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PHYSIO, CHEMICAL AND BIOLOGICAL STUDIES OF *ITRIFAL KISHNEEZI*, A UNANI FORMULATION

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ABSTRACT

India has one of the oldest, richest and most diverse cultural traditions associated with the use of medicinal plants. This traditional knowledge forms the recognized indigenous systems of medicine and exist in the forms of Ayurveda, Unani and Siddha. ***Itrifal Kishneezi*** is a traditional Unani formulation used as Munaqqi Dimagh (Drugs clearing vitiated humour from the Brain), Munaqqi Meda (Drugs clearing vitiated humour from the stomach). High-Performance Thin-layer Chromatography has become the most potent tool for quality control of herbal medicines because of its simplicity and reliability. Therefore in the present study, for the first time HPTLC chemo-profiling was developed for raw materials and formulations. Thus, the HPTLC chemo-profiling of the botanically authenticated raw materials and formulations will serve as primary reference for quality control and quality assurance.

KEYWORDS: *Itrifal Kishneezi, Unani medicine, HPTLC, quality control.*

INTRODUCTION

The Indian systems of medicine viz., Ayurveda, Siddha, Unani and Homoeopathy predominantly use plant-based raw materials in most of their preparations in addition to some materials of minerals, metals and animal origin.

The importance of safety, quality and efficacy in such products has universally been acknowledged. There is an increasing demand for ASU / botanical drugs / dietary supplements in the developing countries and the industrialized developed world is also looking for the standardized botanical products. The need of the time is therefore, to subject Ayurvedic, Sidha & Unani (ASU) drugs / products to rigorous modern scientific testing and develop standards so as to maintain quality for global competitiveness.

Ayurveda, Siddha and Unani drugs which are mainly poly-herbal/herbo-mineral preparations are very different from synthetic molecules of the allopathic system which are produced under controlled laboratory conditions. Both traditional and modern parameters are used for quality testing and standardization of raw materials as well as finished products. Many methods from organoleptic standardization of drugs, chemical analysis, biological assaying for testing of heavy metals, pesticides, Chromatographic fingerprint profiles , use of active therapeutic ingredients as marker compounds and estimating microbial load have been developed for quality control and standardization of ASU drugs.

Unani system of medicine is quite popular among the masses. Today, the Unani system of medicine with its recognized practitioners, hospitals and educational and research institutions, forms an integral part of the national health care delivery system.

Preparation and Standardization of Unani formulation Itrifal Kishneezi, were carried out to know quality standards in this formulation. It is called Trifaloon or Itrifal in Greek. In Ilmul Advia, Itrifaloon and Itrifal words are used to explain Itrifal. Inventor of Itrifal is said to be Indroomakhas. So it is Greek name. But some expert said that it was Triphal, which was made Itrifal in Arabic. Haleela *(Terminelia chebula fruit)*, Balela *(Terminelia belerica fruit)*, Kishneez *(Coriandrum sativum fruit)* are essential ingredients. Its name is due to its Chief Ingredient Kishneez *(Coriandrum sativum fruit).*

Action: Munaqqi Dimagh (Drugs clearing vitiated humour from the Brain), Munaqqi Meda (Drugs clearing vitiated humour from the stomach).

Uses: Amraz Sar (Diseases of Head), Amraz Gosh (Diseases of Ear) due to Cold and Cough.

MATERIALS AND METHODS

Preparation of Itrifal Kishneezi: The formulation was prepared on the basis of the specifications laid down by the standard guidelines. The chief ingrediants for the

formulation is Halela Zard *(Terminelia chebula half ripe* fruit*)*, Halela Kabuli *(Termineliachebula ripe* fruit*)* and Halela Siyah *(Terminelia chebula unripe* fruit*)* and Kishneez *(Coriandrum sativum* fruit*).*

Sr. No.	Ingredients	Weight taken
1.	Kashneez Khushk (*Coriandrum sativum* fruit)	35 gm
2.	Post Balela (*Terminelia belerica* fruit)	35 gm
3.	Post Haleela Kabuli (*Terminelia chebula* ripe fruit)	35 gm
4.	Post Haleela Siyah (*Terminelia chebula* unripe fruit)	35 gm
5.	Post Haleela Zard (*Terminelia chebula* half-ripe fruit)	35 gm
6.	Asl *(Honey)* Three times of all drugs weight.	500 gm
7.	Roghan Badam shirin (For Roasting)	10 ml

In a clean and dry vessel add 500gm honey i.e 3 times weight of formulation. Then the following steps were followed.

- On a slow flame heat the honey collect the honey forth and remove it let the honey, cool.
- Take a separate vessel take first 4 ingredients i.e Post Halela zard, Post Halela siyah, Post Halela kabli, Post Balela.
- First add 10ml Roghan Badamshirin.
- Roast all first 4 ingredients. Dont let it brun roast till smell come.
- After roasting add kishneez khushk. Mix well.
- Add this roasted mixture in cooled honey mix well with clean and moisture free spoon.
- Keep it for a week time store it in clean dry glass bottle.

Storage and preservation: It waspreserved in dried, airtight, fungus free clean glass or china clay container.

Physico-chemical analysis: Acid value was determined (Iyengar, 1995; Trease and Evans Wc., 1989).

Phyto-chemical Analysis: Preliminary tests were carriedout on methanolic extract for the presence / absence of phyto-constituents like alkaloids, carbohydrates, flavanoids, glycosides, saponins, sterols, terpenes and tannins (Sazada*et al.*, 2009).

Microscopic Analysis: The microscopic Character ofeach ingredient and final product were carried out (Anonymous, 1992). Permanent slides were prepared andstained with Safronin (1%) + Glycerin (Selvakumar*et al.*, 2010).

HPTLC Profile

For HPTLC profiling, 1 gm of sample was extractedwith 10 ml of methanol in a reflux **TLC fingerprint profile of formulation and its raw materials with marker compounds.**

Presence of important marker compounds indicates the presence of the respective raw materials in the formulation. Multiple marker based evaluation ensures the quality with respect to the ingredients containing these marker compounds. However, it is practically impossible to have marker compounds representing all the ingredients of a polyherbal formulation. Hence, for TLC, marker compounds present in large quantity (major phytochemical constituent) in most of the herbal raw materials of the formulation, was used.

The different ingredients of the formulation contain important marker compounds namely tannins viz. Gallic acid, Ellagic acid etc. These compounds are biomarkers since they have been shown to have several biological activities. Suitable extraction procedures were adapted to effect complete extraction of the compounds from the samples. For establishing TLC fingerprint profiles, the methanolic extracts of the formulation was used. The presence of the markers in the sample extracts was ascertained by co-chromatography. The resolved bands were observed under UV 254nm and further treated with a suitable detecting reagent. Comparison of R_f values of the herbal raw materials and formulation with that of the standard of the marker compound was done.

Formulation	TLC plate dimension	Standard used	Sample used (Methanolic extracts of raw materials and formulation)	Volume of sample taken (µl)	Order of spotting
1. Itrifal Kishneezi	10x10 cm^2	Gallic acid solution	1. Halela Zard extract 2. Halela Kabuli extract 3. Halela Siyah extract 4. Balela extract 5. Kishneez Khushk extract 6. Itrifal kishneezi extract	10µl each	1. Gallic acid standard 2. Kishneez Khushk extract 3. Halela Kabuli extract 4. Halela Siyah extract 5. Balela extract 6. Halela Zard extract 7. Itrifal kishneezi extract

Development of TLC plates

- **Mobile phase used for all the formulations:** Chloroform**:** Ethyl acetate**:** Formic acid (2.5: 2.0: 0.8).
- Approximately 37 ml of mobile phase was prepared for each formulation in the ratio 17.5:14.0:5.6.
- **Chamber saturation time:** 20 min.
- After chamber saturation, the plates were kept inside and developed till the mobile phase run was up to $3/4^{th}$ of the plate.
- Plates were removed out after development.

Microbial Screening: For the finished product microbialanalysis was done. (Gopala *et al.*, 2008).

Antimicrobial test: Formulation was checked for itsantimicrobial activity against *Klebsiella pneumonia, Proteusvulgaris, Salmonella typhae, Staphylococcus aureus, Escherichia coli* by Agar diffusion method (Gopala *et al.*,2008).

Stability studies: Comparison of the finished product (formulation) stored at room temperature for first, second, third month was carried out by conducting tests for the parameters gallic acid content, using HPTLC technique (Gopala *et al.*, 2008).

RESULT AND DISCUSSION

Botanical parameters revealed that brownish Greyish in color, with bitter odor, spicy taste (Table 1). Biochemical analysis showed the presence of carbohydrate, aminoacids and proteins (Table 2). Phytochemical analysis showed presence of tannins, flavonoides, glycosides and steroids (Table 3 and table 4).Microscopic analysis of sample showed the presence of identifying diagnostic characters, which are not overlapping. It shows presence of xylem thickening, Cork cells, xylem vessels, sclerides (Fig. 1).

Table 1: Organoleptic Characteristics.

Characteristics	Observation
Color	Brown
Taste	Intense Bitter
Odour	Sweet
Texture	Semisolid
consistency	Viscous

Itrifal Kishneezi: Microscopic evaluation of Itrifal Kishneezi showed presence of

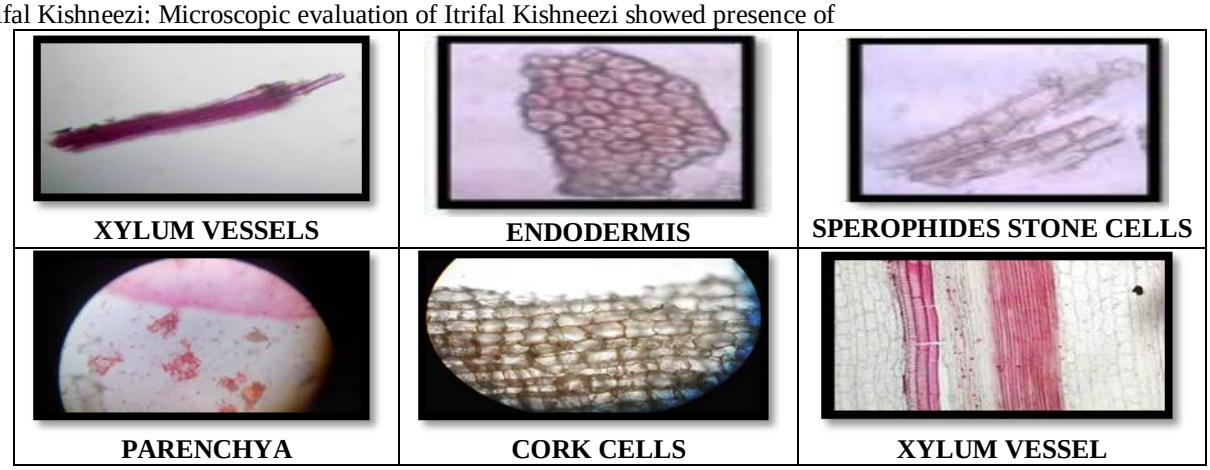

Fig. 1: Microscopic Analysis.

Table 2: Biomolecules tests.

Biomolecules	Test	Itrifal Kishneezi
Carbohydrate test	Fehling test	Present
Amino acid test	Ninhydrin	Present
Proteins tests	Folin-Lowry	Present

Table 3: Phytochemical tests.

Test	Tannins	Flavonoids	Glycosides	Steroids
Itrifal Kishneezi	Present	Present	Present	Present

Table 4: Physico-Chemical Parameters.

Test	Itrifal Kishneezi
1. Acid Value	1.390

TLC fingerprint profiles were established for Itrifal Kishneezi along with its ingredients using the marker component Tannins i.e Gallic acid as standard (Fig. 2).

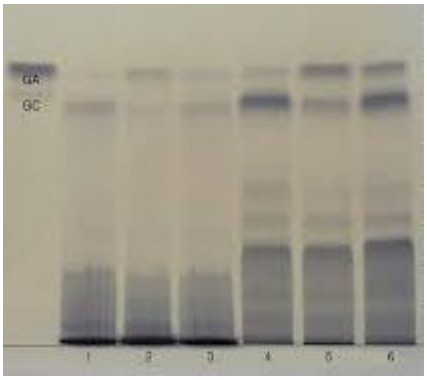

Fig. 2: HPTLC fingerprint of methanolic extracts.

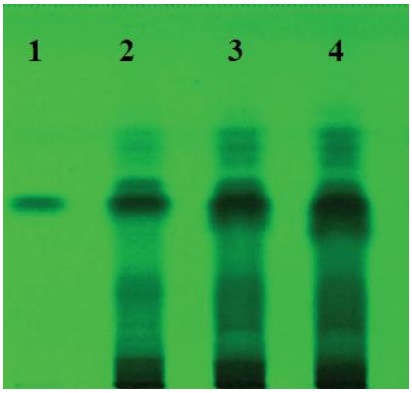

Fig. 3: Stability studies by HPTLC.

Key: 1 – Standard Gallic Acid, 2 –third month sample, 3- second month sample, 4- First month sample.

Itrifal Kishneezi: Kishneez khushk didn't show any spot under UV and even after treatment of the TLC plate with the detecting reagent. Hence, it doesn't contain tannin. Rest all of the raw materials showed the presence of tannins and their R_f values approximately matched to Gallic acid Standard. Formulation extract also showed a faint spot of tannin and a R_f value nearly matching to that of the standard and other raw materials. Thus, it can be concluded that this formulation contains these raw materials as its major ingredients (Fig. 3).

For the finished product, microbial analysis was done. Pathogens *Escherichia coli, Candida albicans*, were found to be inhibited by formulation (Table 5). Total aerobic count was done and bacteria, fungi and coliforms were found to be within limits.

Table 5: Antimicrobial analysis.

Test Organisms	Staphylococcus aureus	Escherichia coli	Salmonella typhae	Candida albicans	Klebsiella pneumonia
Itrifal Kishneezi	Negative	Positive	Negative	Positive	Negative

CONCLUSION

Standardization is maintaining the same physicochemical properties and quality of a product or formulation throughout the process of preparation and utilization leading to identical therapeutic efficacy an all batches.

Standardization of ASU project was done under the AYUSH department. Before starting the preparation of Formulation different pharmacognosis test can be done, like monograph. Different raw material test performed.
The standardization of Itrifal Kishneezi was carried out using various pharmacognostic tools like HPTLC and HPLC fingerprinting, macroscopic and microscopic analysis etc.

Quality control tests were done to analyse the raw material as well as formulation. Powder microscopy for raw materials were performed during and later-on in the process of preparation of ASU formulation. Tests like phytochemical test for saponins, tannins, glycosides, flavonoids, steroids & terpenoids. Biochemical test to check the presence of carbohydrates and proteins. HPLC and HPTLC fingerprinting, total ash value, bulk density of raw materials as well as for formulation to compare and check for the presence of marker compound.

The same protocol may be applied for as a regular development of drug, its quality control and standardization for polyherbal formulations.

Further studies are required to determine its mechanism of action and *in vivo* studies.

REFERENCES

1. Ahmed H, Sofi GD, Tajjudin R. Dang, Kumar N. Unani system of medicine introduction and challenges. Med J Islamic World AcadSci, 2010; 18: 27-30.
2. Butler MS. Natural products to drugs: Natural product derived compounds in clinical trials. Nat Prod Rep, 2005; 22: 162.
3. Chin YW, Balunas MJ, Chai HB, Kinghorn AD. Drug discovery from natural sources. AAPS J, 2006; 8: 239.
4. Eike R, Anne S. High performance Thin-Layer Chromatography for the Analysis of Medicinal Plants, Thieme medical publisher New York, 2006.
5. Gopala, K. R. Simha, V. Laxminzrzyan. Standardization of Navaka Guggulu- An ayurvedic formulation. Indian J of Traditional Knowledge, 2008; 7(4): 542-547.
6. Jain SK, Credibility of Traditional Knowledge-The criterion of multilocational and multiethnic use, Indain J of Traditional Knowledge, 2004; 13(2): 137-153.
7. Harborne JB. phytochemical methods-A guide to Moderen Techniques of Plant Analysis. Springer Private Ltd., Delhi, India, 2007.
8. Mitchell W. Principles and Practice of Dermatology, New York: Churchill Livingstone, 1996; 2: 7-13.

9. Mukherjee PK. (2002). Quality control of herbal drug- an approach to Evaluation of Botanicals Business Horizons publishers, New Delhi, India, 2002.
10. National Formulary of Unani Medicine, Part-1, 1st edn, Government of India, Ministry of Health and Family Welfare, New Delhi-110011, 1995.
11. Patel, P.M., Patel, N.M., Goyal, R.K. Evaluation of marketed polyherbal antidiabetic formulations uses biomarker charantin, *The Pharma Review*, 2006; 4(22): 113.
12. Patel, P.M., Patel, N.M., Goyal, R.K. Quality control of herbal products", *The Indian Pharmacist*, 2006b; 5(45): 26-30.
13. Perumal R. and Gopala KP. Current status of herbal and their future perspectives, Nature Precedings: hdl: 10101/npre, 2007; 1176.
14. Patil Sonali, Zafar S., bapat U., Bhoir M., Standardization and Stability studies of Jawarish – e- Bisbasa, an Unani formulation. Biological Forum-An International Journal, 2011; 3(2): 14-17.
15. Sonali Patil, Sharique Zafar. "Quality Assessment And Standardization Of The MAJOON NAJAH, A Polyherbal Unani Formulation."scijournal, 2017; 1(1).
16. Sonali Patil, Sharique Zafar "Integrated Approaches Towards Drug Development And Standardization Of TIRYAQ-E-PECHICH From Unani System Of Medicines", scijournal, 2017; 1(1).
17. Ram SM, Abdin MZ, Khan MA, Jha P. HPTLC fingerprint analysis: A Quality control of Authentication of Herbal Phytochemicals. Springer Verlag Berlin Heidelberg, 2011; 2011: 105.
18. Rodney C, Tonic M, Kutchan N Lewis G. Natural products. Biochemistry and molecular biology of the plants: Unani Pharmacopoeia of India, Vol I-IV, (Government of India ministry of health and family welfare department of Ayurveda, Yoga-Naturopathy; Unani, Sidha, homeopathy (AYUSH) New Delhi: 1995, 2002.
19. Sagar Bhanu P.S., Zafar, R., Panwar, R. Herbal drug standardization, *The Indian Pharmacist*, 2005; 4(35): 19-22.
20. Sazada, S., Arti, V., Ayaz, A., Faraha, J., Maheswari, M.K. *Preliminary Phytochemical analysis of Some Medicinal andAromatic Plants, Advance in Biological Research*, 2009; 3(5-6): 188-5.
21. Selvakumar, D., Anithakumari, R., Ramesh, R.V. Standardization of polyherbalayurvedic formulation, Mehari Choornam. *International J. of Pharmaceutical Science and Biotechnology*, 2010; 1(1): 43-47.
22. Sethi PD, Identification of Drugs in Pharmaceutical Formulations by Thin Layer Chromatography. CBS Publishers, New Delhi, 1992.
23. Thin Layer Chromatographic Atlas of Ayurvedic Pharmacopoeial Drug, Government of India Department of AYUSH New delhi, 2009.
24. Wagner H, Bladt S, Zgainski EM. Plant Drug Analysis, A thin layer chromatography atlas, Springer – Verlag Berlin Heidenberg New York Yokyo, 1984.