Ingredients identification and quality control evaluation of phalakalyan ghrita: an ayurvedic formulation

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Abstract
Infertility is defined as failure to conceive within one or more years of regular & unprotected coitus. It is the most sensitive and cumbersome problem which haunts every couple. Total 10-15% of world population affected due to this problem. Among them Female is directly responsible about 40%. The sub factors of female infertility are Ovarian, Endometrial, Tubal and Uterine etc. In Ayurved so many formulation are given for Infertility. Phalakalyana Ghrita (PKG) is a commonly used and prescribed Ayurvedic poly herbal formulation in all types of Infertility. However, till date no published data is available on its analytical profile. The main aims and objectives of this study are to develop the pharmacognostical and phytochemical profile of PKG. The pharmacognostical study reveals the presence of Lignified fibres, Prismatic crystals, Borderpitted vessels, Rhomboidal crystal, Scleroids, Stone cells etc. Pharmaceutical analysis showed that the loss on drying value was 0.47% w/w, Specific gravity was 0.9133, Refractive index was 1.46, Iodine value was 48.56, Saponification value was 227.05, and Acid value was 1.29. HPTLC fingerprinting profile of PKG revealed 10 spots at 254 nm and 5 spots at 366nm.

Keywords:
HPTLC, Phalakalyana Ghrita, Pharmacognosy, Physicochemical.

Introduction
Infertility is defined as failure to conceive within one or more years of regular & unprotected coitus. Total 10-15% of world population affected due to this problem. Female is directly responsible in 40% cases in infertility. In female etiological factors like Ovulatory, Endometriosis, Tubal factors, Fibroids etc. responsible to cause infertility [1].

Acc. to Ayurved, Vandhyatva (infertility) has been included in 80 types of diseases caused by the vitiation of Vata [2]. Acharya Kashyapa has mentioned Pushpa being useless Pushpa or menstruation (no conception) and certain others characterized with repeated expulsion of foetuses of different gestational periods[3]. Since in these conditions also the woman fails to get a child, thus it can be included under infertility. Acc. to Acharya Charaka, Infertile couples were considered shade less, branchless, fruitless waste tree or like a lamp in picture or portrait which will not emit any light or brightness[4]. Aim of Womanhood is complete after conception and child birth. In Bhaisajyaratnavali Yoniyaapada Chikitsa chapter one nice formulation is mentioned for infertility i.e. Phalakalyana Ghrita (PKG) [5]. It contains 22 drugs which are commonly used in gynecological disorders. PKG is a commonly used and prescribed Ayurvedic poly herbal formulation in all types of infertility. Prime ingredient of PKG is Shatavari (Asparagus officinalis)
 racemosa Willd.). Kakoli & Ksheerakakoli were replaced with Ashwagandha and Meda were replaced with Shatavari due to it’s unavailability [6]. In spite of its numerous gynecological attributes, till date no published data is available on its analytical profile. Keeping these facts in concern, present study has been undertaken to develop the pharmacognostical and phytochemical profile of PKG.

Materials and methods
Plant material
The raw drug materials were collected from the pharmacy department, GAU, Jamnagar. TABLE- 1

Pharmacognostical Evaluation
The raw drugs are identified and authenticated and powder microscopy was done in the pharmacognosy department, IPGT & RA, GAU, Jamnagar. The study includes organoleptic evaluation and microscopic evaluation[7].

Microscopic Study
The individual powered drug are first examined under distilled water for the observation of calcium oxalate crystals and other cellular materials, then stained with Phloroglucinal and conc. HCl [8] for the lignified characters, then stained with iodine to observe the starch grains. Raw drugs were separately studied under microscope, the diagnostic characters microphotographs are taken by using Carl zeiss trinocular microscope [9].

Method of preparation of PKG
The drugs enlisted in the TABLE 1 were taken and PKG was prepared as per classics.
- Kalka Dravyas - Each12 gm (coarse powder)
- Drava Dravyas – Godugdha -3 litre, Shatavari Swarasa- 3 litre
- Sneha Dravya - Goghrita – 750 ml.

Organoleptic Study  TABLE - 2
Contents of PKG were evaluated for organoleptic characters like taste, odour and colour etc. [10]

Pharmaceutical evaluation
Physico-chemical analysis

Physico-chemical Parameters of PKG like Loss on drying, Specific gravity, Refractive index, Acid value, Saponification value, and Iodine value were determined as per the API guideline.

HPTLC
First of all take a drop of sample and diluted with haxen (as per require) then application of the sample at the one end of the precoated plate through linomat V (150 µl/sec) then on the sample zone again applied 7% alcoholic KOH then leave for 10-15 minutes at 60-80ºc in oven. The plate is then developed by the suitable mobile phase in a chromatographic chamber which was previously saturated with the mobile phase. Then after development it is visualized into day light, short UV (254nm) and/or by derivatization of the plate with suitable reagent. The Rf value and the colors of resolved bands and fingerprinting profiles are recorded.

Results and discussion
Microscopic Study
The diagnostic microscopic characters of individual powder are shown in PLATE – 1 (Figure 1-36)

Pharmaceutical evaluation:
Organoleptic characters of PKG
Organoleptic characters of Contents of Ghrita like colour, taste and odour are recorded separately and are depleted. TABLE-3

Physicochemical tests
Physicochemical analysis of PKG revealed the loss on drying value was 0.47% w/w, Specific gravity was 0.9133, Refractive index was 1.46, Iodine value was 48.56, Saponification value was 227.05, and Acid value was 1.29. TABLE- 4

HPTLC study results
Chromatographic study (HPTLC) was carried out under 254 and 366 nm UV to establish fingerprinting profile. It showed 10 of spots at 254 nm with Rf values and 5 spots at 366nm with Rf values were recorded which may be responsible for expression of its pharmacological and clinical actions. PLATE - 2, TABLE- 5

Original Research Article
PLATE 2: Densitogram of PKG AT 254 AND 366NM
Table 1: Ingredients of *Phalakalyana ghrita*

| CONTENT       | LATIN NAME                        | PART USED | RATIO | FORM       |
|---------------|-----------------------------------|-----------|-------|------------|
| Shatavari     | Aspargos racemosa Wild.           | Moola     | 3 Lit.| Svarasa    |
| Godugdha      | Animal product                    | ----------| 3 Lit.| Liquid     |
| Goghrita      | Animal product                    | ----------| 750 ml.| Liquid     |
| Manjistha     | Rubia cordifolia Linn.            | Root      | 12 gms| Kalka      |
| Yastimadhu    | Glycyrrhiza glabra Linn.          | Root      | 12 gms| Kalka      |
| Kushtha       | Saussurea lappa C.B.clarke        | Root      | 12 gms| Kalka      |
| Triphala      | Emblica officinalis Gaertn.       | Fruit     | 12gms | Kalka      |
|              | Terminalia bellirica Roxb.        |           |       |            |
|              | Terminalia chebula Retz.          |           |       |            |
| Sharkara      | Saccharum officinarum Linn.       | Ghana     | 12gms | Svarasa    |
| Balamoola     | Sida cordifolia Linn.             | Root      | 12gms | Kalka      |
| Shatavari     | Aspargos racemosa Wild.           | Root      | 12gms | Kalka      |
| Ksheervidari  | Ipomoea digitata Linn.            | Kanda     | 12gms | Kalka      |
| Ashwgandha    | Withania somnifera Linn.          | Root      | 24gms | Kalka      |
| Ajamoda       | Carum roxburghianum Craib.        | Fruit     | 12gms | Curna      |
| Haridra       | Curcuma longa Linn.               | Kanda     | 12gms | Kalka      |
| Daruharidra   | Cedrus deodar Roxb.               | Kandsara  | 12gms | Kalka      |
| Ghritabhrusta Hing | Emblica officinalis Gaertn. | Flower | 12gms | Kalka      |
| Katuki        | Picrorhiza kurroa Royle           | Root      | 12gms | Kalka      |
| Neelkamal     | Nelumbo nucifera Gaertn.          | Flower    | 12gms | Kalka      |
| Kumada Puspa  | Nympheaea nouchali Burn.          | Flower    | 12gms | Kalka      |
| Draksha       | Vitis vinifera Linn.              | Flower    | 12gms | Kalka      |
| Rakta chandan | Pterocarpus santalinus Linn.      | Kandasara | 12gms | Kalka      |
| Shweta chandan | Santalum album Linn.              | Kandasara | 12gms | Kalka      |

Table-2: Pharmacognostical study

| Sr. No. | Drug       | Colour     | Taste      | Odour     | Nature of powder | Microscopic Characters Identified                                                                 |
|---------|------------|------------|------------|-----------|------------------|----------------------------------------------------------------------------------------------------|
| 1       | Shatavari  | Creamish   | Sweet      | Characteristic | Coarse                 | Parenchyma cells, Acicular crystals and raphids of calcium oxalate, scalariform vessels, Fragments of cork in surface view. |
| 2       | Manjistha  | Redish     | Sweet, Bitter | Aromatic  | Coarse               | Prismatic and acicular crystal of calcium oxalate, fibres, cork in surface view, simple starch grains, brown contents, pitted vessels. |
| 3       | Yastimadhu | Specific   | Sweet      | Sweet     | Coarse             | Cork in surface view, prismatic crystals of calcium oxalate, crystal fibre, pitted vessels, fibres and starch grains. |
| 4       | Kushtha    | Brownish   | Bitter     | Aromatic  | Coarse             | Oil globules, Prismatic crystals, Starch granules, Spiral vessels, Scleriform vessels, Parenchyma cells, Stone cells |
| 5       | Haritaki   | Greenish   | Astringent | Characteristic | Coarse             | Epicarp cells, epidermal cells, sclereids, group of stone cells, tannin contant and starch grains simple rounded or oval in shape. |
| 6       | Bibhitaki  | Greenish   | Astringent | Characteristic | Coarse             | Epicarp cells, mesocarp cells, Stone cells in groups, simple starch grains, rosettes of calcium oxalate |
### Table-3: Organoleptic Characters of PKG

| Sr. No. | Characters | Observed          | Description                                                                 |
|---------|------------|-------------------|-----------------------------------------------------------------------------|
| 1       | Colour     | Yellowish         |                                                                             |
| 2       | Odour      | Agreeable         |                                                                             |
| 3       | Taste      | Bitter and slight sweet |                                                                             |

| Sr. No. | Characters | Observed          | Description                                                                 |
|---------|------------|-------------------|-----------------------------------------------------------------------------|
| 7       | Amalaki    | Brownish Black    | Sour, Swtish, Coarse, Silica crystals, scleroids, fibres, mesocarp cells, epicarp cells. |
| 8       | Bala       | Greenish          | Astringent, Bitter, Characteristic, Coarse, Prismatic crystal of, Stellate trichome. |
| 9       | Payasya    | Creamishwhite     | Sweet, Sweetish, Coarse, Simple and compound starch grains, fibres, prismatic crystals, parenchyma cells. |
| 10      | Draksha    | Black             | Sweet, Sweetish, Coarse, Epidermis cells, reddish-brown contents, mesocarp cells, prismatic crystals of calcium oxalate, endosperm cells, oil globules and cluster crystals of calcium oxalate. |
| 12      | Ajmoda     | Brown             | Pungent, Aromatic, Coarse, Vittae cells, oil globules, alouerone grains, lignified parenchyma cells, spiral, scalariform vessels, epidermal cells. |
| 13      | Madhuka    | Brown             | Sweet, Sweet, Sticky, Brown content, starch grains, trichomes oil globules. |
| 14      | Chandana   | Creamish          | Bitter, Aromatic, Coarse, Border pitted vessels, lignified fibres, oil globule, starch grains, prismatic crystals. |
| 15      | Rakta-Chandana | Red        | Tikta, Fragrant, Coarse, Vessels large border pitted, prismatic crystals of calcium oxalate occur in a few cells, red colouring matter, fibres abundant, and starch grains. |
| 16      | Ashwa Gandha | Whitish cream    | Slightly bitter, Characteristic, Coarse, Cork in surface view, simple starch grains. |
| 17      | Haridra    | Yellow            | Astringent, Bitter, Aromatic, Coarse, Epidermal cells, cubical cells of various dimensions, oleo-resin contents, cork in surface view, starch grains, oil or amorphous resinous matter, vessels mainly spirally thickened. |
| 18      | Daru Haridra | Yellow          | Bitter, Characteristic, Coarse, Epidermal cells, unicellular hairs, multibranched lignified trichomes, fragments of spongy parenchymatous cells, stomata, tanniferous contents, pollen grains, annular vessels. |
| 19      | Hingu      | Brown             | Bitter, Foetid, Coarse, --- Epidermal cells, cubical cells of various dimensions, oleo-resin contents, cork in surface view, starch grains, oil or amorphous resinous matter, vessels mainly spirally thickened. |
| 20      | Katakiki   | Brown             | Bitter, Sweetish, Coarse, Tannin content, pitted vessels. |
| 21      | Neelkamal  | Pinkish white     | Sweet with stringent, Slightly aromatic, Coarse, Unicellular hairs, spongy parenchymatous cells, tannin content, lignified multilobed trichomes, pollen grains, epidermal cells. |
| 22      | Kumuda Pushpa | Sky blue        | Sweet, Sweetish, Coarse, Multibranched trichome, pollen grains. |
| 23      | Sharker    | Sugar             | -                                                                             |
| 24      | Godugdha   | Cow milk           | -                                                                             |
Table 4: Pharmaceutical Evaluation of PKG

| Sr. No. | Test                | Result               |
|---------|---------------------|----------------------|
| 1       | Loss on drying      | 0.47% w/w            |
| 2       | Specific gravity    | 0.9193               |
| 3       | Refractive index    | 1.46                 |
| 4       | Iodine value        | 48.56                |
| 5       | Saponification value| 227.05               |
| 6       | Acid value          | 1.29                 |

Table 5: HPTLC of PKG

| Wavelength | Number of spots | Max. Rf values     |
|------------|-----------------|--------------------|
| 254nm      | 10              | 28.85, 3.69, 5.62, 5.31, 7.79, 4.62, 3.34, 1.73, 3.66, 5.39 |
| 366nm      | 5               | 55.90, 6.99, 6.59, 22.71, 7.81 |

Conclusion

Pharmacognostical and phyto-chemical evaluation of PKG illustrated the specific characters of ingredients which were used in the preparation. All the Pharmaceutical parameters analyzed within the permissible range. On the basis of observations and experimental results, the evaluation research of PKG may be used as standard reference for further research work and clinical studies.

Conflict of interest statement

We declare that we have no conflict of interest.

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