared drug was found acidic i.e.5.5 which resembles the normal vaginal pH and thus helps to normalise the vaginal flora. The results obtained by conducting the preliminary qualitative analysis reveal the presence of Carbohydrates, tannins, fanolic compound, glycosides, steroids and flavonoids are present in the prepared drug. The quantitative pharmaceutical analysis was in normal range and in accordance with those mentioned in reference books. Tannins etc. are known as anti-oxidants and blood purifiers with anti-inflammatory actions. It also possess antimicrobial properties against the *Candida albicans* and phytopathogenic fungi species¹⁴.

CONCLUSION

Pharmacognostical findings confirm the ingredients present in the finished product and there is no major change in the microscopic structure of the drug during the pharmaceutical processes of preparation of *Kwatha* for *Yonivarti*. Identified phytochemical components like Tannins, steroids etc. are astringents and anti-inflammatory, thus prevent the inflammation and reduce the secretion of discharge, pain or tenderness, redness, swelling of vulva and vagina. It is inferred that the formulation meets minimum qualitative standards as prescribed by API at preliminary level. The results of this study may be used as the reference standard in further research undertakings of its kind.

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