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Research Article

Integrated Approaches towards Drug Development and Standardization of TIRYAQ-E-PECHICH from Unani System of Medicines

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Abstract:

AYUSH is an Indian system of medicine which comprises of Ayurveda, Yoga, Unani, Siddha and Homeopathy. Unani medicines are an alternative approach to medical treatment that is practiced on a large scale in India. Tiryaq-E-Pechichis one of the Unani formulation which is known for its Abdominal disorders, Anti-pyretic, Heart ailment. Keeping in view the high medicinal importance of drug, it has been studied on the basis of physico-chemical and biological aspects. The parameters applied for the present study include qualitative and quantitative phytochemical tests, TLC fingerprinting, antimicrobial activity and stability studies.

In the light of results obtained it can be concluded that this study play very important role in the herbal drugs development and standardization. Present study is based on integrated approaches to standardize physico-chemical and biological basis that can be useful to supplement information with regards to its identification and shall be helpful in establishing the medicinal use.

Keywords: Physico-chemical studies, Phytochemical studies, Standardization, Quality control.

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ISSN: Awaited
Volume 1, Issue 1, 2017

Introduction:

With global realization that use of synthetic drugs is not safe on the long run, the medical fraternity at large is looking at alternatives from natural sources to combat diseases particularly those wherein conventional modern system of medicine has little to offer. On one hand, this realization has increased demand for herbal drugs and on the other hand need for quality standardization of these drugs has increased. WHO has emphasized the need to ensure quality control of medicinal plant products by using modern techniques and by applying suitable parameters and standards.

Standardization of herbal formulations is essential in order to assess the quality of drugs, based on the concentration of their active principles. The Department of AYUSH (Ayurveda, Siddha, Unani &Homoepathy) renamed in November, 2003 is responsible for designing, formulating & implementing policy for promoting & propagating the Indian systems of Medicines. It emphasis on upgradataion of AYUSH eductaional standards, quality control & standardization of drugs, improving the availability of medicinal plant material, research & development & awareness about the efficacy of the systems domestically & internationally.

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 (Sagar *et al.*, 2005).

The Unani system of medicine originated in Greece. Hippocrates is known as the father of this system of medicine. The theoretical framework of Unani medicine is based on his teachings. There were other Greek scholars who followed in his footsteps to enrich this system considerably.

In the 9th century, the Arabic and Persian physicians imbibed Unani system of medicine. In Unani medicine, single drugs or their combinations in raw forms are preferred over compound formulations. The naturally occurring drugs used in this system are usually free from any side effects while drugs that are toxic in crude

ISSN: Awaited
Volume 1, Issue 1, 2017

form are first processed and purified in many ways before use so as to make them free of any kind of side effects.

Supplements in the developing countries and the industrialized developed world are 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 (Patel *et al.*, 2006)

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

The importance of safety, quality and efficacy in such products has universally been acknowledged. There is an increasing demand for ASU / botanical drugs / dietary

and standardization of ASU drugs (Bhutani, 2003).

Preparation and Standardization of Unani formulation, **Tiryaq-E-Pechich** were carried out to know quality standards in this formulation. It is used to abdominal disorders, Anti-pyretic, Heart ailment.

Materials and methods:

Preparation of Tiryaq-E-Pechich: The formulations were prepared on the basis of the specifications laid down by the standard guidelines. The chief ingredients for the formulation are: Safed jeera, Ajwain desi, Halelazard (Each 40 gms) in powder form.

Mix all three ingredients i.e.

1. Post halelazard
2. Ajwain desi
3. Zerasafed

Mix well the safoof.

Storage and preservation: It was preserved in dried, airtight, fungus free clean glass or chinaclay container.

ISSN: Awaited
Volume 1, Issue 1, 2017

Experimental procedure: Physico-chemical analysis and Biological analysis

Organoleptic analysis, Total Ash value, acid insoluble Ash, water soluble extractive value, ethanol soluble extractive value, fixed oil content, loss on drying were determined (Iyengar, 1995; Trease and Evans Wc., 1989).

Phyto-chemical Analysis: Preliminary tests were carried out on methanolic extract for the presence / absence of phyto-constituents like alkaloids, carbohydrates, flavanoids, glycosides, saponins, sterols, terpenes and tannins (Sazada\ et al., 2009). ***TLC was performed for tannins with mobile phase*** Chloroform: Ethyl acetate: Formic acid (2.5:2.0:0.8) and detecting reagent as 5% FeCl3 solution.

1) Test for phytochemical constituents

Sr. No	Test	Observation
1	**Test for Tannins:-** Methanolic extract + water (1:1). boil for 5 minutes and filter. Filtrate + Ferric Chloride (feCl3)	Dark colour/ppt
2	**Test for Flavanoids :-** Methanolic extract + Lead acetate solution (1:1)	Yellow colour
3	**Test for Saponin glycoside (Foam Test) :-** Shake the drug extract or drug powder vigorously with water.	Peristant Form
4	**Test for Steroids :-** Methanolic extract + 2ml Chloroform (CHCl3) + 2ml Conc. Hydrochloric acid (HCl). Shake well	$CHCl_3$ layer appear red and acid layer shows greenish yellow fluorescence.

2) Biochemical Analysis

Solution	Reagents added	Observation
Standard	2ml of Std. + 5ml of Fehling's A + 5ml of Fehling's B	Blue coloured obtained
Tiryaq-E-Pechich	2ml of sample + 5ml of Fehling's A + 5ml of Fehling's B	Blue coloured obtained

ISSN: Awaited
Volume 1, Issue 1, 2017

Test for Proteins

Solution	Reagents added	Observation
Standard	1ml of Std. + 5ml of Alk. $CuSO_4$+ 0.5ml of F.C reagent	Blue coloured obtained
Tiryaq-E-Pechich	1ml of sample + 5ml of Alk. $CuSO_4$+ 0.5ml of F.C reagent	Blue coloured obtained

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

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

Standard plate count: This method was followed in order to enumerate the total aerobic count in a sample (Gopala *et al.*, 2008).

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

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

1 gm of sample was extracted with 10 ml of methanol in a reflux condenser for 1 hr, filtered and concentrated. Plates were developed using a mobile phase (consisting of Toluene: Ethyl acetate: Methanol (7:2:1 v/v), for comparing finished product with raw material.

For stability studies, Toluene: Ethyl Acetate: GAA (7.5:3:0.2) was used as the mobile phase. After that Densitometric scanning was performed on Camag TLC

ISSN: Awaited
Volume 1, Issue 1, 2017

scanner III in the operated by win CATS planer chromatography version 1.4.3.

Formulation	TLC plate dimension	Standard used	Sample used (Methanolic extracts of raw materials and formulation)	Volume of sample taken (µl)
Tiryaq-e-Pechish	10x10 cm^2	Gallic acid solution	1. *Ajwain Desi extract* *2. Halelazardextract* *3.Zeerasafed* extract 4.Tiryaq-e-Pechish extract	10µl each

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, cells of mesocarp, sclerides (Fig. 1). TLC fingerprint profiles were established for Tiryaq-e-Pechish along with its ingredients using the marker component Tannins i.e. Gallic acid as standard (Fig. 2).

Tiryaq-e-Pechish**:** Ajwain Desi 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 aR_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.

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

ISSN: Awaited
Volume 1, Issue 1, 2017

and coli forms were found to be within limits.

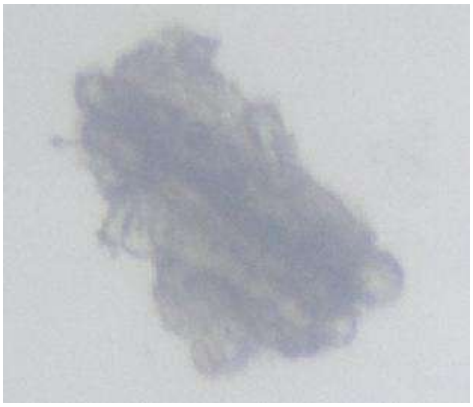
Organoleptic Characteristics (Table 1)

Characteristics	Observation
Color	Grayish
Taste	Bitter
Odour	Spicy
Texture	Fine
Consistency	Powder

Microscopic analysis (fig. 1)

Tiryaq-e-Pechish: Microscopic evaluation of Tiryaq-e-Pechish showed presence of

(a) Cells of mesocarp surface view (macerate)



(b) Xylem

(c) Rectangular sclereids

(d) Cork Cells

ISSN: Awaited
Volume 1, Issue 1, 2017

A. Biomolecules tests (Table 2)

Biomolecules	Test	Tiryaq-e-Pechish
Carbohydrate test	Fehling test	Present
Amino acid test	Ninhydrin	Present
Proteins tests	Folin-Lowry	Present

B. Phytochemical tests (Table 3)

Test	Tannins	Flavonoids	Glycosides	Steroids
Tiryaq-e-Pechish	Present	Present	Present	Present

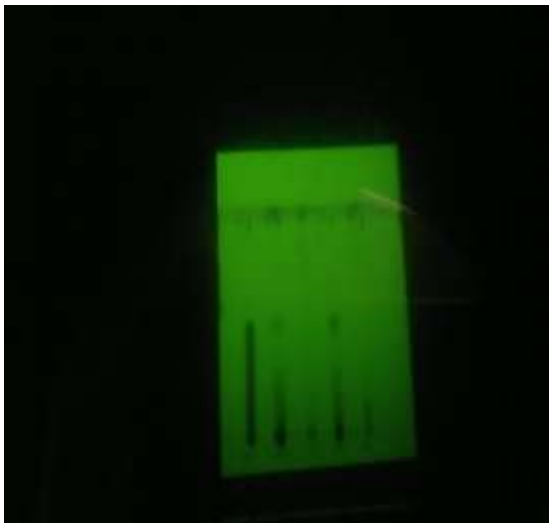
C. Physico-Chemical Parameters (Table 4)

Test	Tiryaq-e-Pechish
1.Acid Value	1.900
2.Saponification	256.90%

Thin layer chromatography and stability studies –

TLC plate was developed with suitable solution system and was made to run under 254 nm. The results are as follows:

TLC fingerprint of methanolic extracts under UV at 254 nm (Fig. 2).



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D. Antimicrobial analysis (Table 5)

Observation table:

Test Organisms	*Staphylococcus aureus*	*Escherichia coli*	*Salmonella typhae*	*Candida albicans*	*Klebsiella pneumonia*
Tiryaq-e-Pechish	Negative	Positive	Negative	Positive	Negative

Conclusion:

The analysis of sample Tiryaq-e-Pechishby different integrated approaches including physico-chemical and biological parameters like total ash, acid insoluble ash, water soluble extractive, alcohol soluble extractive, microscopic analysis, biochemical phytochemical analysis, HPTLC chromatogram, microbial screening and stability studies showed reproducible fingerprints between batches. So it can be concluded that these parameters can be used for the evaluation of Tiryaq-e-Pechish. 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.

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ISSN: Awaited
Volume 1, Issue 1, 2017

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