International Journal of Herbal Medicine
Available online at www.florajournal.com

E-ISSN: 2321-2187
P-ISSN: 2394-0514
www.florajournal.com
IJHM 2022; 10(1): 58-64
Received: 22-11-2021
Accepted: 25-12-2021

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Assessment of organoleptic, physico chemical and microbial variation on *Triphala* decoction: A comparative study

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Abstract
Triphala decoction (TD) is one of the most popular poly herbal formulations (A collection of *Phyllanthus emblica* L., *Terminalia bellerica* (Gaertn.) Roxb and *Terminalia chebula* Retz.) prescribed by the Ayurveda practitioners in Sri Lanka due to its precious medicinal properties, high effectiveness and convenience of administration. But decoction is advised to use when it is freshly prepared due to its short shelf life, but due to the inconvenience of preparing decoction, specially the TD at every time before consumption, various storage methods are advised to the patients by the practitioners at least to maintain the quality parameters. The study was conducted to reveal the variations in organoleptic parameters (colour, odour, taste and appearance), physico - chemical parameters (pH, specific gravity and refractive index) and microbial contamination (fungus, salmonella, coliform & *staphylococcus aureus*) of both standard (S) and pharmacy (p) samples of *Triphala* decoction throughout the 14 days. Each sample was divided into 4 samples by kept them under the room temperature (RT) and refrigerating (RF) temperature in transparent (T) and amber (A) colour bottles. Organoleptic parameters tested in the sample P/RT/TD has shown remarkable variances when comparing them with the samples of S/RT/TD according to the time period of 14 days. In physico-chemical parameters; both P and S samples depicted remarkable variances in; pH by becoming more acidic, a gradual increment of specific gravity and as well, mild changes in refractive index. And microbial assessment has shown absence of Coliform, Salmonella and *Staphylococcus aureus* and significant development of fungal growth of Yeast and Moulds that has influenced more in sample P/RT than S/R. Time duration and container have shown less significant in microbial growth.

Keywords: Thriphala decoction, physico-chemical parameters, organoleptic parameters, microbial growth

1. Introduction
Ayurveda is science of life (Ayuh+veda) [1]. It deals with prevention and treatment of various diseases [2]. Throughout history, all cultures have employed a variety of plants or plant derived materials for the prevention and treatment of disease [3]. The World Health Organization (WHO) survey indicated that about 70-80% of the world population particularly in the developing countries rely on non-conventional medicines mainly of herbal medicines are relatively accessible and cheaper than the synthetic drugs [4]. Many plants are used in traditional medications as herbal preparations for human health care [5]. And they are being promoted as natural and safe without any side effects. Widespread use of herbal medicines, calls for the assurance of sustainable availability of quality and safe herbal medicines to ensure continued access especially for rural communities, without compromising patients safety. [6]. *Triphala* decoction is a very popular, potent and widely practiced Ayurveda formulation for different disease conditions. This is a combination of Nelli (Amalki/ *Phyllanthus emblica* L.,), Bulu (Vibhitaka/ *Terminalia bellerica* (Gaertn.) Roxb.) And Aralu (Haritaki/ *Terminalia chebula* Retz.). *Triphala* is classified as a tridoshic rasayana, that the energetic are appropriate for *vata*, *pitta* and *kapha* or all type of diseases. Acharya Charaka describes *rasayanas* as having the qualities of supporting strength and immunity [7]. The raw materials collected using unscientific method are commonly exposed to many pathogenic contaminants and are often deteriorated by pathogenic microorganisms before harvesting, and also during handling and storage. Therefore lack of regulation for herbal supplements presents potential health risk, largely their contamination chances with pathogenic microorganisms. It can render the raw materials for herbal drugs prone to fungal infestations [8]. According to the WHO technical guidelines for the assessment of microbial quality of herbal preparations, determination of microbiological contaminations and limit testes for total viable aerobic bacteria and fungi indicate the quality of herbal preparations [9].

According to the British pharmacopoeia (2004) standards, salmonella and shigella species must not be present in herbal medicines intended for internal use, at any stage [3].

1.1 Problem statement

Decoction preparations are one among basic ayurvedic dosage forms which are highly effective. *Triphala* decoction is a well-recognized poly herbal medicine. It is classified as *tridoshic rasayana* in Ayurvedic medicine as it promotes longevity and rejuvenation in patients of all constitutions and ages. Numerous references in well-respected ayurvedic medical texts make clear that *Triphala* is referred as multiuse therapeutic and perhaps even panacea historically [7]. In Ayurveda system, *Triphala* decoction is prescribed to indoor and outdoor patients for 7 – 14 days [10]. With ever increasing use of *Triphala* decoction, safety has become a concern for both health authorities and the public. This is because many contaminants and residues that may cause harm to the consumers have been reported [11].The quality assessment of this medication is therefore very important. It is thus necessary that the microbiological limit tests of *Triphala* decoction should be done to ensure that the product is free from risk. Microbial contamination is the preclusion of unacceptable substance or impurities (microbiological or chemical or foreign matter) onto raw material to be used, intermediate product or finished herbal preparations during production processes, packaging, storage/perseveration or transport of this preparations or products (WHO, 2007). The microbial contamination can be avoided by applying best practice guidelines such as Good Manufacturing Practice (GMP) (WHO, 2007). Furthermore, many microbial contaminants can alter the physicochemical features which can then lead to mischievous changes to the quality of herbal preparations [12]. So, it is important that physicians have the knowledge about the microbiological safety of these preparations [13]. Some of the formulations are not subject to aseptic conditions during various stages of preparation, packaging, storage and transportation which aim at achieving high standards of quality of drug. Plants and plant materials also carry huge number of organisms mainly originating from the soil. Microbial growths occur during harvesting, handling and production [12]. In Sri Lanka, *triphala* decoction is given as a prepared decoction for outdoor patients for the period of 7- 14 days. Given dose was 30ml twice a day (morning and evening). And for all these days ask the patient to refrigerate the medicine.

1.2 Background and Justification

Triphala decoction is used as laxative in chronic constipation, colon cleansing, digestion problems and poor food assimilation, cardiovascular diseases, high blood pressure, to reduce serum cholesterol, poor liver function, large intestine inflammation, ulcerative colitis. It is good rejuvenator, tonic, hair tonic and good for digestion, purgative, cure all diseases of eyes, heal ulcer, remove diseases of skin, fat, diabetes, blood and fever. Therefore it is important to have the knowledge about the safety of the preparation [14]. The microbial contamination of medications can result in clinical infection. And the fungal contamination has been reported to affect the chemical composition of the raw materials and thereby decrease the medicinal potency of herbal drugs [9]. However, only a few surveillance studies have been reported to assess this threat. And some studies figured out the biological threats in herbal medicines and added the knowledge of proliferating bacteria, yeast and moulds in such medicines [9]. The large number of isolated microorganisms from the samples could pose a risk of acquisition of pathogenic microbial agents to those taking these herbal mixtures. This observation prompted the authors to conduct this study in order to establish the stage at which contamination takes place during the processing of herbal medicines by traditional herbalists [3].

In this study, is planning to conduct comparative study with pharmacy sample and standard sample to conclude, at which stage the decoction becomes contaminate/shows changes with the period of 14 days.

2. Objectives

2.1 General Objective

- To determine the organoleptic, physicochemical and microbial variation of *Triphala* decoction for the period of 14 days.

2.2 Specific objectives

- To determine the nature of microbial contamination of *Triphala* Decoction.
- To determine the extent of microbial contamination of *Triphala* Decoction.
- To determine the suitable storage method for the *Triphala* Decoction.
- To compare the prepared standard sample with pharmacy sample of *Triphala* decoction

1. Materials and methods

This is a comparative study on *Triphala* decoction between the decoction prepared in Bandaranaike Memorial Ayurvedic Research Institute (BMARI) pharmacy and decoction prepared according to standard method mentioned in ayurvedic text *Sri Sharngadara Samhitha* to figures out the physicochemical and microbial variation throughout the period of 14 days. The study was carried out at Research laboratory of BMARI, Navinna, Maharagama from 20th January to 20th February 2020.

3.1 Sample preparation

3.1.1 Preparation of *Triphala* Decoction

3.1.1.1 According to Sri Sharngadhara Samhita [15]

- The ratio of raw materials, initial water quantity and final decoction quantity is 1:16:2 (1/8th part of initial volume)
- Altogether aralu, bulu and nelli were taken 1350g in equal quantity of each 450g and cleaned.
- Washed and dried under sunshade.
- Ground into coarse powder.
- Measured 21.6 litres of water. (Required quantity for the preparation)
- Ingredients were transferred into a vessel and added 2.7 litres water and measured the height with a scale and noted the initial water level. (Which is the final water level of decoction when reduced to 1/8th part from 16 part of initial volume).
- Add remaining water in to the vessel and boiled in mild flame until the water level reduced to 2.7 litre of water level.
- Allowed the preparation to cool down.
- Filtered with white cloth and transferred into cleaned sterile container.

According to pharmacy method

- The ratio of raw materials, initial water quantity and final decoction quantity is 1:40:10 (1/4th part of initial volume)

- Altogether aralu, bulu and nelli were taken 300g in equal quantity of each 90g.
- Cleaned and washed
- Measured 10.8 litres of water. (Required quantity for the preparation)
- Ingredients were transferred into a vessel and added 2.7 litres of water and measured the height with a scale and noted the initial water level. (Which is the final water level of decoction when reduced to 1/4th part from the initial volume).
- Added remaining water in to the vessel and boiled in mild flame until the water level reduced to 2.7 litre of water level.
- Allowed the preparation to cool down.
- Filtered with white cloth and transferred into cleaned sterile container.

3.1.2 Storage

- 150ml decoction from both samples was taken for 1st day analysis.
- Then each sample was divided into four in required amount.
- Two samples from each preparation were stored in sterile transparent bottles and other two were stored in sterile amber bottles separately.
- One transparent bottle and one amber bottle samples from each preparation kept in room temperature and remaining samples kept in refrigerator continuously for 14 days.
- Each sample bottles were shaken and opened for a while (about a minute) then closed in every morning and evening to give similar environment of *Triphala* decoction which is used by the patient.
- Assessments of physicochemical and microbial variation were carried out on 1st, 7th and 14th day duration.

3.2 Statistical Analysis

Univariate Analysis of variance (ANOVA) and Logistic Regression were used to check for significant variation of microorganism and physicochemical analysis.

4. Results and Discussion

Decoction is a liquid preparation that preserves their potency up to 24 hours and advised to prepare freshly for daily uses. Due to its short life span, need to develop which could remain its potency for a long duration without deteriorating its therapeutic properties [16]

Organoleptic parameters showed considerable variations in taste, colour, odour and appearance in all samples of pharmacy and standard throughout fourteen days of study except S/RF/A/TK. Standard sample showed minimum changes than the pharmacy sample. The most affected sample throughout 14 days was P/RT/T/TK sample and it was more rapidly varied according to the time duration due to rotten affect and fungal growth was reported on the 5th day of study. It was appeared as a floating, in little whitish ash colour which converted to brownish frothy colour throughout the 14 days of study. The most unaffected sample was S/RF/A/TK and it did not change its all the organoleptic parameters throughout the study period. (Table 1) And also the ambient standard sample in room temperature did not show any organoleptic changes of TK. People who haven't the facilities of refrigeration can suggest using sterile ambient bottles to store TK for the period of seven days.

Considering about most affected and unaffected study samples, method of preparation, temperature and transparency of bottle are the factors can be considered. In method of preparation, standard sample (16:2) took more time and heat than pharmacy sample (4:1) to make the decoction. Exposure to mild temperature in longer time duration may cause more fungal destruction in standard sample. Refrigeration (below 8^{o}C) was given unfavourable environment for fungal growth may cause to preserve the standard sample; and amber coloured bottles blocked sun rays pass through and was given unfavourable environment for fungal growth.

In physicochemical parameters revealed that pH value was differ between standard and pharmacy fresh samples from the very 1st day of study and standard was more acidic than pharmacy sample. Standard samples showed significant reduction in pH than pharmacy samples with the time duration of 14 days. Duration ($p<.001$) and method of preparation ($p=.001$) are significantly influenced in pH. Acidic environment may cause to reduce fungal growth and helps to preserve the standard sample. (Figure 1.1, 1.2)

The specific gravity was influenced significantly only by the method of preparation ($p=0.005$) which revealed standard samples were higher specific gravity than pharmacy samples ($p=0.005$). Simply means that the solution is more concentrated than pharmacy sample. Time taken to prepare decoction (method) and the particle size (coarse powder) may be given more surface area to react with water.

The RF index was influenced significantly only by method of preparation which revealed standard samples were higher RF index than pharmacy samples. Higher concentration with more particles may cause to increase RF index than pharmacy sample. (Figure 3)

In microbiological study showed, absence of E- coli, *salmonella* and *staphylococcus aureus* of both the pharmacy and standard samples and found significant development of fungal growth in *triphala* decoction of pharmacy samples. The fungal growth (yeast and moulds) were identified in the incubated petri dishes observed by naked eye from 5th day of study. Fungus can be present in herbal preparations because of unscientific method of collection of raw materials, method of preparation, storage method, and transportation and congenial climatic condition; these can be render the raw materials for herbal drugs prone to fungal infestation [16]. Among the herbal preparations, the liquid forms are prone to develop fungal growth because of its moisture content than other forms According to the results, the method of preparation and storage temperature were significantly influenced on fungal growth; and duration of storage, type of container and dilution of samples did not show any significant effect. In pharmacy method of preparation, the raw materials are directly added just after washing and in standard method of preparation, the raw materials first washed then dried in sunlight and make them in to coarse power and finally mixed with water. As mentioned above, standard sample took more time and more heat to reduce in to the required amount of decoction. And this may help to destroy more fungus and may inhibit the fungal growth in standard sample than the pharmacy. (Table 5.3)

Room temperature gives good environment for better fungal growth and optimum temperature was 30 ^{o}C. Studies showed maximum toxin production occurred at temperature in the range of 15-25 ^{o}C. Refrigeration (4-8 ^{o}C) was given unfavourable environment for fungal growth and refrigerated samples given significant reduction of fungal growth. (Table 5.4)

A. Organoleptic variations

Table 1: Organoleptic variations of *triphla* decoction

Time duration	Samples of triphala kwatha		Organoleptic parameters			
			Colour	Odour	Taste	Appearance
Day – 01	Pharmacy(P) TK		Brownish	Herbal decoction	Acrid	Clear with homogenous
	Standard(S) TK		Dark brownish	Herbal decoction	Acrid	Clear with homogenous
Day – 07	RT	P/RT/T/TK	SC	C	SC	Frothy, fungal growth and turbid
		P/RT/A/TK	NC	SC	SC	NC
		S/RT/T/TK	NC	SC	NC	NC
		S/RT/A/TK	NC	NC	NC	NC
	RF	P/RF/T/TK	NC	NC	SC	Slightly Frothy
		P/RF/A/TK	NC	NC	SC	NC
		S/RF/T/TK	NC	NC	NC	NC
		S/RF/A/TK	NC	NC	NC	NC
Day – 14	RT	P/RT/T/TK	C	C	C	Precipitation with turbid, more frothy Fungal growth with layer separation
		P/RT/A/TK	C	C	C	Frothy with turbid
		S/RT/T/TK	SC	SC	SC	NC
		S/RT/A/TK	NC	SC	SC	NC
	RF	P/RF/T/TK	SC	SC	SC	Frothy but no fungal growth
		P/RF/A/TK	SC	SC	SC	Turbid
		S/RF/T/TK	NC	SC	SC	NC
		S/RF/A/TK	NC	NC	NC	NC

B. Physicochemical variation

PH

Only Duration (p<.001) and Method (p=.001) significantly influenced pH

1.1. Duration

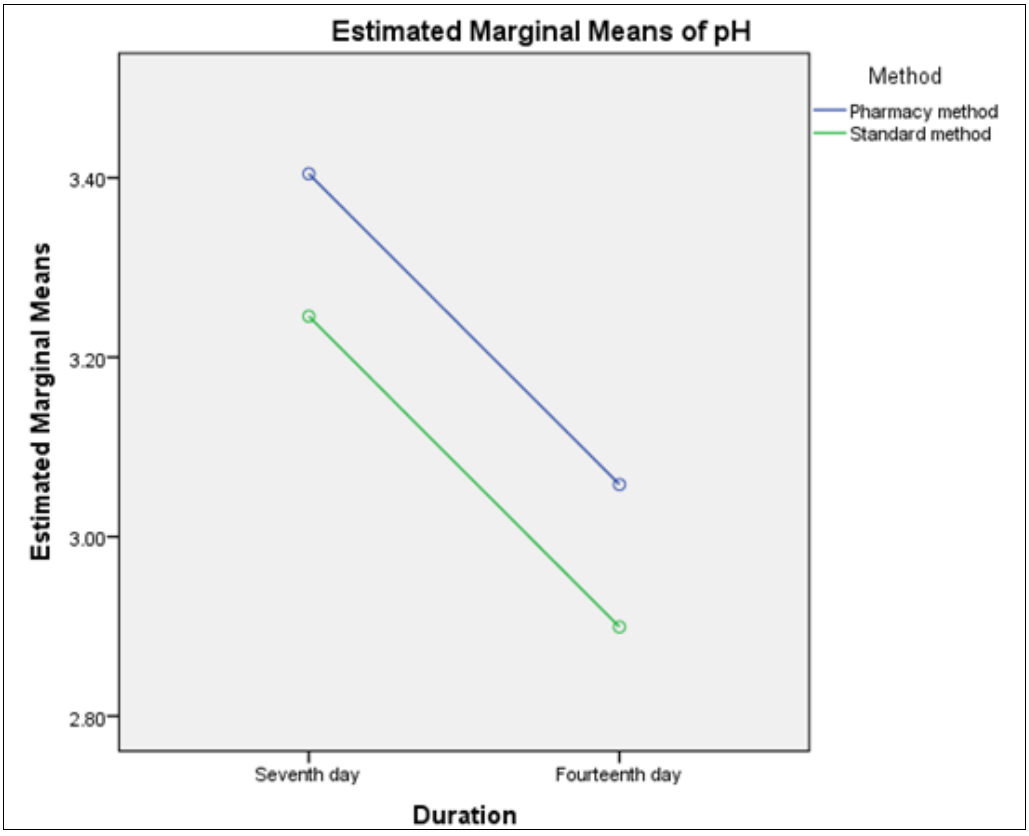

Fig 1: Estimated marginal means of PH

1.2. Method

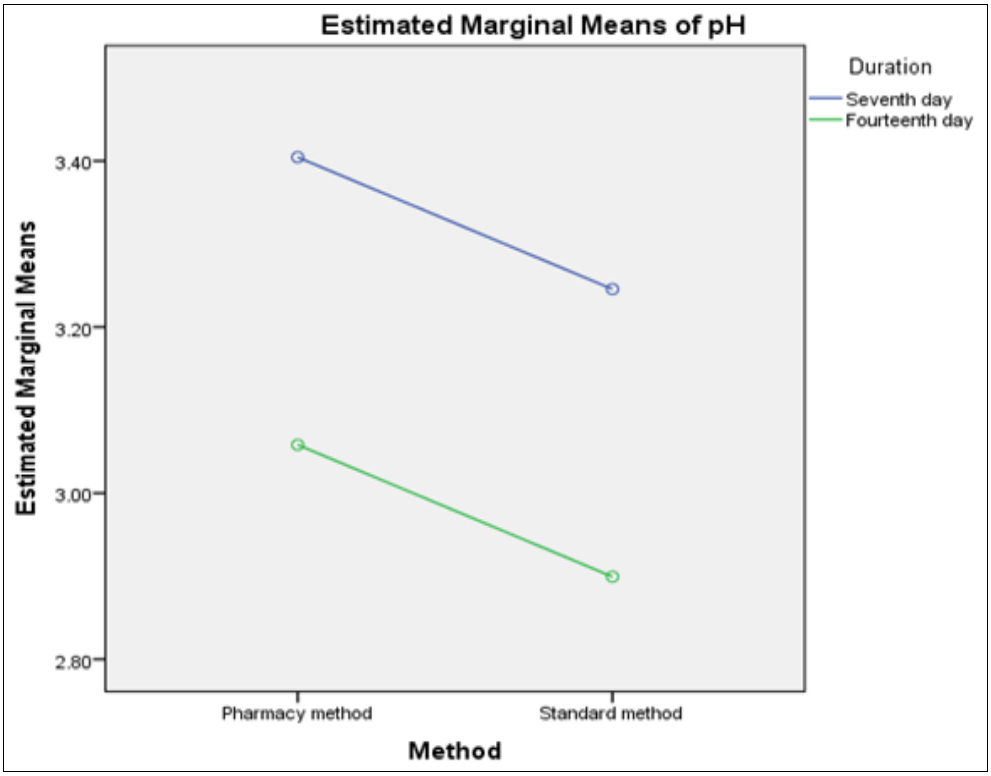

Fig 2: Estimated marginal means of PH

2. Refractive index

Only the Method of Preparation significantly influenced the RF ($p<.001$)

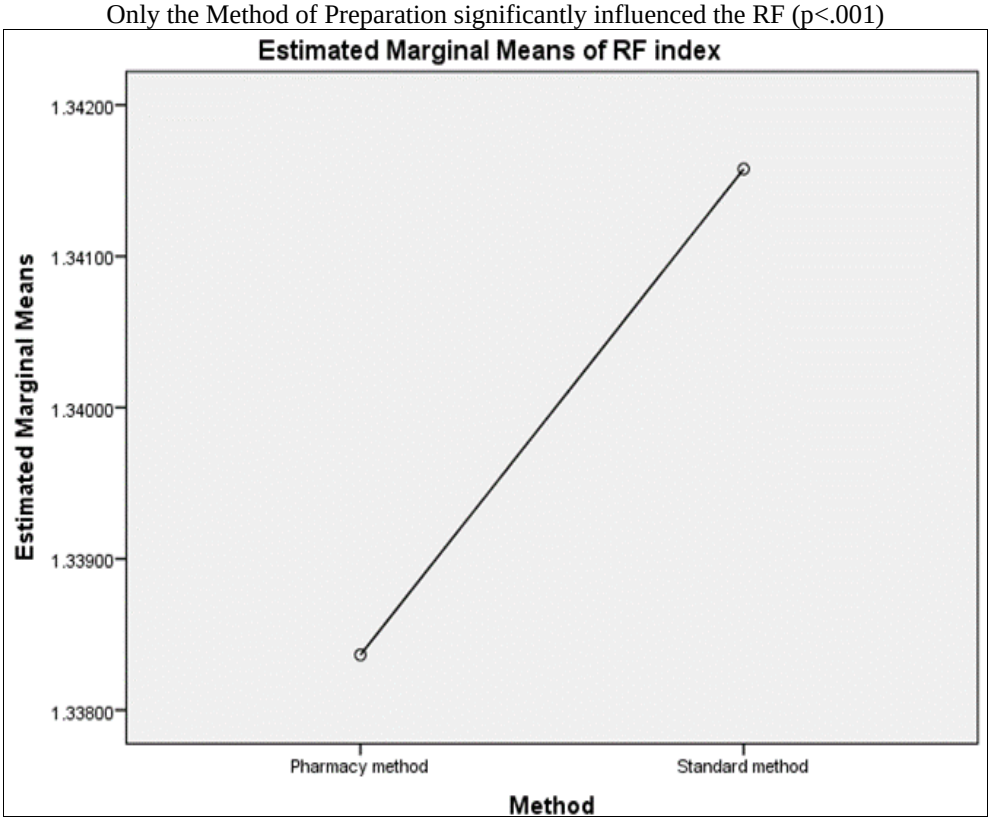

Fig 3: Estimated marginal means of RF index

C. Microbial variation

Study was shown absents of E- coli, salmonella and *Staphylococcus aureus* but there was significant development of fungal growth.

3.1. Storage Duration & Fungal Growth

Table 3.1: Storage duration and fungal growth

			Fungal Growth		Total
			Absent	Present	
Storage Duration	Seventh day	Count	14	10	24
		% within Storage Duration	58.3%	41.7%	100.0%
	Fourteenth day	Count	13	11	24
		% within Storage Duration	54.2%	45.8%	100.0%

Storage Duration did not significantly influence Fungal Growth (p>.05)

3.2. Method of Preparation & Fungal growth

Table 3.2: Method of preparation and fungal growth

			Fungal Growth	
			Absent	Present
Method of Preparation	BMARI Pharmacy method	Count	10	14
		% within Method of Preparation	41.7%	58.3%
	Standard method	Count	17	7
		% within Method of Preparation	70.8%	29.2%

Method of preparation (pharmacy/standard) significantly influences fungal growth (p=.042)

3.3. Storage temperature & fungal growth

Table 3.3: Storage temperature and fungal growth Cross tabulation

			Fungal Growth	
			Absent	Present
Storage Temperature	Room temperature	Count	10	14
		% within Storage Temperature	41.7%	58.3%
	Refrigerated	Count	17	7
		% within Storage Temperature	70.8%	29.2%

Storage temperature significantly influences fungal growth (p=.042)

3.4. Container & fungal growth

Table 3.4: Container and fungal growth

			Fungal Growth	
			Absent	Present
Storage Container	Transparent	Count	14	10
		% within Storage Container	58.3%	41.7%
	Ambient	Count	13	11
		% within Storage Container	54.2%	45.8%

Storage container did not significantly affect fungal growth

3.5. Dilution & fungal growth

Table 3.5: Dilution and fungal growth

			Fungal Growth	
			Absent	Present
Dilution	Neat sample	Count	6	10
		% within Dilution	37.5%	62.5%
	10 dilution	Count	9	7
		% within Dilution	56.3%	43.8%
	100 dilution	Count	12	4
		% within Dilution	75.0%	25.0%

Dilution did not significantly affects fungal growth (p>.05)

5. Conclusion

Standard method of *kwatha* preparation mentioned by *Sharngadhara samhita* was given more promising results than pharmacy method and the study revealed that keeping the standard decoction in a refrigerator using amber coloured bottle helps to preserve its quality throughout fourteen days period without changing its organoeptic characters also.

Standard sample was given RF index (1.34), Specific gravity (1.079) and the pH (3.43)

2. Suggestions

- A microbial colony count assessment through the microscopic view including biochemical test will be revealed further microbial contamination.
- Estimation of pathogenic levels and microscopic identification of fungi will help to reveal the effect to the human body.
- This Study should be carried out as a day by day study up to 14 days to identify the exact starting point of deterioration.

3. Acknowledgement

The research team is really grateful to Dr. S. Kisholorjan of BMARI for helping for statistical analysis and Mrs. Thamara Nawaratne, scientific officer of standardization laboratory of BMARI for giving full support to fulfil our task on time.

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