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Standardization and microbial quality assessment of antidiabetic polyherbal formulations

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Abstract

In the present experiment, seven polyherbal formulations were developed using fruits, vegetables, medicinal herbs and spices by considering their anti-diabetic potential. Polyherbal formulations were carefully composed using thirteen medicinal herbal powders (*Syzigium cumini* – jamun seeds, *Embelica officinalis* – Aonla fruits, *Punica granatum* – pomegranate peel, *Artocarpus heterophyllous* – jackfruit matured bulb, *Momordica charantia* – bitter gourd fruits, *Allium sativum* – garlic clove, *Murraya koenigii* – curryleaves, *Trigonella foenum graecum* – fenugreek seeds, *Cinnamomum verum* – cinnamon bark, *Curcuma longa* – turmeric rhizome, *Zingiber officinale* – turmeric rhizome, *Costus igneus* – insulin leaves and *Azadirachta indica* – neem leaves).

Methods: Microbial load and phyto-chemical parameters such as total bacteria, yeast, moulds, antioxidant were determined according to standard procedure.

Results: The range of total bacterial count in seven polyherbal formulations had range from 21.92 to 102.18 cfu/g. The mould count varied from 24.60 to 62.67 cfu/g. On the other hand, the yeast counts were in the range of 22.25 – 62.65 cfu/g.

Conclusion: The microbial load analysis of polyherbal formulations revealed relatively low level of microbial contamination.

Keywords: Antidiabetic, polyherbal formulation, fruits, spices, vegetables, microbial quality

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia, insulin resistance and relative insulin deficiency as well as disruption in carbohydrate, protein and lipid metabolism (Nagja *et al*., 2017) [1]. It is estimated that 25 per cent of the world's population is affected by this disease, owing primarily to a shift to a sedentary lifestyle that includes unhealthy eating habits and less physical activity (Arumugam *et al*., 2013) [2]. To manage blood sugar levels, a number of synthetic medications such as oral hypoglycemic agents along with insulin are available, but their expense, complications, limited tolerance and other side effects prevent widespread use. As a result, diabetes is notable one of the refractory diseases noticed by the Indian Council of Medical Research for which alternative medical treatment is desperately needed (Aziz *et al*., 2019) [3].

One of the most significant advantages of medicinal plants is that they are readily available and have fewer side effects than synthetic medicines (Thillaivanam and Samraj, 2014) [4]. Plants have always been an excellent source of drugs, and many of the drugs that are currently available are derived directly or indirectly from them (Arumugam *et al*., 2013) [2]. Plant-based diets, which include vegetables, fruits, medicinal herbs and spices are thought to be a good source of phytonutrients that benefit people’s health (Raza *et al*., 2017) [5]. Considering the facts, Phytotherapy is the most commercially successful and widely used branch of alternative or complementary medicine, acquiring a ‘synergy’ that is more effective than the sum of its parts. Phytotherapy flourishes when more than one herb is included in the formulation to achieve the additional therapeutic effectiveness known as polyherbalism. Either pharmacodynamic or pharmacokinetic synergism is necessary to achieve the synergistic effect, *i.e.*, the herb will either target the therapeutic activity to a receptor or it will improve absorption, distribution and metabolism (Falzon and Balabanova, 2017) [6].

Some previous clinical human and animal studies of vegetables, fruits, medicinal herbs and spices *viz*., Anola fruit, bitter gourd, cinnamon bark, curry leaf, fenugreek seeds, garlic clove, ginger rhizome, insulin leaves, jamun seeds, neem leaves, pomegranate peel, jackfruit bulb and turmeric rhizome have shown anti-diabetic property (Osmani *et al*., 2009; Shetty *et al*., 2010; Dholi *et al*., 2011; Santoshkumar *et al*., 2013; Ahmed *et al*., 2014; Waheed *et al*., 2014; Al-Qudah *et al*., 2016; Ahmed *et al*., 2017; Saeed *et al*., 2017; Sidana *et al*., 2017; Srinivasan *et al*., 2018 and Afrose *et al*., 2018) [7-18]. Seven polyherbal formulations were developed considering their anti-diabetic potential as per the review of literature. Polyherbal formulations were carefully composed using thirteen medicinal herbs (Table 1 and 2). Therefore, the present study was made with the idea to develop polyherbal formulations (PHF) based on fruits, vegetables, medicinal herbs and spices and to assess the microbial load in polyherbal formulations.

2. Materials and Methods

2.1 Plant ingredients: Fresh bitter gourd fruits (variety Abhiram), curry leaves (variety Suhasini), neem and insulin leaves used in the present research were collected from a well-maintained field at the Main Horticultural Research and Extension Center, University of Horticultural Sciences (UHS), Bagalkot. Fenugreek seeds, dried turmeric rhizome and garlic were procured from the local market of Bagalkot. Cinnamon bark was collected from the local market of Sirsi (Taluk). Jamun seed powder, mature jack fruit bulb powder and aonla fruit powder were collected from the Department of Post Harvest Technology, College of Horticulture, UHS Campus, GKVK Post, Bengaluru. Pomegranate fruits were procured from well-maintained orchard (Chilagod village, Hagrebommanahalli Taluk, Bellary District). Matured fresh ginger rhizomes used in the present investigation (variety Himachal) were procured from a well-maintained ginger field (Jogipura village, Channarayapatna Taluk, Hassan District).

2.2 Preparation of fruits, vegetables, spices and medicinal plants powder: Pomegranate, bitter gourd fruits and ginger rhizomes harvested at optimum maturity were brought to the laboratory and thoroughly washed with tap water. After washing with tap water, pomegranate fruits were cut into two parts. Then fruit peel was separated manually and cut into pieces. Bitter gourd fruits and peeled ginger rhizomes were sliced by using a sharp stainless steel knife. Freshly harvested curry leaves, neem and insulin leaves were washed with tap water. Later these leaves were dried under the ceiling fan to remove the surface moisture. After preparation, pomegranate peel pieces, bitter gourd slices, ginger slices, peeled garlic, curry leaf, neem and insulin leaves were dried in electrical (Eazy) drier at 50 °C till reaching a safe final moisture level of 7-9 per cent. These dehydrated products and purchased fenugreek seeds, cinnamon bark and dried turmeric rhizomes were ground in a mixer to obtain a fine powder. Later these powders were passed through a 0.5 mm mesh size sieve and packed in aluminium pouches for further preparation of polyherbal formulation and biochemical estimations.

2.3 Preparation of polyherbal formulation (PHF)

Different proportions of herbal powders of jamun seed, aonla fruit, pomegranate peel, bitter gourd fruit, garlic clove, matured jackfruit bulb, curry leaf, fenugreek seed, cinnamon bark, ginger rhizome, turmeric rhizome, insulin and neem leaves were mixed different proportions for preparation of polyherbal formulations. The details of herbs used in the study and the treatment details of polyherbal formulations are given below (Table 1 and 2).

Table 1: List of ingredients used in polyherbal formulation (PHF)

Botanical name	Common name	Family	Parts used
Syzigium cumini	Jamun	Myrtacease	Seeds
Embelica officinalis	Aonla	Euphorbiaceae	Fruits
Punica granatum	Pomegranate	Lythraceae	Fruit peel
Momordica charantia	Bitter gourd	Cucurbitaceae	Fruits
Allium sativum	Garlic	Amaryllidaceae	Clove
Atrocarpus heterophyllous	Jackfruit	Moraceae	Matured bulb
Murraya koenigii	Curryleaf	Rutaceae	Leaves
Trigonella foenum graecum	Fenugreek	Fabaceae	Seeds
Cinnamomum verum	Cinnamon	Lauraceae	Bark
Curcuma longa	Turmeric	Zingiberaceae	Rhizome
Zingiber officinale	Ginger	Zingiberaceae	Rhizome
Costus igneus	Insulin plant	Costaceae	Leaves
Azadirachta indica	Neem	Meliaceae	Leaves

Table 2: Composition of polyherbal formulations (PHF)

Sl. No.	Herbal powder	Quantity of herbs in different formulations (grams)						
		T_1-PHF$_1$	T_2-PHF$_2$	T_3-PHF$_3$	T_4-PHF$_4$	T_5-PHF$_5$	T_6-PHF$_6$	T_7-PHF$_7$
1	Jamun seed	-	10	20	10	10	10	10
2	Aonla fruit	-	8	5	5	5	5	5
3	Pomegranate peel	-	5	5	5	5	5	5
4	Bitter gourd fruit	20	10	10	20	10	10	10
5	Garlic clove	10	5	5	5	5	5	5
6	Jackfruit bulb	10	5	5	5	5	5	5
7	Curry leaf	-	8	5	5	5	5	5
8	Fenugreek seed	15	8	9	9	20	9	8
9	Cinnamon bark	15	10	10	10	9	20	9
10	Turmeric	10	8	8	8	8	8	20
11	Ginger rhizome	10	8	5	5	5	5	5
12	Insulin leaf	-	8	8	8	8	8	8
13	Neem leaf	10	7	5	5	5	5	5

2.4 Physico-chemical investigation: According to standard procedure microbial loads were analyzed. The microbial count in the polyherbal formulations was carried out as per the method of Harrington and McCane (1966) [19]. One gram of powder was taken for analysis. The samples were plated on the
Nutrient Agar media for total bacteria, Martin's Rose Bengal Agar (MRBA) for mould count and Yeast peptone glucose agar for yeast count following serial dilution technique. Plates were incubated for one week at 25±1 °C. Colonies were counted and expressed as cfu per gram.

2.5 Statistical data analysis: Statistical analysis was performed using Web Agri Stat Package (WASP) Version 2.0 (Jangam and Thali, 2010) [20]. All data collected (7 treatments with 3 replications) were analyzed by one-way analysis of variance (ANOVA). Significant differences among means at $p \leq 0.05$ were determined by using Duncan's multiple range tests.

3. Results

3.1 Microbial load (cfu/g): Examination concerning to microbial load in different polyherbal formulations of this study revealed significant differences (Table 3, Figure 1). All the polyherbal formulations differed significantly. The range of total bacterial count in seven polyherbal formulations had range from 21.92 to 102.18 cfu/g. The mould count varied from 24.60 to 62.67 cfu/g. On the other hand, the yeast counts were in the range of 22.25 – 62.65 cfu/g. Significantly maximal bacterial count was observed in T_1 (102.18 cfu/g) and the least bacterial count was noticed in T_3 (21.92 cfu/g). Statistically, the highest mould count was observed in T_3 (62.67 cfu/g), whereas the minimum mould count was noted in T_6 (24.60 cfu/g). The maximum yeast count was noted in T_5 (62.65 cfu/g) which was at parity with T_1 (62.25 cfu/g) and the lowest yeast count was registered in T_2 (22.25 cfu/g).

Table 3: Effect of different compositions on total bacteria, mould and yeast count of polyherbal formulations

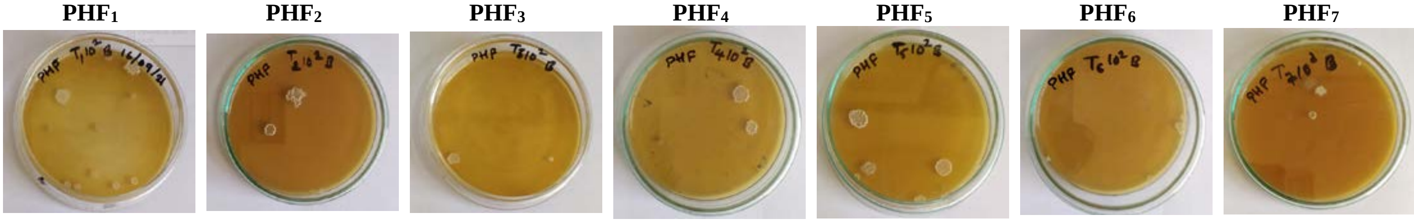
Treatments	Microbial load (cfu/g)		
	Total bacteria	Mould	Yeast
T_1	102.18^{a}	52.42^{b}	62.25^{a}
T_2	51.83^{d}	41.92^{c}	22.25^{d}
T_3	21.92^{e}	62.67^{a}	35.08^{c}
T_4	52.58^{d}	32.90^{d}	24.10^{d}
T_5	84.17^{b}	34.42^{d}	62.65^{a}
T_6	62.75^{c}	24.60^{e}	24.25^{d}
T_7	54.08^{d}	48.08^{b}	45.81^{b}
Mean	61.36	42.43	39.48
S.Em±	1.50	1.55	1.51
CD at 5%	4.58	4.72	4.58

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

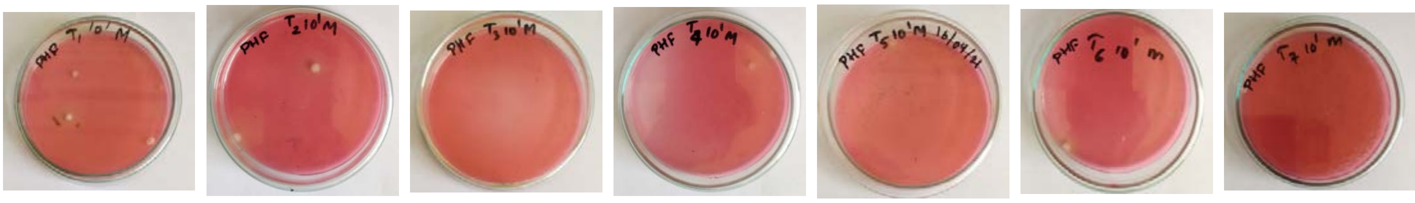
4. Discussion

4.1 Microbial load (cfu/g): Microbial quality assessment is one of the most important parameter for the standardization of herbal drugs. Medicinal plant may come in contact with a broad variety of microbial contaminants, represented by bacteria, fungi, yeast and moulds (Borah and Saikia, 2020) [21].
Nakajima *et al.* (2005) [22] opined that the presence of microbial contaminants in non-sterile pharmaceutical products can reduce or even inactivate the therapeutic activity of the drugs and have the potential to adversely affect patients taking the medicines. The microbial quality of pharmaceuticals is influenced by the environment and the quality of the raw materials used during formulation. Thus, manufacturers should ensure the lowest possible level of microorganisms in the raw material, finished dosage forms and the packaging components to maintain the appropriate quality, safety and efficacy of the herbal products (Okunlola *et al*., 2007) [23].
According to World Health Organization (WHO) standards for microbial load analysis, the limits for the total aerobic count and yeast and mould count should be <1000 and <100 cfu/g, respectively. In the present investigation, the total bacterial count of seven polyherbal formulations had range from 21.92 to 102.18 cfu/g (Table 3). However, the mould count varied from 24.60 to 62.67 cfu/g, while the yeast counts were in the range of 22.25 – 62.65 cfu/g. Furthermore, low levels of microbes in all seven polyherbal formulations indicate hygienic preparation. From the results, it is shown that all seven polyherbal formulations comply with the WHO standards for microbial load analysis and hence it is safer to be taken internally. Wakkumbura *et al*. (2020) [24] reported absence of *Escherichia coli*, Coliforms and *Salmonella* and aerobic plate counts revealed few (<10 cfu/g) yeasts and moulds (<10 cfu/g) and *Staphylococcus aureus* (<10 cfu/g) in ayurvedic polyherbal formulation. Similarly Francis and Sudha (2016) [25] noticed absence of bile-tolerant gram negative bacteria, *Escherichia coli*, *Salmonella* and total aerobic microbial count. Total combined yeast/mould counts revealed less than 10 cfu/g in polyherbal formulation.

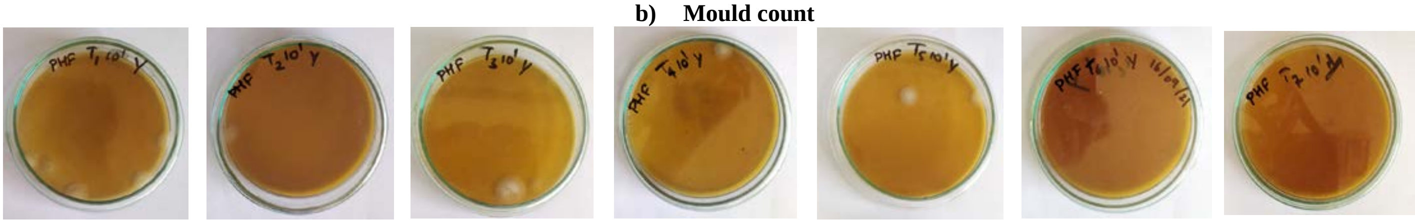
PHF_1 PHF_2 PHF_3 PHF_4 PHF_5 PHF_6 PHF_7



a) Total bacterial count



b) Mould count



c) Yeast count

Fig 1: Picture indicating microbial load a) total bacteria, b) mould and c) yeast count in polyherbal formulations (PHF_1 –PHF_7)

5. Conclusion

The microbial load analysis indicated relatively low level of microbial contamination. These polyherbal formulations can be used to develop pharmaceutical products such as tablets and capsules.

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7. Author conflict of interest statement: The authors declare they have no conflict of interest.

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