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Original Research Article

Pharmacognostical, physico-chemical and contamination studies of polyherbal formulation "Qurse pudina"

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ABSTRACT

Qurse pudina (QP), a poly herbal formulation, is used for the treatment of constipation and gas problems in both Unani and Ayurvedic System of Indian Medicine. In the present article, an attempt has been made to standardize the poly herbal formulation, QP. The QP was standardized with the help of microscopic, physico-chemical and contamination evaluation. This study revealed that total bacterial and fungal count and *Staphylococcus aureus* were found under limit, while *E. coli* was absent in the formulation. Pesticide residues, aflatoxin and toxic metals such as cadmium and mercury were not found in the formulation. Lead (0.75 mg/kg) and arsenic (0.50 mg/kg) were found under limit. The present outcome from contamination results suggests that QP could be considered as a good polyherbal formulation without showing any contamination or toxicity. The data evolved in the present work will aid in identifying the raw drugs used in finished product and will help to fix the scientific standards for QP.

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1. Introduction

The demand of traditional (herbal) medicine is increasing day by day due to their efficacy, rare chances of side effects in the treatment, good faith of society on herbal medicine and also their products (Rawat et al., 2003). India has a vast heritage of conventional systems of medicine for various ailments. Due to the lack of standardization and quality control measurements, people are unable to utilize the benefits of the conventional systems of medicine. Regarding this scientific awareness, a scenario has been created to undertake the research activities like standardization of conventional medicines and to develop the scientific methods for the manufacturing of quality medicines. Qurse pudina (QP) is one of the important Unani medicine categorized under the qurse categories, listed in the National Formulary of Unani Medicine, part-V. The QP is being used for the treatment of Zof-e-Meda (weakness of the stomach) and Muqawwi-e-Meda Hazim (stomachic) ailments (Panda, 2013). Qurs-e-Pudina is prepared from eight herbs, i.e., *Trachyspermum ammi* (L.), *Zingiber officinale* Roscoe, *Carum carvi* (L.), *Cuminum cyminum* (L.), *Mentha arvensis* (L.), *Foeniculum vulgare* Mill, *Cinnamomum zeyleniculum* Blume and *Piper nigrum* L.

It has been reported that thymol, a biomarker monoterpenic phenolic compound of *Trachyspermum ammi* has potential antioxidant, anti-microbial, anti-obesity activity, blood pressure lowering action as well as hypocholestrolemic behavior through inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity (Aftab et al., 1995; Haque et al., 2014; Haque et al., 2018). Cuminaldehyde, a quality biomarker compound of *Cuminum cyminum* L and *Carum carvi* L. has been reported to have antiobesity, antioxidant activity, anti-hypergly-

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cemic and antihyperlepidemic effects (Haque et al., 2013; Haque and Ansari, 2018; Haque and Ansari, 2019). *Zingiber officinale* extract has been investigated to possess different pharmacological activities such as anti-inflammatory, antiplatelet, antioxidant, antihypercholesterolemia, hypolipidaemic and hepatoprotective impacts (Chan et al., 2008; Liao et al., 2012; Al-Noory et al., 2013; Shivashankara et al., 2013; Montserrat-de la Paz et al., 2018; Kumar et al., 2018). *Cinnamomum zeyleniculum* typically contains high amounts of cinnamaldehyde and small amounts of eugenol, among many other aromatic compounds. *Cinnamomum zeyleniculum* and cinnamaldehyde have been studied for their antibacterial (Bardají et al., 2016), anti-diabetic (Anderson et al., 2013; Sartorius et al., 2014), anti-inflammatory (Mendes et al., 2016; Chen et al., 2016) and anticancer (Yang et al., 2015) activities, among others. The essential oil of *Foeniculum vulgare* finds application as additive in the food, pharmaceutical, cosmetic, and perfume industries (Tinoco et al., 2007); it also has important medicinal properties, such as diuretic, anti-inflammatory, analgesic, antioxidant, antiseptic, sedative, carminative, stimulant, and vermifugal activities (Tinoco et al., 2007; He and Haung, 2011). Biological activities of *Piper nigrum* have been known as insecticidal (Upadhyay and Jaiswal, 2007; Vinturelle et al., 2017), antioxidant (Nahak and Sahu, 2011), and antimicrobial activities (Morsy and Abd El-Salam, 2017). The leaves of *Mentha arvenisis* L. are extensively used in conventional system for various medicinal purposes such as stomachic, expectorant, inflammation of liver, peptic ulcer, diarrhea, bronchitis, cardiotonic, diuretic, dentifrice, jaundice, hepatalgia, and skin diseases (Chopra and Chopra, 1994; Khare, 2004). QP containing all herbs is very active therapeutically as per above mentioned literature. However, it is necessary to check the quality, purity and safety profile of QP. Therefore, an attempt has been made to scientifically evaluate the Unani compound formulation, QP.

2. Experimental

2.1. Procurement of plant materials

All the ingredients were procured from Unani raw drug dealers (Shamsi dawakhana, Ballimaran, New Delhi) at the month of December 2011 and identified by expert botanical taxonomist Dr. H.B. Singh (scientist B), from National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, India. Voucher specimen and identification certificate reference number NISCAIR/RHMD/Consult/2011-12/1753/53 was obtained and kept in the Department of National Institute of Science Communication and Information Resources (NISCAIR) for future reference.

2.2. Chemicals and reagents

Pesticide residues standards, e.g. organochlorides, organophosphates and pyrethins groups were procured from Sigma (Aldrich). Aflatoxins standards solutions were obtained from Sigma (Aldrich) and kept at 20 ºC in a colored amber vial.

2.3. Method of preparation

The QP was prepared as per the formulation composition given in Table 1. All the ingredients were taken of pharmacopeial quality and quantity. The ingredients 1 to 11 of the formulation were accurately weighed and powdered by grinder and passed through No. 80 mesh sieves. Qiwam was prepared by dissolving 2.25 kg of sugar in 1.5 L of purified water and boiling till two tar consistency (68%) obtained. The consistency was recorded using a standard refractometer. The powder was then mixed with qiwam made a semisolid mass (lubdi) and granulated by passing through No. 12 mesh sieve. The granules were dried and passed through No.16 mesh sieve and powdered saang jarhat saeeda and magnesia fahmi were added. Finally, the granules were subjected to tableting machines to make tablet (Panda, 2013).

2.4. Powder microscopy

Finished product (5 g) was mixed with 50 mL of water in a beaker with gentle warming, till the sample completely dispersed in water. This solution was centrifuged and the supernatant was decanted. The sediment washed several times with distilled water, centrifuged again and the supernatant was decanted. A 5 mg of the sediment was taken and mounted in glycerin and the salient features and drawings of the drug were observed using camera lucida fitted in compound microscope (Magnification 100X) (Meena et al., 2010).

2.5. Physico-chemical studies

The prepared three batch samples were subjected for analytical parameters such as physico-chemical studies like total ash, acid insoluble ash, water soluble ash, together with alcohol and water extractive and loss on drying at 105 ºC. The pH value for 1.0% aqueous solution was also monitored. All physicochemical parameters were determined as per official standard methods (Dutt et al., 2020).

2.6. Contamination studies

Pesticide residue was carried out by official standard methods (Weaver et al., 2010). For pesticide residues determination, Thermo Finnigan GCMSMS was used with DB-5 fused silica capillary column (30 m X 0.25 mm i.d., 0.25 µm film thicknesses). Helium was used as the carrier gas at a flow rate of 1.0 mL/min and mass spectrometer ion trap detector type was used. The injection port was maintained at 250 ºC, and the split ratio was 40:1, while oven temperature programming was done from 60 ºC hold 1.5 min, 60 to 120 at 15 ºC/min, 120 to 220 at 8 ºC/min, 220 to 280 at 5 ºC/min, hold 5 min. Interface temperature was maintained at 250 ºC. Ionization source temperature was at 230 ºC, and 70 eV electron impact modes were employed. Ionization mode was electron impact ionization and the scanning range was from 40 amu to 400 amu. For aflatoxins es-

Table 1
Formulation composition of QP.

S. No.	Unani name	Botanical name/English name	Parts	Quantity
1	Ajwayin Desi	*Trachyspermum ammi* (L) Sprague, API	Fruit	200 g
2	Badiyan	*Foeniculum vulgare* Linn, UPI	Fruit	200 g
3	Podina Khusk	*Mentha arvensis* Linn, UPI	Arial part	200 g
4	Darchini	*Cinnamomum zeyleniculum* Blume, UPI	Bark	25 g
5	Zanjabeel	*Zangiber officinal* Rose, UPI	Rhizome	200 g
6	Zeera Siyah	*Carum carvi* Linn, UPI	Fruit	150 g
7	Zeera Safaid	*Cuminum cyminum* Linn, API	Fruit	50 g
8	Filfil Siyah	*Piper nigrum* Linn.UPI	Fruit	80 g
9	Gond	Gum, IP	-	125 g
10	Namak Siyah	Kala namak, IP	Crystal	200 g
11	Naushadar	Sal Ammonium chloride, UPI	Crystal	80 g
12	Qiwam Shakar Safaid	Sugar, IP	Qiwam	700 g
13	Saang Jarhat Saeeda	Hydrated Magnessium Silicate, IP	Crystal	25 g
14	Magnesia Fahmi	Magnesia, IP	Crystal	5 g

timation, Agilents LCMSMS (Model: 6410B) was used with RRLC column: C18, 50 mm X 2.1 mm, 1.8 µm particle size and maintained 40 ºC. Mobile phase was used as 0.1% formic acid + 5 mM ammonium acetate in water and methanol at a flow rate of 0.2 mL/min. Mass spectrometer QQQ detector type was used for analysis.

2.7. Microbial load determination

2.7.1. Total fungal count

One mg sample powder was mixed in 100 mL of phosphate buffer (pH 7.2). One mL of the preparation was added to 15 mL of the liquefied potato dextrose agar medium in two petri dishes at not more than 45 ºC and incubated at 25 ºC for seven days. Thereafter, the dishes were observed and numbers of colonies were counted (Haque et al., 2012a).

2.7.2. Total bacterial count

One mg of QP powder was suspended in 100 mL of buffered sodium chloride-peptone solution (pH 7). Polysorbate 80 (0.1% w/v) was added to assist the suspension of poorly wettable substances. One mL of the preparation and about 15 mL of the liquefied casein soyabean digest agar were added to two petri-dishes at not more than 45 ºC and incubated at 30 ºC to 35 ºC for 4 days. The dishes were then observed and numbers of colonies were counted (Haque et al., 2012a).

2.7.3. Test for *Escherichia coli*

One mg of the QP powder was suspended in 100 mL of buffered lactose broth by shaking in a sterile screw-capped jar. Polysorbate 80 (0.1% w/v) was added to assist the suspension of poorly wettable substances. 1 mL of the preparation was transferred to a sterile screw-capped container and 50 mL of nutrient broth was added. Preparation was then shaken and allowed to stand for 1 hour and shaken again. The cap was loosened and jar was incubated at 37 ºC for 24 h. The dishes were tested to test the presence of acid and gas as per standard procedure (Haque et al., 2012a).

2.7.4. Test for *Staphylococcus*

One mg of the QP powder was suspended by shaking with 100 mL of nutrient broth in a sterile screw-capped jar and allowed to stand for 4 h and shaken again. The cap was loosened and jar was incubated at 35 ºC to 37 ºC for 24 h.1.0 mL of the enrichment culture was added to soyabean-casein digest medium. Medium was examined for the presence of growth. A portion of medium was streaked on the surface of Vogel-Johnson agar and Mannitol-salt agar medium. Plates were incubated at 36 ºC to 38 ºC for 18 to 24 h and observed for the presenceof black and yellow colonies surrounded with yellow zones (Fig.s 1-4) (Haque et al., 2012a).

2.7.5. Toxic metal analysis

Analysis of these toxic metals was performed as per standard method (Badea, 2015). A Perkin Elmer Elan 6000 ICP-OES quipped with an As-91 auto sampler was used. The instrument was calibrated using refer-

ence standards of 1 ppm and 10 ppm. Approximately, 0.1 mg of sample was accurately weighed into a metal free container and dissolved in 1 mL of aquaregia and heated on a hot plate to extract the metal. Then, the solution was filtered in a volumetric flask and washing of deionized water was added to it and volume made up to 10 mL. The solution was used for toxic metal analysis.

2.8. Thin layer chromatography

2.8.1. Preparation of extracts

QP powder (5 g) was macerated in alcohol (100 mL) for 18 hours, refluxed for ten minutes on water bath and filtered. The filtrates were concentrated on water bath and made up to 5 mL in a standard flask, separately.

2.8.2. Development of thin layer chromatography

The methanolic extract was applied on prepared pre-coated silica gel 60 F254 TLC plate (E. Merck) as absorbent and developed the plate using solvent systems, toluene: ethyl acetate (9:4). The methanolic extract of QP was spotted in the form of a band (3.0 mm) with a CAMAG microlitre syringe (width 8 mm) on a TLC plate (20 × 10 cm with 0.2 mm thickness, E. Merck, Berlin, Germany) using a CAMAG Linomat V sample applicator (CAMAG, Muttenz, Switzerland) attached to CAMAG HPTLC system and TLC scanner III, controlled by win-CATS-IV software.
A constant application rate of 120 nLs^{-1} was employed and the space between two bands was 0.8 cm.The slit dimension was kept at 0.3-0.02 cm and a scanning speed of 20 mms^{-1} was employed. The development was carried out in a linear ascending manner in a twin trough glass chamber (20 × 10 cm) and the optimized chamber saturation time for the mobile phase was 30 min at room temperature. The chromatogram was developed up to the length of 8.5 cm (Haque et al., 2012b). After developing, the plates were dried and observed the colour spots at UV-254, UV-366 nm and vanillin-sulphuric acid as spraying reagent. Anisaldehyde-sulphuric acid was used as spraying reagent in the quantification of thymol (Fig. 5 and Fig. 6).

2.8.3. Calibration curve of thymol

A stock solution of thymol (1 mg/mL) was prepared in methanol. 1, 2, 3, 4 and 5 µL from the stock solution were spotted on TLC plate in six (n=6) to obtain final concentration range of 1000-5000 ng/spot. The data of peak area versus drug concentration were treated by linear least-square regression. QC samples chosen for the study were 1000, 3000 and 5000 ng/pot (Fig. 7).

2.9. Tablet testing parameters

All tablet parameters such as average weight, thickness, hardness, weight variation, friability and disintegration were determined as per described in Indian Pharmacopoeias (Manjula et al., 2020).

3. Results and Discussion

3.1. Organoleptic properties

Appearance, color, and texture play an important role such as in quick identification. Appearance, color, smell, taste, and texture of tablets prepared as per the standard protocol were found acceptable. The presence of a particular smell/odor could be characteristics of a drug and indicates quality and identity of particular drug. QP is a solid powder preparation, yellowish brown in colour having sweet taste and characteristics odour. The results obtained in organoleptic studies reported herein established the macroscopic parameters that characterize the genuine QP. These organoleptics characteristics can be utilized for quick identification of the QP and are particularly useful in the case of powdered materials.

3.2. Microscopically observation

Microscopically observations of the QP were shown in Fig. 8. A few mg of the powder was taken and mounted in glycerin. Then, few drops of phloroglucinol and concentrated hydrochloride acid were added. The salient features and drawings of the drug were observed using Motic microscope. The preparation examined under high magnification showed starch grain and stone cell (Filfil Siyah), unicellular warty trichome (Ajwayin), glandular trichome and covering trichome (Podina Khusk), fibre, (Zanjabeel), oil cell (Darchini) and scalerides (Zeera safaid). The results obtained in powder microscopy studies reported herein established the microscopic parameters that characterize the genuine QP. These microscopic characteristics can be utilized for identification and purity of the formulation and are particularly useful in the case of powdered materials.

3.3. Physico-chemical analysis

The physico-chemical constant determination of the formulation is an important parameter in detecting adulteration. One of the important parameters in the determination of herbal formulation is the ash valuethe total ash content and the acid insoluble ash value. The total ash is particularly important in the evaluation of purity of drugs, i.e., the presence or absence of foreign inorganic matter such as metallic impurities and/or silica (Bhagyanathan and Thoppil, 2015). Quantitative standards exposed that the ash content was 13.47 % and 2.8% of acid insoluble siliceous matter was detected in the drug.
The percent extractives in different solvents indicate the quantity and nature of constituents in the extracts. The extractive values are also helpful in estimation of specific constituents soluble in a particular solvent. Extractive values of the plant with different solvents give a preliminary idea of the percentage of the compounds extracted (Yi-Zeng et al., 2004). Water and alcohol yielded 67.68 and 7.55% extractive (Table 2), among this water is more efficient to extract most of the phytoconstituents from the formulation. The aqueous extraction isthe most common and effective method for the preparatio-

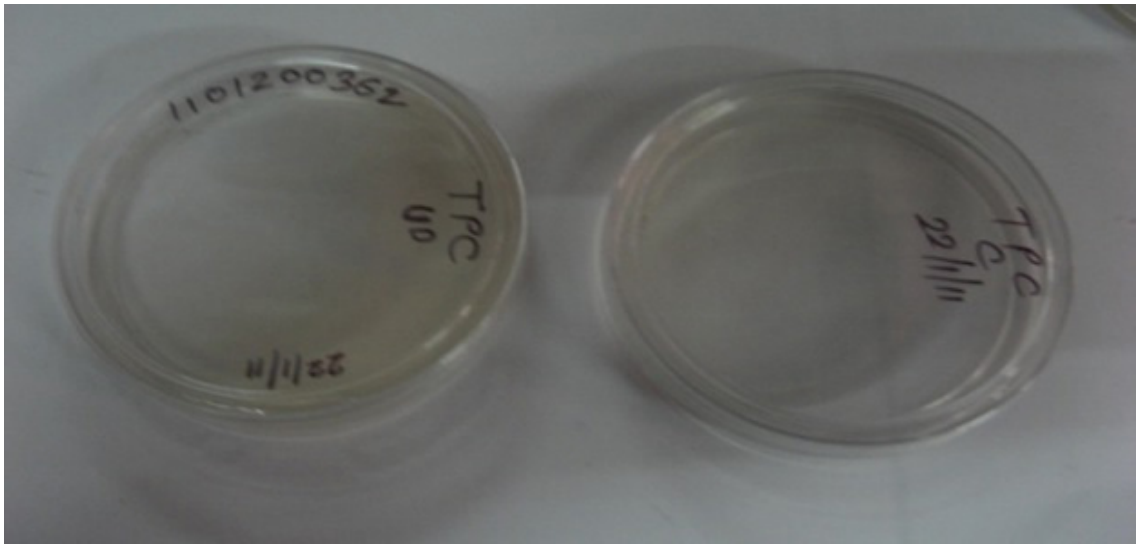

Fig. 1. Observation of growth of total potential counts (TPC) in petriplate dish containing liquefied casein soya bean digest agar.

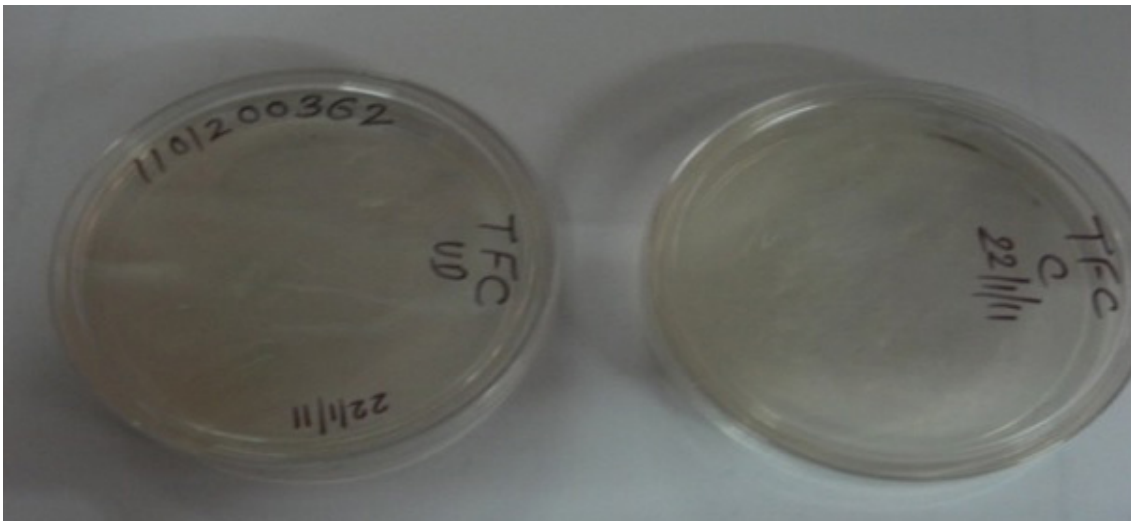

Fig. 2. Observation of growth of total fungal counts (TFC) in petriplate dish containing liquefied potato dextrose agar medium.

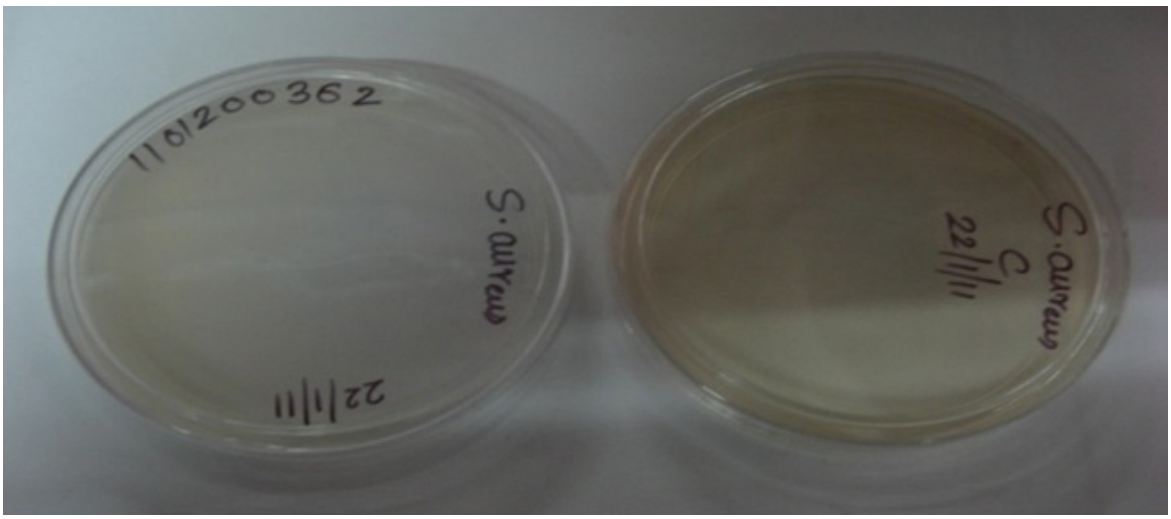

Fig. 3. Observation of growth of *S. aurea* in petriplate dish containing bismuth sulphite agar and brilliant green agar medium.

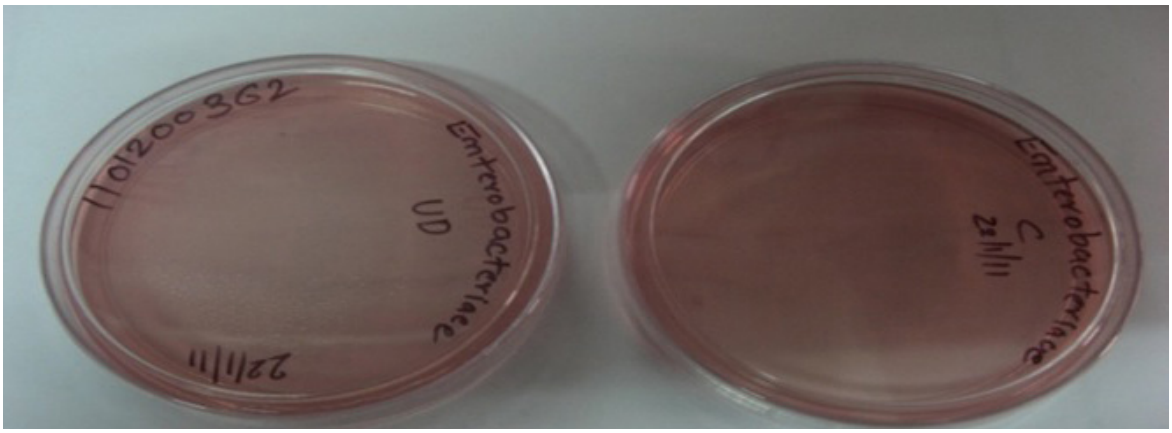

Fig. 4. Observation of growth of *E. coli* in petriplate dish containing vogel-johnson agar and mannitol-salt agar medium.

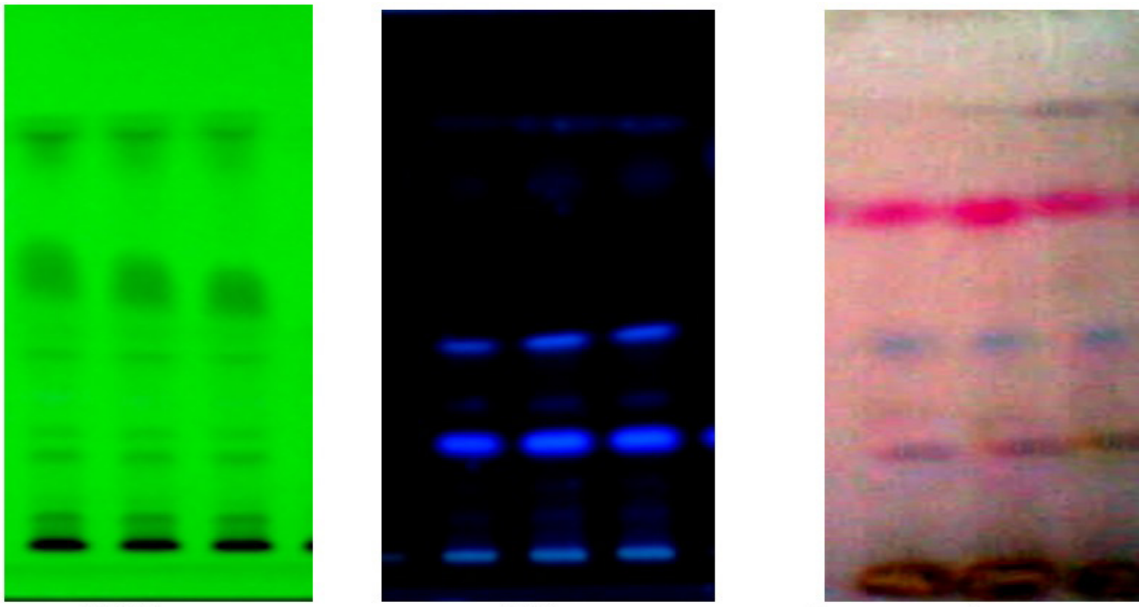

Fig. 5. TLC plate of alcoholic extract of three batches at 254 nm, 366 nm and vanillin. sulphuric acid agent.

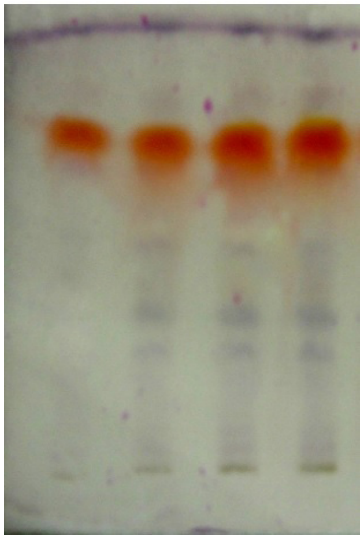

Fig. 6. TLC plate of alcoholic extract of three batches and standard thymol at anisaldehyde sulphuric acid agent.

Table 2
Physico-chemical parameters of the QP.

S. No	Parameters	Batch 1	Batch II	Batch III
1	LOD (%W/W)	5.13	5.15	5.2
2	Total Ash (%W/W)	13.47	13.5	13.4
3	Acid insoluble ash (%W/W)	2.85	2.8	2.75
4	Alcohol extractive (%W/W)	7.56	7.5	7.6
5	Water extractive (%W/W)	67.69	67.65	67.7
6	pH (1%)	4.7	4.7	4.7

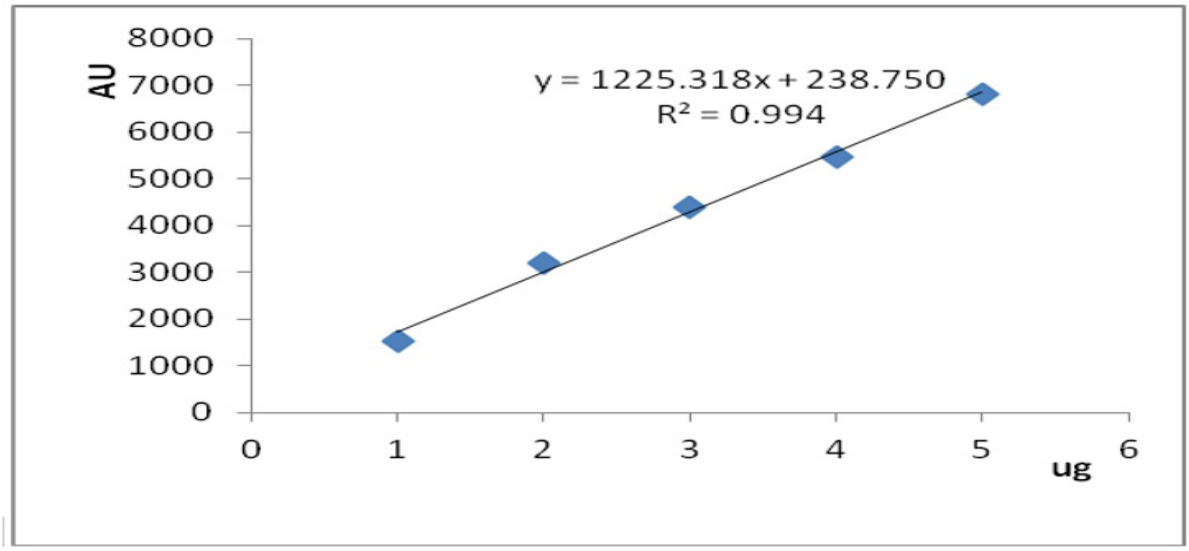

Fig. 7. Calibration curve of standard thymol.

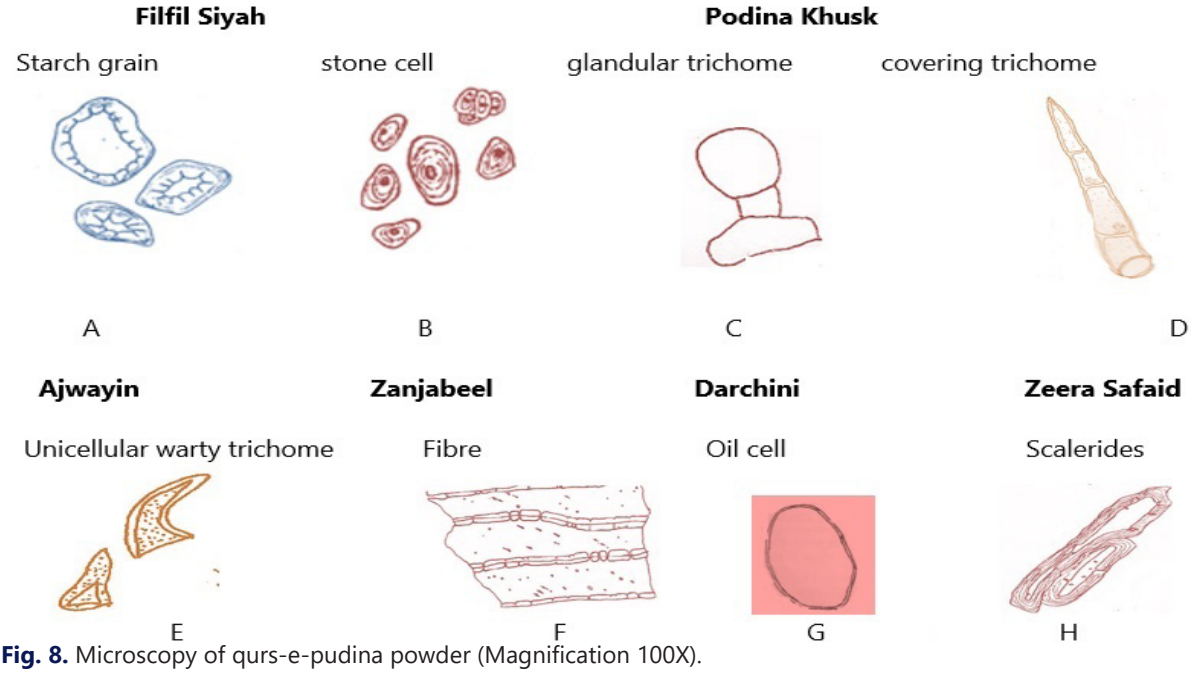

Fig. 8. Microscopy of qurs-e-pudina powder (Magnification 100X).

ns of medicinal plant based drugs.

3.4. HPTLC profile and quantification of thymol

The outcomes from HPTLC fingerprints study indicated that the formulation contain an appreciable amount of bioactive compounds. TLC analysis is one of the best methods for characterization and standardization of polyherbal formulations. The number of spots and R_f value of each spot in a particular mobile phase is an index of identity, purity, strength and quality of a drug and plays a major role to determine adulteration in drug.

The alcoholic extract of all the three batch samples showed identical spots at UV-254 nm, UV-366 nm and vs. reagent. Ethanolic extract on silica gel G plate using toluene: ethyl acetate (9:4) as mobile phase showed six spot under (254 nm) at R_f 0.02 (dark green), 0.08 (light green), 0.13 (light green), 0.32 (light green), 0.37 (dark green) and 0.80 (light green). Ethanolic extract on silica gel G plate using toluene: ethyl acetate (9:4) as mobile phase showed six spot under (366 nm) at R_f 0.04 (light blue), 0.08 (light blue), 0.12 (dark blue), 0.17 (light blue), 0.32 (dark blue), 0.86 (light blue). After spraying the plate with vanillin-sulphuric acid reagent followed by heating at 110 for 10 min, it showed three spots at R_f 0.10 (light violet), 0.32 (light blue) and 0.79 (dark blue). The HPTLC profiles can be used for the identification and evaluation of the quality of the herbal formulation. The densitometric HPTLC fingerprint profiles (Fig. 2 and Fig. 3) may be used as marker for quality evaluation and standardization of the drug. Thus, HPTLC fingerprint profile along with their R_f values were recorded, which would serve as a reference standard for the scientist engaged in research on the medicinal properties of plant (Haque et al., 2012b). The compounds corresponding to the spots observed may be responsible to render various bioactivities possessed by the herbal formulation. Thymol content was found 0.856 ± 0.43%.

3.5. Contamination analysis

3.5.1 Aflatoxins and pesticide residue analysis

As some secondary metabolites produced by moulds could be toxic to humans, the European legislation has set maximum levels of mycotoxins (aflatoxin B1 and sum of B1, B2, G1 and G2) for a variety of foostuffs and spices. Microbiological and mycotoxicological quality assessment of medicinal herbs should include mycotoxin contamination, especially of the parts at higher risk of contamination or herbs from hot and humid climates. Aflatoxins were differentiated as B (blue fluorescence) and G (green fluorescence). Generally, aflatoxins produce liver toxicity when it accumulates in the product higher than limits (McPartland, and McKernan, 2017). Aflatoxin was absent in the QP (Table 3). Thus, this formulation may be protected from acute toxicity and liver carcinogenicity due to the lack of aflatoxins toxicity. Pesticides are chemical compounds used to control or eradicate pests. The main adverse effects associated with overexposure to pesticides are symptoms of the nervous system, including headache, dizziness, paraesthesia, tremor, discoordination, or convulsions. Pesticide residue such as *o*,*p*-DDD, *p*,*p*′-DDD, *o*,*p*-DDE, *p*,*p*′-DDE, *o*,*p*′-DDT, *p*,*p*′-DDT, endosulfan, α-HCH, β-HCH, γ- HCH, δ-HCH etc was absent in all the drug (Table 4).

3.5.2. Toxic metal analysis

Metals are widely distributed throughout nature and occur freely in soil and water. As they are likely to be present in many foods, it is important to reduce the total population exposure to toxic elements by minimizing contamination of herbal products. Nevertheless, limits for toxic elements in herbal products are yet to be set at the global level (McPartland, and McKernan, 2017). However, the European Pharmacopoeia has issued a draft monograph herbal drugs, proposing the following limits for heavy metals in herbal drugs: 5 mg kg^{-1} for lead, 0.5 mg kg^{-1} for cadmium, and 0.1 mg kg^{-1} for mercury. For herbal drugs known to accumulate toxic metals, the European Pharmacopoeia has set the following limits: 90 mg kg^{-1} for arsenic, 0.5 mg kg^{-1} for cadmium, 5 mg kg^{-1} for lead and 0.1 mg kg^{-1} for mercury in some medicinal plants (Haque et al., 2012a). Toxic metals like arsenic and lead were found under limits in the product, while cadmium and mercury were not detected in the QP (Table 5).

3.5.3 Biological contamination (microbial contamination)

Biological contamination refers to impurities in medicinal herbs, their preparations and products, and may involve living microbes such as bacteria and their spores, yeasts and moulds, viruses, protozoa, insects (their eggs and larvae), and other organisms. However, products of microbial metabolism such as toxic, low-molecular-weight metabolites from moulds are chemical contaminants. Microbial contamination of herbs and/or products may result from improper handling during production and packaging. The most likely sources of contamination are microbes from the ground and processing facilities, e.g. contaminated air and microbes of human origin. Cross contamination is also possible from extraneous materials such as plastics, glass, and other materials which come in contact with medicinal herbs, herbal preparations or products (McPartland, and McKernan, 2017). Hypothetically, sources of biological contamination could be human excrement, animal manure and faeces used as fertilizers. Medicinal plant material generally carry a various number of microbes, often originating in the soil, while a large range of microbes form the naturally occurring micro flora of herbs, aerobic spore forming bacteria frequently predominate. Current practice of harvesting, handling, storage and production may cause additional contamination and microbial growth (Haque et al., 2012a). Microbial count is just one of medicinal herb quality indicators. All products must be clear of true bacterial pathogens. Study results revealed that total bacterial count and total fungal

Table 3
Observations of aflatoxins residues in QP.

S. No.	Test parameter	Test method	Result
1	Aflatoxin B1	AOAC 990.33	Not Detected
2	Aflatoxin B2	AOAC 990.33	Not Detected
3	Aflatoxin G1	AOAC 990.33	Not Detected
4	Aflatoxin G2	AOAC 990.33	Not Detected

Table 4
Observations of pesticides residues in QP.

S. No.	Pesticides	Test method	Result
1	α-BHC	AOAC 970.52/EPA 525.2	Not Detected
2	β-BHC	AOAC 970.52/EPA 525.2	Not Detected
3	γ-BHC(Lindane)	AOAC 970.52/EPA 525.2	Not Detected
4	δ-BHC	AOAC 970.52/EPA 525.2	Not Detected
5	Heptachlor	AOAC 970.52/EPA 525.2	Not Detected
6	Heptachlor_Epoxide	AOAC 970.52/EPA 525.2	Not Detected
7	α-Chlordane	AOAC 970.52/EPA 525.2	Not Detected
8	α-Endoulfan	AOAC 970.52/EPA 525.2	Not Detected
9	β-Chlordance	AOAC 970.52/EPA 525.2	Not Detected
10	Endrin	AOAC 970.52/EPA 525.2	Not Detected
11	Total DDE	AOAC 970.52/EPA 525.2	Not Detected
12	Total DDD	AOAC 970.52/EPA 525.2	Not Detected
13	Total DDT	AOAC 970.52/EPA 525.2	Not Detected
14	β-Endoulfan	AOAC 970.52/EPA 525.2	Not Detected
15	Endrin aldehyde	AOAC 970.52/EPA 525.2	Not Detected
16	Endoulfan sulfate	AOAC 970.52/EPA 525.2	Not Detected
17	Aldrin	AOAC 970.52/EPA 525.2	Not Detected
18	Endrin ketone	AOAC 970.52/EPA 525.2	Not Detected
19	Methoxychlor	AOAC 970.52/EPA 525.2	Not Detected
20	Dieldrin	AOAC 970.52/EPA 525.2	Not Detected
21	Alachlor	AOAC 970.52/EPA 525.2	Not Detected
22	Butachlor	AOAC 970.52/EPA 525.2	Not Detected
23	Monocrotophos	AOAC 970.52/EPA 525.2	Not Detected
24	Phorate	AOAC 970.52/EPA 525.2	Not Detected
25	Mevinphos	AOAC 970.52/EPA 525.2	Not Detected
26	Dimethoate	AOAC 970.52/EPA 525.2	Not Detected
27	Malathion	AOAC 970.52/EPA 525.2	Not Detected
28	Methyl-parathion	AOAC 970.52/EPA 525.2	Not Detected
29	Chlorpyrifos	AOAC 970.52/EPA 525.2	Not Detected
30	Ethion	AOAC 970.52/EPA 525.2	Not Detected
32	Simazine	AOAC 970.52/EPA 525.2	Not Detected
33	Diazinon	AOAC 970.52/EPA 525.2	Not Detected
34	Phosphamidon	AOAC 970.52/EPA 525.2	Not Detected
35	Fenitrothion	AOAC 970.52/EPA 525.2	Not Detected
36	Fenthion	AOAC 970.52/EPA 525.2	Not Detected

Table 4 Continued

37	Phosalone	AOAC 970.52/EPA 525.2	Not Detected
38	Quinalphos	AOAC 970.52/EPA 525.2	Not Detected
39	Coumaphos	AOAC 970.52/EPA 525.2	Not Detected
40	Parathion	AOAC 970.52/EPA 525.2	Not Detected
41	Malaoxon	AOAC 970.52/EPA 525.2	Not Detected
42	Dichlorvos	AOAC 970.52/EPA 525.2	Not Detected
43	2,4-D	PAM Vol I/EPA 515.3	Not Detected

Table 5
Observations of toxic metal residues.

S. No.	Test parameter	Test method	Results	WHO limits
1	Cadmium (Cd)	ICP-OES	Not Detected	0.3 mg/kg
2	Lead (Pb)	ICP-OES	0.75 mg/kg	10 mg/kg
3	Arsenic (As)	ICP-OES	0.50 mg/kg	-
4	Mercury (Hg)	ICP-OES	Not Detected	-

Table 6
Observations of microbial load in QP.

S. No.	Parameter analyzed	Results	WHO limits
1	Total Bacterial Count	682	10^5 CFU/gm
2	Total Fungal Count	< 10	10^3 CFU/gm
3	*Staphylococcus aureus*	<10	Nil
4	*E. coli*	Absent	Nil

Table 7
Observations of tablet testing parameters in QP.

S. No.	Parameter analyzed	Results
1	Average weight	762 ± 0.34 mg
2	Thickness	0.565 ± 0.64 mm
3	Hardness	4.02 ± 0.84 Kg/cm^2
4	Weight variation	-0.057 to + 0.039 % W/W
5	Friability	0.562 ± 0.064 % W/W
6	Disintegration time	28 ± 1.64 minute

count were found to be within the permissible limit (Table 6). Bacteria *Staphylococcus aureus* was found under limit and *E. coli* was absent in the formulation.

3.6. Pharmaceutical quality

Friability is an important parameter to measure the strength and quality of tablets. Friability of QP was found to be within the acceptable limit, i.e. 0.5-1%. Hardness was found to be 4.02 ± 0.084 kg/cm (Table 7). It was within the acceptable limit of 4 kg. It is essential to hold up mechanical distress during manufacturing, packaging, storage and transportation (Ali et al., 2016). Hardness is one of the issues in herbal tablets and special attention was given to improve hardness of QP, hardness of 4 kg was achieved by binder 20% of total wt. of powder (16% in the formulation). Disintegration time in aqueous media was found to be 28.00 ± 1.64 min. This test represents breakdown of tablets into smaller particles and shows that QP disintegrate within the permissible limit (maximum 30 min) (Yi-Zeng et al.,

2004; Ali et al., 2016) when placed in liquid medium under the experimental circumstance.

4. Concluding remarks

The microscopic features, physic-chemical parameters along withTLC profiles altogether may be used for quality evaluation and the standardization of the compound formulation of QP. The data generated in this analysis will help in setting up regulatory limit viz. microscopy, physico-chemical properties, HPTLC fingerprints and pharmaceutical quality to ensure the quality of an Indian medicine. A routine use of such scientific techniques will lead to standardization of the herbal products to a certain extent and would definitely help in building confidence in use of such polyherbal products for medication.

Conflict of interest

The authors declare that there is no conflict of interest.

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