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AYUSH KWATHA FOR THE MANAGEMENT OF URT SYMPTOMS IN PRE-SYMPTOMATIC TO MILD COVID-19 INFECTION: PHARMACEUTICAL STUDY TO IMPROVE COMPLIANCE

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ABSTRACT

Ayurveda: The science of traditional medicine practiced abundantly in South East Asia has holistic approach and aims principally to prevent the onset of the disease. Ayush Kwatha one of the Ayurvedic formulation advised by Ministry of AYUSH, helps in improving the health of upper respiratory tract can be used in COVID-19. It comprises of *Oscimum sanctum*, *Cinnamomum zeylanicum*, *Zingiber officinale*, *Piper nigrum.* These drugs are known for its pharmacological activity like immuno-modulatory activity, anti-tussive activity, anti-asthamatic activity, analgesic, adaptogenic activity/anti-stress activity, antipyretics activity and anti-inflammatory activity. Ayush Kwatha is used as hot decoction but, every time making decoction is time consuming and tedious procedure leading to reduction in compliance. Thus the present study is designed to prepare Ayush Kwatha in such form to improve the palatability and potency of the formulation. Ayush Kwatha was prepared in four different forms viz. AKC- conventional method, AKV-DW with distilled water, AKV-NS with Nimbu swarasa and AKV-Aq. Ex. with aqueous extract. The finished product of each batch was subjected to analytical tests on parameters obligatory to assure its acceptable quality (by Central Council for Research in Ayurvedic Sciences-CCRAS). All the batches were found analytically optimal on Quality control parameters. It can be used safely to manage symptoms of upper respiratory tract in mild to moderate illness of COVID-19 therapeutically. Batch IV AKV-Aq. Ex. is more potent as it is made up of aqueous extract and can be used orally along with any of the other three batches in the form of decoction.

KEYWORDS: Ayurveda, traditional medicine, AYUSH Kwatha, COVID-19, CCRAS.

INTRODUCTION

COVID-19 also known as Coranavirus disease has emerged as one among the deadliest pandemic. As the disease is a novel one, lot of speculations has been made about its clinical presentation, pathological findings and possible treatment.

Clinical presentation of COVID-19 may vary per-se. Most persons experience fever, cough, fatigue, anorexia, shortness of breath, myalgia etc. Sore throat, nasal congestion, headache, diarrhea, nausea and vomiting are some of the non-specific symptoms. Loss of sense of smell and loss of sense of taste has also been reported. Reduced alertness, reduced mobility, diarrhea, loss of appetite, delirium, are also seen in immune-suppressed and older people.[1]

Severity of illness of the patients with COVID-19 can be grouped into the following categories

- **Asymptomatic or Pre-symptomatic Infection:** Individuals who are positive for COVID-19 but, has no symptoms.
- **Mild Illness:** Individuals who have any of the signs and symptoms of COVID-19 without shortness of breath, dyspnea, or abnormality in chest imaging.
- **Moderate Illness:** Individuals who have lower respiratory disease and oxygen saturation (SpO_2) ≥94%.
- **Severe Illness:** Individuals with respiratory rate >30 breaths per minute and SpO_2 <94% ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO_2/FiO_2) <300 mmHg, or lung infiltrates >50%
- **Critical Illness:** Individuals with respiratory failure, septic shock, and/or multiple organ dysfunctions.[2]

Affirmed treatment by NIH includes azithromycin, chloroquine, hydroxychloroquine, remdesivir[3] and COVID-19 convalescent plasma.[4] But specific antiviral medicine is still to be developed for which many organizations are working vigorously.

In current obvious situation arising due to COVID-19, role of prevention and prophylactic treatment becomes the front line management strategy.

Ayurveda: The science of traditional medicine practiced abundantly in South East Asia has a lot to contribute in this scenario and people are seeking Ayurvedic treatment for preventive,

prophylactic and curative purpose as well. Ministry of AYUSH (MoA) has issued advisories from time to time. Some of the recommended measures of the advisories are practices of Yogasana, pranayama, meditation, intake of spices like turmeric, garlic etc, chyawanprasha, Ayush Kwatha, Golden milk, nasal application, oil pulling, steam inhalation with mint leaves and caraway seeds, clove powder with honey.[5]

Ayush Kwatha helps to improve the health of Upper Respiratory Tract (URT) comprises of Tulasi (Basil), Tvak (Cinnamon), Shunthi (Dry Ginger) and Maricha (Black pepper). All the ingredients to be taken in dry form, made in to coarse powder, mixed and to be consumed like tea by dissolving 3 gm of powder in 150 ml of boiled water. Tablet of Aqueous extract can also be made. Jaggery/Raisin/Lemon Juice can be added to taste. This formulation can be followed to the extent possible as per the individual's compatibility and feasibility.

Table no. 1: Contents of Ayush Kwatha.

S. No.	Name of Drug	Botanical Name	Family	Common Name	Part used	Quantity (Parts)
1.	*Tulasi*	*Oscimum sanctum*	Lamiaceae	Basil	Leaves	4
2.	*Tvak*	*Cinnamomum zeylanicum*	Lauraceae	Cinnamon	Stem Bark	2
3.	*Shunthi*	*Zingiber officinale*	Zingiberaceae	Dry Ginger	Rhizome	2
4.	*Maricha*	*Piper nigrum*	Piperaceae	Black pepper	Fruit	1

Pharmacological activity of the content drugs of Ayush Kwatha as per Ayurvedic science is mentioned in Table No. 2

Table no. 2: Ayurvedic categorization of contents of Ayush Kwatha.

S. No.	Drug	CharakaSamhita	SushrutSamhita	Ashtangahridya	Indications
1.	***Tulsi***	*Shwasahar Mahakshaya*	*Surasadi gana Shirovirechana*[6]	*Surasadi gana*	*Kasa, Shwasa, Kshaya*[7] *Parsvasula, Krimi, Visama Jvara.*[8]
2.	***Tvak***	*Sheetnashak lepa*	*Eladigana*	*Trijata*	*Pinasa, Kasa, Shwasa, yakshma*[9] *Aruci, Hridroga,*
3.	***Shunthi***	*Triptighana Mahakshaya, Arshoghana Mahakshaya, Deepaniya Mahakshaya, Shoolaprashamana, Trishnanigrah Mahakshaya*[10]	*Pippalyadi gana, Trikatu*	*Pippalyadi gana*	*Kasa, Shwasa, Hridroga, Raktapitta, Jvara, Atisara*[11]
4.	***Marich***	*Deepaniya Mahakshaya, Shoolaprashamana, Krimighan Mahakshaya, Shirovirechan Mahakshaya*[12]	*Pippalyadi gana, Trikatu*	*Aushadh varga*	*Visama Jvara, Kasa, Shwasa*[13]

Authenticated and established pharmacological activity of content drugs on contemporary parameters relevant to COVID-19 are enlisted in Table No. 3

Table no. 3: Pharmacological activities of content drugs related to COVID-19 symptoms.

S. No.	Drug	Pharmacological activity
1.	***Tulsi***	Antioxidant activity, immunomodulatory activity, analgesic,[14] adaptogenic activity/antistress,[15] antipyretics,anti-inflammatory,[16] anticoagulant, [17] decreases the severity and duration of the swine flu [18] antimicrobial. [19] Used for common cold, coughs, sore throat and respiratory disorders.[20]
2.	***Tvak***	Anti-inflammatory,[21] antipyretic and analgesic,[22] antioxidant, anti-coagulant.[23]
3.	***Shunthi***	Anti-platelet[24], anti-coagulant, immunomodulatory, anti-inflammatory, antioxidant[25] and anti-tussive.[26]
4.	***Marich***	Anti-asthamatic,[27] anti-oxidant,[28] anti-inflammatory,[29] immunomodulatory,[30] analgesic.[31]

The present study aims to make use of Ayush Kwatha more palatable for its easy, best and to gain its maximum benefit as *Sanskara* of the drugs may provide opportunity to design new compound with improved palatability, therapeutics and potency.[32]

Primary Objectives: To prepare Ayush Kwatha by four different methods:

- Ayush Kwatha Churna by conventional traditional method-**AKC**
- To prepare Ayush Kwatha Vati by using Distilled water-**AKV-DW**
- To prepare Ayush Kwatha Vati by using Nimbu Swarasa- **AKV-NS**
- To prepare Ayush Kwatha Vati of aqueous extract of the contents-**AKV-Aq. Ex.**

Secondary Objectives

- To assure the quality of the four samples of Ayush Kwatha on Analytical Parameters recommended by CCRAS for churna and vati.[33]

MATERIAL AND METHODS

The raw drugs were collected from authentic source and were subjected to various tests for authentication viz. botanical name, part used, organoleptic characters (colour, texture, odour and taste), identification (macroscopy, microscopy, powdered drug analysis), physicochemical parameters (loss on drying, total ash, acid insoluble ash, alcohol soluble extractive, water soluble extractive, pH(10%) and TLC.

Ayush Kwatha was prepared in four batches with modification to improve potency and palatability of the formulation:

AKC: Ayush Kwatha Churna as recommended by MoA. Tulasi, Tvak, Shunthi and Maricha were cleaned separately and foreign particles were removed. Dried and powder was made. Powder in ratio of 4:2:2:1 were added respectively to get homogenous mixture AYUSH Kwatha Churna (**AKC)**.

AKV-DW: Ayush Kwatha Churna was levigated with distilled water to get homogenous blend of dough suitable for making pills. Pills of 500 mg was made manually, shade dried and kept in air tight container and named **AKV-DW**.

AKV-NS: Ayush Kwatha Churna was levigated with Nimbu swarasa to get homogenous blend of dough suitable for making pills. Pills of 500 mg was made manually, shade dried and kept in air tight container and named **AKV-NS**.

AKV-Aq.Ex.: Ayush Kwatha Churna was soaked in water (8 times) for four hours. The mixture was cooked on medium flame till the water was reduced to half of the initial volume. The mixture was filtered through muslin cloth. The obtained filtrate was again kept on low flame and cooked to reduce in volume. When the mixture started sticking on the side of the vessel, it was kept in water bath with intermittent stirring to get semisolid mixture. The mixture was made into pills of 500 mg, dried and kept in air tight container and named **AKV-Aq. Ex. (Fig.1)**

The finished product of each batch was subjected to analytical tests viz: organoleptic characters (colour, texture, odour and taste), identification (macroscopy & microscopy), physicochemical parameters (hardness, disintegration time, loss on drying, total ash, acid insoluble ash, alcohol soluble extractive, water soluble extractive, pH (10%),TLC, Mesh size, Pesticide residues, Heavy Metals, Microbial limits and Aflatoxins.

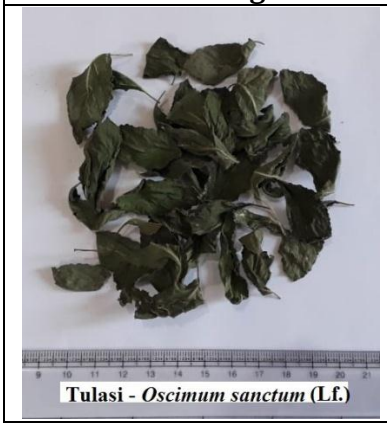
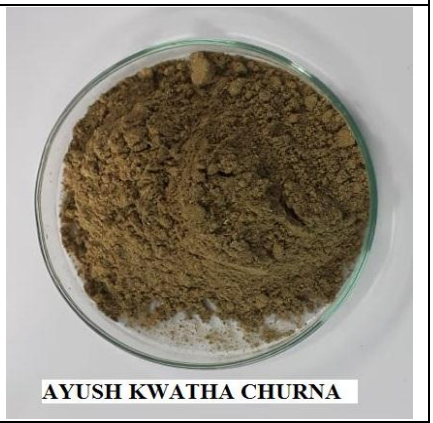
Raw Drugs	Process	Final Batches
 Tulasi - *Oscimum sanctum* (Lf.)	 POWDER OF CONTENTS	 AYUSH KWATHA CHURNA

Fig.1 Pictures of raw drugs and pharmaceutical procedures.

RESULTS

Tulasi-*Oscimum sanctum* (Lf.)

Macroscopic characters: Dried leaves are greenish brown in colour, oblong in shape, 2.5-5 cm long, serrated, pubescent on both surfaces, petiole 1.5-3 cm long.

Microscopic characters: Powder shows wavy thin walled epidermal cells; numerous uni-serrate unicellular or multicellular non-glandular trichomes, with diacytic stomata and few glandular multicellular trichomes with globose gland at the tip; a septate fiber; pitted xylem vessels in group; prismatic crystals of calcium oxalate and rounded to oval, simple starch grains.

Tvak - *Cinnamomum zeylanicum* (St. Bk.)

Macroscopic characters: Dull yellowish-brown bark pieces, with inner surface darker in color, about 0.5-2 mm thick, brittle, occurs as single or double closely packed compound quills, up to about 1-1.5 cm in diameter, outer surface, marked with pale wavy longitudinal lines with occasional small scars, striated with longitudinally elongated reticulation, fracture, splintery and occasional traces of cork.

Microscopic characters: Transverse section shows pericyclic sclerenchyma devoid of cork, 3 or 4 rows of isodiametric cells, inner and radial walls often being thicker than the outer, occasional pericylicfibres embedded in the sclerenchyma, phloem of tangential bands of sieve tissue present alternatively with parenchyma, secreting cells containing volatile oil or mucilage is present. Intermittent crystals of calcium oxalate present.

Shunthi-*Zingiberofficinale* (Rz.)

Macroscopic characters: Yellowish buff colored Rhizomes laterally compressed, ovate, oblique and irregularly branched, Length: 4-7cm, Width: 2.5-4 cm and Thickness: 0.8-2cm. Form: longitudinally striations, ends of branches with depressed stem scars. A well-marked endodermis and a wide stele showing numerous scattered fibro-vascular bundles. Fracture: brittle, uneven and fibrous with aromatic and characteristic odour and pungent and spicy taste.

Microscopic characters: Fine powder shows light yellow to reddish brown oleoresin; shows thin-walled parenchymatous cells, arranged radially around numerous scattered, collateral vascular septate fibers with oblique, elongated pits on their walls, with scattered vascular strands, single starch grains of varying shapes with eccentric hilum, measuring 5-25 µ in diameter.

MARICHA- *PIPER NIGRUM* (FR.)

Macroscopic characters: Grayish black wrinkled, hard, oval shape, 4-5 mm thick in diameter, with minute scar of stalk; with aromatic smell and aromatic and strongly pungent taste.

Microscopic characters: Fruit consists of a thick pericarp for about one third of fruit and an inner mass of perisperm, enclosing a small embryo; pericarp consists of epicarp, mesocarp and endocarp. Epicarp: single layered epidermis and 1 or 2 layers of radially elongated, stone

cells are present below. Mesocarp: wide, band of tangentially elongated parenchymatous cells present with elongated oil cells in outer region and a few fibro-vascular bundles. Endocarp: a row of beaker shaped stone cells; yellow coloured testa, single layered, thick walled sclerenchymatous cells. Grayish black powder with iso-diametric elongated stone cells, thin-walled, hypodermal cells, Endocarp packed with minute compound starch grains with aleurone grains and oil globules.

Table no. 4: Organoleptic characters of content drugs.

S.No.	Name of the Drug	Colour	Texture	Odour	Taste
1.	***Tulasi***	Greenish brown	Rough in touch on either surface	Aromatic	Pungent
2.	***Tvak***	Dull yellowish-brown	Outer surface rough, inner surface smooth	Fragrant	Sweet, aromatic with sensation of warmth
3.	***Shunthi***	Externally creamish with pale brown patches, internally bright cream	Uneven with smooth striations, thin folds and scars	Aromatic	Agreeable pungent, characteristic
4.	***Maricha***	Externally dark grevish-brown to black, internally bright yellowish cream	Warty	Aromatic and characteristic	Spicy, pungent

Table no. 5: Physicochemical parameters of content drugs.

S.No.	Parameters	*Tulasi* [34]	*Tvak* [35]	*Shunthi* [36]	*Maricha* [37]
1.	Loss on Drying	9.5	10.3	10.3	12.2
2.	Total Ash	13.4	2.6	4.1	3.7
3.	Acid insoluble ash	2.4	1.6	0.2	0.4
4.	Alcohol soluble extractive	7.0	2.8	3.3	9.5
5.	Water soluble extractive	15.5	9.6	14.2	8.5
6.	pH (10% Aq. suspension)	7.1	5.8	5.1	6.7

High Performance Thin Layer Chromatography (HPTLC)

Sample preparation : 1gm of the sample was subjected to reflux with methanol, for 1 hour and extract was filtered using filter paper. The filtrate was concentrated and taken for the following TLC profile.

Stationary Phase: Pre-coated (Support on Aluminum Sheets) Silica Gel Plate, Specification: TLC Silica Gel $60F_{254}$, Mfg. by Merck, 26.09.16, Batch No.1.05554.0007

Sample application: Applied volume 5 µl as 8 mm band and at 15 mm from the base of the plate, Plate size was 5x10 cm.

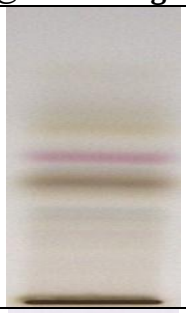
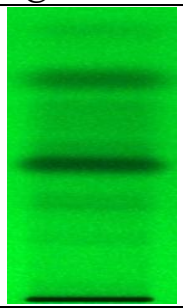
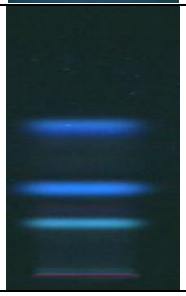
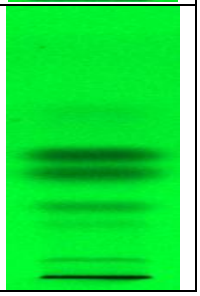
Development: Developed up to 90 mm in CAMAG Twin trough chamber, Plate Pre-conditioning Temp 25°C and relative average humidity was 42%)

Visualisation: Images of the developed plate were captured under 254 nm and 366nm UVlight.

Derivatisation: Developed plate was dipped in 20% aq. Sulphuric acid and charred at 105°C and visualized at white light.

Table No. 6: R_f Values of content drugs at different light (Fig.2).

Drugs	Mobile Phase:	White light	366 nm	254 nm
Tulsi	Ethyl acetate: Methanol (10:1, v/v)	0.03, 0.12, 0.21, 0.33, 0.45, 0.85, 0.93	0.30, 0.33, 0.45, 0.83, 0.85, 0.93, 0.98	0.33, 0.45, 0.55, 0.75, 0.83, 0.88, 0.93
Tvak	Hexane: Ethyl acetate (7:3, v/v)	0.06, 0.10, 0.28, 0.35, 0.39, 0.46, 0.58, 0.63	0.03,0.17, 0.21, .034, 0.59	0.06, 0.21,0.27,0.40, 0.48, 0.63
Shunthi	Hexane: Ethyl acetate: Methanol (5:4:1, v/v)	0.05, 0.08, 0.29, 0.52	0.03, 0.08, 0.20, 0.46, 0.53, 0.58, 0.69, 0.81, 0.97	0.30, 0.49, 0.58, 0.67, 0.83
Marich	Hexane: Ethyl acetate: Formic acid (5:4:1, v/v)	0.05, 0.07, 0.27, 0.54	0.18, 0.27, 0.33, 0.46, 0.54, 0.61, 0.67, 0.78	0.45, 0.54, 0.61, 0.64, 0.77

Name of the raw drugs	@ Visible light	@ 366 nm	@ 254 nm
Tulasi ***Oscimum sanctum***			
Tvak ***Cinnamomum zeylanicum***			

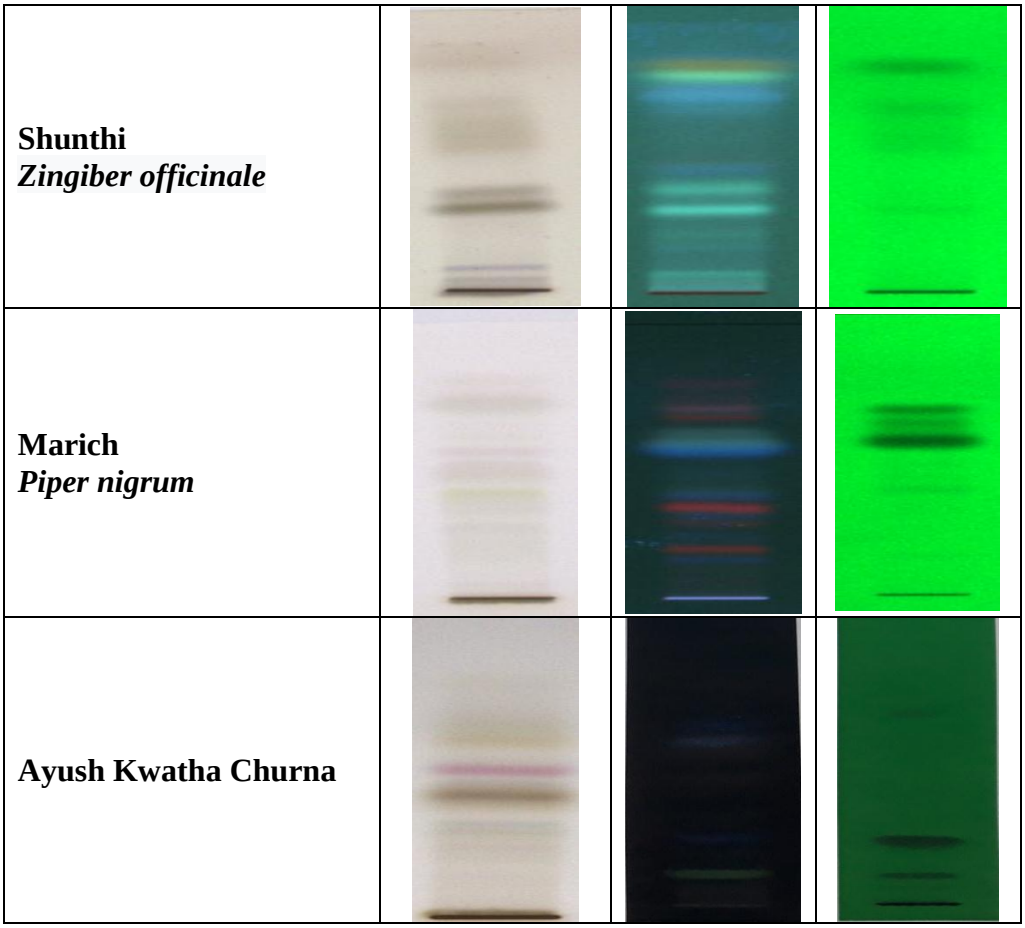


Fig.2 HPTLC profile of drugs at Visible light, 366 nm and 254 nm.

Organoleptic characters: Ayush Kwatha Churna is pale brown fine powder with characteristic odour and pungent taste with tingling feeling in the end.

Macroscopy: Brown-coloured powder with pleasant strong odour, bitter, spicy and pungent taste.

Microscopy: Non-glandular trichomes, thin walled epidermal cells, septate fiber, crystals of calcium oxalate and occasional starch grains (Tulasi); thick fibers walls with narrow lumen, lignified stone cells with thick inner walls, crystals of calcium oxalate and pitted parenchyma (Tvak); fragment of septate non-lignified fibres, large simple oval shaped starch grains with eccentric hilum with broad spiral and reticulate vessels, parenchymatous cells with yellow coloured oleo-resin (Shunthi); fragments of epidermis of pericarp with groups of beaker shaped stone cells in different shapes, sizes and thickness, interspersed among parenchymatous hypodermis (Maricha).

Table No. 7: Physicochemical Properties of Ayush Kwatha Churna.

S.No.	Parameters	AKC	AKV-DW	AKV-NS	AKV-Aq. Ex.
1.	Hardness (Kg)	-	2.0	2.20	2.80
2.	Disintegration Time (sec.)	-	3.0	5.00	42.00
3.	Loss on Drying	3.28	2.4	2.02	2.14
4.	Total Ash	5.46	1.58	1.50	3.54
5.	Acid insoluble ash	0.77	0.36	0.35	0.43
6.	Alcohol soluble extractive	10.89	6.04	6.26	8.55
7.	Water soluble extractive	14.33	8.16	9.81	12.63
8.	pH (10% Aq. suspension)	-	5.88	4.32	5.14

Mesh size: 92% of the Ayush Kwatha Churna passed through #85 mesh size.[38]

Heavy Metals i.e. Lead, Arsenic, Mercury and Cadmium were absent.[39]

Aflatoxins B_1, B_2, G_1, G2 and **pesticides** were also not detected in Ayush Kwatha Churna.[40,41]

Table No. 8: Microbial Load and pathogen analysis of Ayush Kwatha Churna.[42]

Tests	Results (in cfu/gm)	Permissible Limit (in cfu/gm)
Total Bacterial Count	4.1×10^3	$< 10^5$
Total Fungal Count	210	$<10^3$
Escherichia coli	Absent	Absent
Salmonella sp.	Absent	Absent
Staphylococcus aureus	Absent	Absent
Pseudomonas aeruginosa	Absent	Absent

DISCUSSION

Ayush Kwatha is one of the many remedies recommended by the MoA to be included in day to day practice to improve the general health and boost the immunity. But looking thoroughly to its content and pharmacological activity on Ayurvedic as well as contemporary principles, a lot more can be attributed to it. On the basis of evidence available for its pharmacological activity i.e. antioxidant activity, immuno-modulatory activity, analgesic, adaptogenic activity/antistress, antipyretics, anti-inflammatory, anticoagulant, anti-tussive and anti-asthamatic; it can also be used to manage the symptoms associated with upper respiratory tract in mild to moderate COVID -19 illness.

Ayush Kwatha is to be taken as hot infusion, which is a quite tedious procedure and time consuming to follow every time. There, arises a compliance issue.

Thus, the present study was conducted to prepare Ayush Kwatha in different forms to improve the palatability, potency and compliance of the medicine. Ayush Kwatha was

prepared in four batches (AKC, AKV-DW, AKV-NS and AKV-Aq. Ex.) with slight modification.

Batch I: AKC was prepared strictly complying MoA advisory which is to be dissolved in hot water (3 gm in 150 ml) and to be taken as hot decoction. Flavoring agents like honey, sugar, jaggery, lemon can be added to taste.

Batch II: AKV-DW was prepared again complying MoA advisory. Distilled water was used to make vati (500 mg each). This vati can be taken orally in dose of two tablet TDS or can be dissolved in 150 ml of boiling water and consumed as herbal tea.

Batch III: AKV-NS was prepared by levigating AKC with Nimbu Swarasa to make vati (500 mg each). As Nimbu swarasa is used it has better taste (mild sour) than Batch II. This can be taken orally in dose of two tablet TDS or can be dissolved in 150 ml of boiling water and consumed as herbal tea.

Batch IV: AKV-Aq. Ex. was prepared by aqueous extract of AKC and making vati (500 mg each). This can be taken with hot/luke warm water. The dose of vati is one tablet TDS as this is aqueous extract of AKC.

In Quality control parameters each batch was found absolutely within the limit laid down by CCRAS, Govt. of India. Thus, it is suitable and safe for use as far as quality control parameters are concerned.

CONCLUSION

Ayush Kwatha as advised by Ministry of Ayush helps to improve the health of Upper Respiratory Tract (URT). Though this is used as preventive measures to improve the immunity, it can also be used to manage symptoms of upper respiratory tract in pre-symptomatic to mild illness of COVID-19. Along with Ayush Kwatha-Batch I, II and III (AKC, AKV-DW and AKV-NS) Batch IV (AKV Aq. Ex.) can be added to increase its pharmacological activity like immuno-modulatory activity, anti-tussiveactivity, anti-asthamatic activity, analgesic, adaptogenic activity/antistress activity, antipyretics activity and anti-inflammatory activity.

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