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Development of Quality Control Parameter of PratishyayghnaKwath

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Abstract
Standardization of herbal formulation is essential in order to assess the quality, Purity, Safety & efficacy of the drug based on the amount of their active principles. Pratishyayghnakwath is a polyherbal formulation containing *Glycyrrhizaglabra* (Roots), *Vitisvinifera* (Fruits), *Cordiadichotoma* (Fruits), *Viola odorata* (Flowers, Leaves), *Saccharumofficinarum* (Sugar crystals), *Pipernigrum* (Fruits) as ingredients. It is most widely effective and used in treatment of cough and cold. In present investigation Pratishyayghnakwath was prepared as per official Ayurvedic text "SarSangarh". The formulation was standardized for organoleptic characters, physico-chemical parameters, phytochemical screening and Thin layer chromatographic studies. A RP-HPLC method was developed for the standardization of Pratishyayghnakwath by qualitative estimation of Glycyrrhetinic acid as markers. The developed method was validated with respect to linearity, precision, accuracy & robustness. The set of parameters were found to be sufficient to evaluate the Pratishyayghnakwath and can be used as reference standards for the quality control/quality assurance purposes.
Keywords: Pratishyayghnakwath (PK), Polyherbal formulation, Standardization etc.

Contents

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1. Introduction
Pratishyayghnakwathan Ayurvedic polyherbal formulation consists of *Glycyrrhizaglabra* (Mulethi) and other 6 ingredient in kwath. (liquid dosage form) .Preparation of PK is based on traditional method in according with the procedures given (1). Due to lack of modern Pharmacopoeial standards laid down and followed for processing of PK using traditional methods ,the medicine prepared may not have the desired quality and batch to batch consistency. Hence there is a need for standardization of PK following scientific parameter including organoleptic characters, physicochemical analysis,phytochemical screening, and Thin layer chromatography .The work was undertaken to standardize and validate Ayurvedic medicine, Pratishyayghnakwath used in treatment of cough and cold. Kwatha is widely accepted for therapeutic purpose due to feasibility in preparation and convenience of administration.

Therefore, for establishing the rationality of its usage, present work has been carried out. For the purpose of research work on standardization of herbal formulations, a profound knowledge of the important herbs found in India and widely used in Ayurvedic formulation is of outmost importance.

2. Materials and Method

Procurement of crude drug

Pratishyayghnakwath consists of 7 ingredients *Glycyrrhizaglabra* (Mulethi), *Vitisvinifera* (Munnakaa), *Cordiadichotoma* (Lisoda), *Viola odorata* (Banafsha), *Saccharumofficinarum* (Sugar crystal), *and Pipernigrum* (Kalimarich) (2-6). All these 7 ingredients were procured from local market and identified morphologically and microscopically and compared with standard pharmacopoeial monograph*. Sample of crude drug were also authenticated by Botany department from Saifia science collage Bhopal (M.P).

Preparation of Pratishyayghnakwath

Pratishyayghnakwath was prepared according to the procedure to the mentioned in "Sarsangarh". The ratio of ingredient has been showed in (Table 1).Each ingredients were coarsely powered individually and weighed and mixed.Then boiled 1 tola(11.66gm) of vegetable substance in coarse form with 16 part with portable water in earthen pot until the volume reduced up to $1/4^{th}$ from the initial quantity. After desirable reduction of volume, the Kwath was filtered through muslin cloth and collected in separate vessel. The residue remained above cloth was discarded. The results obtained during the preparation of kwath have been tabulated in (Table 2).

Organoleptic characteristics: Colour, odour, taste of kwath were observed and mentioned in (Table 3).

Physicochemical analysis:

Determination of Density: Density was determined using specific gravity bottle and calculated the density by the following formula (7).

Density of kwath at 25^0C = mass\volume

Determination of specific gravity: Specific gravity was determined using specific gravity bottle and calculated the by the following formula.

Specific gravity of kwath(liquid) at 25^0C = density of liquid/density of water

The result is showed in Table 4.

Determination of pH: pH was determined by the digital pH meter[7].The result is showed in Table 4.

Total solid content: - Accurately weight quantity of the kwath being was placed in tarred dish, evaporate at as low tempeture as possible until the solvent is removed and heated on a water bath until the residue is apparently dry. Transfer to oven and dry to constant weight at 105^0C the residue was weight and recorded. The result is showed in Table 4

Determination of Viscosity: -Viscosity was determined by Brookfied viscometer (7-9).

Temperature - 25^0C, Spindle no – 2, Factor – 8, Viscosity = Dial reading × Factor, Speed of the spindle in kwath – 50 rpm

Average dial reading of the three determinations- 2,the result is showed in Table-1

Table 1. Quantity of Each Ingredient of PK

S.No	Parameter	Observation
1.	Initial qty of churna for kwath preparation (gm)	11.66±0.078
2.	Total qty of water (ml)	186.56±0.024
3.	Temp during preparation of Kwath^{0}C	97±0.707
4.	Total time for $1/4^{th}$ reduced (min)	45±0.707
5.	Total qty of Kwath obtained (ml)	45±0.282

Table 2. Observation obtained during the preparation of PK

S.No	Common Name	Parts	Ayurvedic system	In metric system
1.	Mulethi	Roots	1 Tola	11.66 gm
2.	Munnakaa	Fruits	1 Tola	11.66 gm
3.	Lisoda	Fruits	1 Tola	11.66 gm
4.	Adusapatr	Leaves	1 Tola	11.66 gm
5.	Banafsha	Leaves	1 Tola	11.66 gm
6.	Kalimarich	Fruits	6 Masa	5.83 gm
7.	Mishri	Sugar crystal	6 Masa	5.83 gm
8.	Gulbanafsha	Flowers	1 Tola	11.66 gm
9.	water	-	16 Tola	186.56 gm

Table 3. Organoleptic characteristics of PK

S.No.	Organoleptic characteristic	Observation
1.	Colour	Reddish brown
2.	Odour	Sweetish
3.	Taste	Sweet

Table 4. Physicochemical Parameter of PK

S.No	Parameter	Observation
1.	Density (g/ml)	1.17±0.0003
2.	Specific gravity	1.034±0.0007
3.	pH	4.52±0.034
4.	Total solid content (% w/v)	12.3±0.014
5.	Viscosity (cp)	16.0±0.00

n=3 (mean ± S.D.)

Table 5. Phytochemical Screening of PK

S.No	Chemical constituents	PK
1.	Alkaloids	+
2.	Carbohydrates	+
3.	Glycosides	+
4.	Saponins	+
5.	Tannins and Phenolic compound	+
7.	Proteins and Amino acid	+
8.	Gum and Mucilage	+
9.	Flavonoid	+

+ (Present), - (Absent)

Identification of marker constituents of *Glycyrrhizaglabra* by TLC

Test Solution: 0.5g of powdered drugwas extracted with 50ml methanol. Methanolic extract was filtered and concentrated and made up the volume to 25ml with methanol.

Solvent system: Toluene: Ethyl acetate: Glacial acetic acid (12.5: 7.5: 0.5)

Procedure: 10µl of standard solution and sample solution was applied by capillary tube on precoated silica gel G plate of uniform thickness. Develop the plate in the solvent system (10-13)

Detection: Iodine vapour

Evaluation: A band corresponding to Glycyrrhetinic acid is visible in standard and test solution tracks.

Table.6

S.No	Name of Drug	Solvent system	No. of Spots	Rf value
1.	Standard Drug (Glycyrrhetinic acid)	Toluene: Ethyl acetate: Glacial acetic acid (75:45:3.0)	01	0.44
2.	Methanolic Extract	_	5.0	0.25,0.42,0.5,0.64, 0.87

High Performance Liquid Chromatography

Reagents and Materials

Glycyrrhetinic acid (GA) was purchased from Yuccoo Enterprises, Mumbai. Methanol and Water (50:50) HPLC grade used as mobile phase (14-19).

Preparation of mobile Phase

The mobile phases were prepared by mixing methanol and water in ratio 50:50 and filtered through syringe filter.

Preparation of Calibration curve of Gycyrrhetinic acid:

Preparation of Standard Stock Solution of Glycyrrhetinic acid

Standard Glycyrrhetinic acid (100 mg) was dissolved in 100 ml of methanol to prepare stock solution with concentration of 1000µg/ml.

Preparation of Dilution: For the preparation of calibration curve a series of dilution with concentration of 10, 20, 30, 40, 50µg/ml were prepared by taking aliquots of 0.1, 0.2, 0.3, 0.4, 0.5ml of stock solution (1000µg/ml) and

diluted up to 10 ml with methanol in 10 ml volumetric flask. Calibration curve was constructed by plotting area against concentration (20).

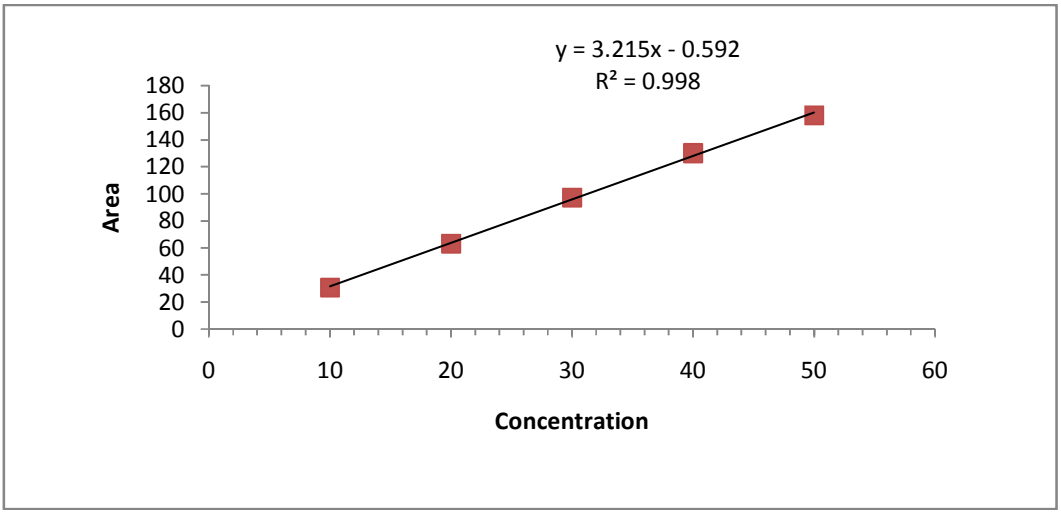

Figure 1. Calibration curve of Glycyrrhetinic acid at 204

Preparation of Sample Solution:
Weighed 11.66 gm Pratishyayghnachurna and extracted with 186.56ml methanol for 45 min by decoction method. Filter the extract and concentrated the methanolic extract till semisolid mass is obtained.Then it dried to obtain 0.820 mg extract. Taken 100 mg extract was dissolved in 100 ml methanol to preparation sample stock solution. For determination of concentration of Glycyrrhetinic acid in formulation taken 0.2 ml stock solution in 10 ml volumetric flask and make the volume with methanol. Then it was filtered through a syringe filter and analysed with HPLC.

Quantitative estimation of Glycyrrhetinic acid:
HPLC chromatograms were obtained using Shimadzu HPLC system with a 20µl sample loop. The HPLC analysis was completed using a C-18 reversed phase column, LC UV detector. The detector was connected to a computer and the data were analyzed by analchromesoftware. Determination of Glycyrrhetinic was done by using methanol and Water, at flow rate 1ml/min. The detector wavelength was 204 nm (19,21,22,23).

Table 7. Estimation of Glycyrrhetinic acid (Mean ± S.D., n= 3)

S.No	Name	GA Content % w/w
1.	PratishyayghnaKwath	1.36± 0.02

The regression equation was y=3.215x-0.592, where X is the concentration of standard sample (mg/ml).The correlation coefficient is equal to 0.998 which shows a very good linearity within the range of 10-50 µg/ml.

3. Results and Discussion

As part of standardization procedure, all batches of the finished product of PK were tested for relevant physical, chemical and phytochemical analysis and also subjected to Thin layer chromatography which give valuable information for further investigation for PK. TLC studied showed the present of active principle like Glycyrrhetinic acid on 0.44 Rf value for sample with was more close to standard Glycyrrhetinic acid Rf 0.44.Rf with prominent brown coloration in both. This is further suggested that a TLC Plate gives perfect and close result which can be repeated in next future. This is best method for qualitative evaluation of Glycyrrhetinic acid on lab scale with very less equipment and expenses.The concentration of GA in PratishyayghnaKwath was analysed by HPLC and the concentration of GA was found to be 1.36±0.02 % w/w in 11.66gm (1 tola) Pratishyayghnachurna.

4. Conclusion

The formulation was standardized for organoleptic characters, physico-chemical parameters, phytochemical screening and Thin layer chromatographic studies. A RP-HPLC method was developed for the standardization of Pratishyayghnakwath by qualitative estimation of Glycyrrhetinic acid as markers. The developed method was validated with respect to linearity, precision, accuracy & robustness.

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