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Comprehensive Study and Impact of Microbial Growth on Treated and Un-Treated Herbal Extracts

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ABSTRACT

Purpose of Study: The purpose of the study is to determine the presence of microorganisms in the herbal thick extracts which are being used in different phyto pharmaceutical industries for manufacturing of different dosage form designs to cure a wide range of disorders in humans. No doubt, medicinal herbs have been effectively used by our ancestor's as remedy for curative functions but the high growth of natural and pathogenic microorganisms impede their efficiency, rather present safety issues. In present study, therefore the effect of microbial activity has been determined in untreated and treated conditions.

Methodology: This study was carried out at the research lab, Department of Microbiology, Federal Urdu University, Karachi and R&D unit, Herbion Pharmaceutical (Pvt.) Limited, Karachi Pakistan. The effect of microbial activity has been determined in untreated (without any treatment) and different treated conditions (heat treatment, addition of preservatives and heat treatment along with preservatives) to find out the most satisfactory technique for microbial control without harming natural constituents of plants thick extracts.

Result: A high rate of bacterial and fungal count was observed in untreated raw plant extract. After treatment with heat, however, led to remarkable reduction of microbial growth (>10^3cfu/g). It is observed after heat treatment for an hour and heat treatment along with preservatives for 30 minutes.

Conclusion: A remarkable effect on microbial growth can be observed in the herbal extracts through use of combination of preservatives and treatment for 30 minutes at 80°C temperature. However, no significant depletion in microbial activity was visualized after application of preservation and evaporation technique alone.

Keywords: Herbal thick extracts, preservatives, heat treatment, microbial growth.

INTRODUCTION

Medicinal Herbs are used as a valuable source of medication worldwide due to presence of significant potential for different therapeutic activities-antimicrobial, immunomodulatory, provision of general health, and healing and other therapeutic potentials; thus, providing cure and prevention for several diseases. Thus extracts obtained from these plants of medicinal value are being used for formulating a wide range of Herbal formulations [1].

Initially, these herbs are used to prepare a herbal extract which is obtained as the result of action of solvent on plant parts and dissolving some of its soluble components, resulting in a solution [2]. The obtained solution is then separated from the rest of the insoluble plant ingredients. On separation, the extract left in liquid form can be kept in the same form of solution or the excess liquid can be converted to a

semi solid extract through some process [3]. The assumption of the study was that the natural plant extracts produce considerable influence on increasing the rate of microorganism's especially total aerobic count, total yeast and mold count. Many studies have been reported about the beneficial as well as detrimental effects of herbal thick extracts. Many herbal thick extracts have this property of providing an excellent activity against different Gram +ve or Gram –ve bacteria and molds but some are providing good source of harboring group of different species [4]. Some microorganisms are treatable and some are mutagenically resistant to different treatment procedures. The behavior and effect of microbial growth is being influenced by plant type.

Plants are naturally known to harbor endophytic microbes and some gets infected from other sources for example environment flora (dust particle), water (raw or waste water), soil and human activity. Synthesis of biologically active compounds by plants is important for microbial promotion and that result hidden microbial spoilage in plants significantly used in pharmaceutical products [5].

MATERIALS AND METHODS

Instruments, Reagents and Chemicals

Disinfected gloves, S.S testing table (Laminar air flow), media bottles with Isopropyl alcohol (IPA)**.** Tryptone Soy Agar (TSA), Tryptone Soy Broth (TSB), Lactose Broth (LB), Peptone water (PW), test tubes, ATCC cultures were the major chemicals used.

Plant Extracts

Insty granules herbal thick extract and grinded Bonjigar extract of raw herbs were taken for the study purpose. Bonjigar extract is a polyherbal plant extract which acts as liver tonic. On the other hand, Insty Granules is a blend of herbs commonly used for flu, cold and cough. The first step of manufacturing is the extraction of desired parts of plant eventually employed for dosage form design. The extracts obtained were taken for study purpose for any possible microbial contamination.

Procedure

Herbal extracts were tested by first transferring 10 ml / 10gm of the extract in 250ml glass bottle containing 90 ml /100 ml of sterilized fluid soybean casein digest medium (TSB) or buffered sodium chloride peptone water with pH 7.0 or phosphate buffer with pH 7.2 or normal saline to make 1:10 dilution (i.e. master dilution) [6]. If herbal thick extract to be inspected holds antimicrobial activity, this so far as possible removed or neutralized (Soy lecithin 0.5% polysorbate20 4.0%). Diluted further if necessary, so that it was expected to yield between 30-300 colonies [7, 8]. Pipette 1 ml of last prepared dilution into each of for sterile petri plates with the help of sterile pipette/ micropipettes [9]. Immediately poured into two petri plates about 15-20 ml of sterilized tryptone soy agar (TSA) and sabouraud dextrose agar medium (SDA) which had been melted earlier and then cooled to nearly 45°C. The petri plates are covered and then the sample is mixed with the media through tilting or rotating the plates and the contents are allowed to solidify at room temperature. Inverted the petri plates, and incubated tryptone soy agar (TSA) containing plates for 72 hours at 32.5 ± 2.5°C and sabouraud dextrose agar medium (SDA) for 5 to 7 days at 20° to 25°C [10]. After incubation period, examined the plates for growth, counted the number of colonies i.e. average no of colonies × dilution factor and expressed the results in terms of the colony-forming unit per ml of sample [11]. If no microbial colonies are observed from the plates representing the first 1:10 dilution of sample, it dictated the results as less than 10 colony forming unit per ml of sample [12].

Detection of Pathogens

The presence or absence of pathogenic species have been examined along with their positive and negative controls in herbal plants extracts, for example *Escherichia coli*, Gram negative bacteria, *Salmonella* species, *Candida albicans*, *Aspergillus niger* [13].

RESULTS AND DISCUSSION

The herbal thick extracts are observed in different conditions and activity of microbes is different in different conditions but mutual conditions are also determined to find out microbial activity in heat treatment alone for one hour and heat treatment 30 minutes along with preservatives methyl paraben 282 gm and 0.45% and Propyl paraben 56gm and 0.09%. Bonjigar raw herb was tested at different dilutions but results did not meet the recommended criteria and high bacterial and fungal growth is observed, as shown in Fig. **1**, Fig. **2** and Fig. **3**.

Fig. (1). Grinded Bonjigar extract raw herb.

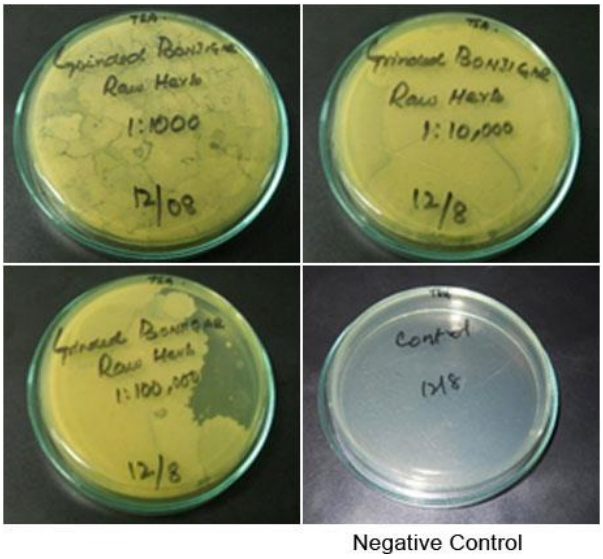

Fig. (2). Bacterial growth in grinded Bonjigar extracts raw herb.

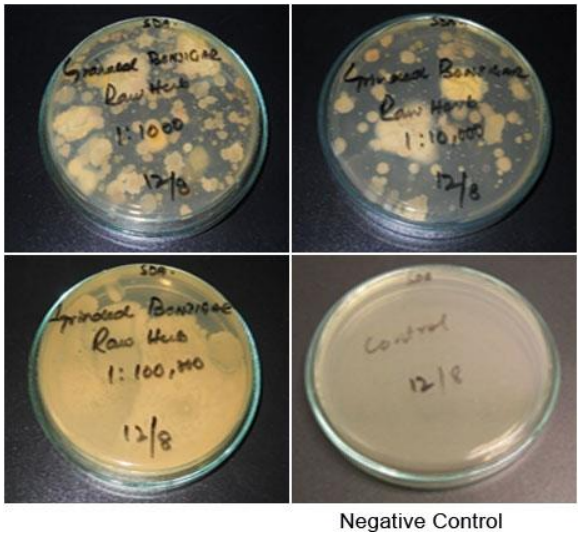

Fig. (3). Mold growth in grinded Bonjigar extracts raw herb.

Insty granules thick extract after addition of preservatives and heat-treatment were tested on different dilutions and the results met the standard criteria of microbial limit of botanical ingredients and products in as shown in Fig. **4**, Fig. **5** and Fig. **6**.

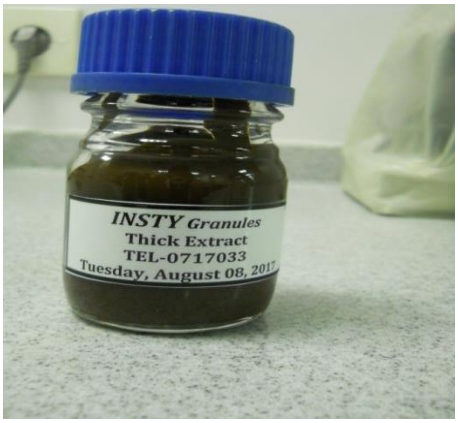

Fig. (4). Insty granules thick extract after HT treated and addition of preservatives (30mins).

Fig. (5). Bacterial growth in Insty granules after HT treated and addition of preservatives (30mins).

Fig. (6). Mold count in Insty granules after HT treated and addition of preservatives (30mins).

Preservatives are, however, were found not enough to control growth unconditionally and inhibited the growth when provided with additional 80°C temperature for 30 minutes, which worked synergistically for the limitation of bacterial and fungal growth (Table **1** and Table **2**).

Table 1. Effects of microbial growth in untreated bonjigar thick extract.

S. No.	Test Description	BP Specification	Observation
			Bonjigar Raw Herb
1	**Total Aerobic Microbial Count**	Not more than 10^4 *(=50,000 CFU/gm)*	10,00,00,000 cfu/gm
2	**Total Yeast and Mold Count**	Not more than 10^2 *(=500 CFUgm)*	5000,000 cfu/gm
3	**Bile-tolerate Gram negative bacteria**	1000 cfu/g or Cfu/mL	Absent
4	***E. coli***	Absent (1g or 1mL)	Absent
5	***Salmonella***	Absent (25g or 25mL)	Absent
6	**Media Used:**	**Tryptic Soy Agar** (For Total Aerobic Microbial Count) **Sabaroud Dextrose Agar** (For Yeast and Mold Count)	-
7	**Incubation temperature and period for Microbial Count:**	30-35 ℃ for 72 hours	-
8	**Incubation temperature and period for Yeast and Mold Count:**	20-25 ℃ for 5-7 days	-
9	**Method Used:**	By Pour Plate	-

Table 2. Effects of microbial growth in treated bonjigar thick extract.

S. No.	Test Description	BP Specification	Observation			
			Bonjigar caspules after HT treated and addition of preservatives	**Bonjigar capsules after addition of preservatives**	**Bonjigar capsules after decantation primary evaporation**	**Bonjigar capsules after HT treated**
1.	**Total Aerobic Microbial Count**	Not more than 10^4 *(=50,000 CFU/gm)*	Nil cfu/gm	100,000 cfu/gm	60,000 cfu/gm	Nil cfu/gm
2.	**Total Yeast and Mold Count**	Not more than 10^2 *(=500 CFUgm)*	Nil cfu/gm	10,000 cfu/gm	7,000 cfu/gm	Nil cfu/gm
3.	**Bile-tolerate Gram negative bacteria**	1000 cfu/g or Cfu/mL	Absent	Absent	Absent	Absent
4.	***E. coli***	Absent (1g or 1mL)	Absent	Absent	Absent	Absent
5.	***Salmonella***	Absent (25g or 25mL)	Absent	Absent	Absent	Absent
6.	**Media Used:**	**Tryptic Soy Agar** (For Total Aerobic Microbial Count) **Sabaroud Dextrose Agar** (For Yeast and Mold Count)	**-**	**-**	**-**	**-**
7.	**Incubation temperature and period for Microbial Count:**	30-35 ℃ for 72 hours	**-**	**-**	**-**	**-**
8.	**Incubation temperature and period for Yeast and Mold Count:**	20-25 ℃ for 5-7 days	**-**	**-**	**-**	**-**
9.	**Method Used:**	By Pour Plate	**-**	**-**	**-**	**-**

Insty granules after addition of preservative, tested on different dilution resulted in both aerobic microbial count and yeast and mold counts which exceeded to its limit. This shows preservatives were alone not sufficient to minimize and inhibit microbial multiplication that's why high growth is noticeable in as shown in Fig. **7**, Fig. **8** and Fig. **9**.

Fig. (7). Insty granules thick extract after addition of preservatives.

Fig. (8). Bacterial growth in Insty granules after addition of preservatives.

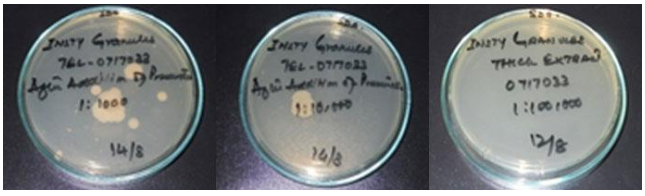

Fig. (9). Mold count in Insty granules after addition of preservatives.

Another possible reason could be the resistance of mutagenic microorganism that could not be controlled by preservatives alone. High temperature is compulsory for removal of microbes. Insty granules herbal thick extract after evaporation were tested on different dilution conc, total aerobic count was found within the limit but yeast and mold exceeded to its acceptance limit as shown in Fig. **10**, Fig. **11** and Fig. **12**.

Fig. (10). Insty granules thick extract after evaporation.

Fig. (11). Bacterial count in Insty granules after evaporation.

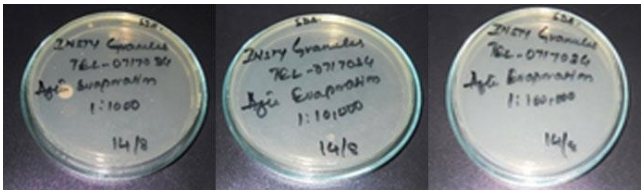

Fig. (12). Mold count in Insty granules after evaporation.

Evaporation is not simply an efficient technique to eliminate mold count from the thick extract. Insty granules after an hour heat treated tested on different dilution results meets the standard criteria of microbial limit of botanical ingredients and products. According to several different treatments, it is clear that the high temperature for an hour is the best way for killing microorganism from the thick extracts as shown in Fig. **13**, Fig. **14** and Fig. **15**.

Fig. (13). Insty granules thick extract after HT treatment (1hr).

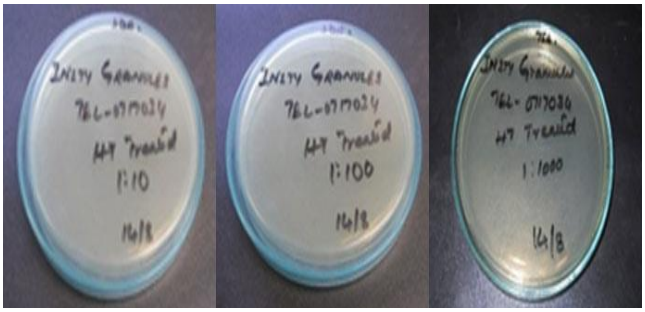

Fig. (14). Bacterial count in Insty granules after HT treated (1hr).

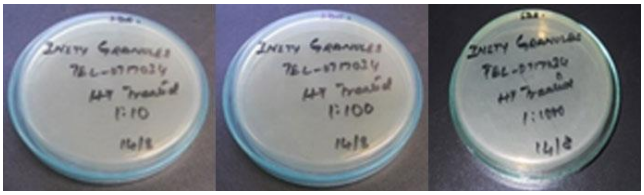

Fig. (15). Mold count in Insty granules after HT treated (1hr).

Combination of preservatives and 30 minutes 80°C temperature is sufficient enough and control growth (Table **3** and Table **4**).

Table 3. Effects of microbial growth in untreated Insty thick extract.

S. No.	Test Description	BP Specification	Observation
			Insty Raw Herb
1	**Total Aerobic Microbial Count**	Not more than 10^4 *(=50,000 CFU/gm)*	30,00,00,000 cfu/gm
2	**Total Yeast and Mold Count**	Not more than 10^2 *(=500 CFUgm)*	4000,000 cfu/gm
3	**Bile-tolerate Gram negative bacteria**	1000 cfu/g or Cfu/mL	Absent
4	***E. coli***	Absent (1g or 1mL)	Absent
5	***Salmonella***	Absent (25g or 25mL)	Absent
6	**Media Used:**	**Tryptic Soy Agar** (For Total Aerobic Microbial Count) **Sabaroud Dextrose Agar** (For Yeast and Mold Count)	**-**
7	**Incubation temperature and period for Microbial Count:**	30-35 ℃ for 72 hours	-
8	**Incubation temperature and period for Yeast and Mold Count:**	20-25 ℃ for 5-7 days	-
9	**Method Used:**	By Pour Plate	-

Table 4. Effects of microbial growth in treated Insty thick extract.

S. No.	Test Description	BP Specification	Observation			
			Insty granules after HT treated and addition of preservatives	**Insty granules after addition of preservatives**	**Insty granules after decantation primary evaporation**	**Insty thick extract after HT treated**
1.	**Total Aerobic Microbial Count**	Not more than 10^4 *(=50,000 CFU/gm)*	Nil cfu/gm	200,000 cfu/gm	40,000 cfu/gm	Nil cfu/gm
2.	**Total Yeast and Mold Count**	Not more than 10^2 *(=500 CFUgm)*	Nil cfu/gm	20,000 cfu/gm	10,000 cfu/gm	Nil cfu/gm
3.	**Bile-tolerate Gram negative bacteria**	1000 cfu/g or Cfu/mL	Absent	Absent	Absent	Absent
4.	***E. coli***	Absent (1g or 1mL)	Absent	Absent	Absent	Absent
5.	***Salmonella***	Absent (25g or 25mL)	Absent	Absent	Absent	Absent
6.	**Media Used:**	**Tryptic Soy Agar** (For Total Aerobic Microbial Count) **Sabaroud Dextrose Agar** (For Yeast and Mold Count)	**-**	**-**	**-**	**-**
7.	**Incubation temperature and period for Microbial Count:**	30-35 ℃ for 72 hours	-	-	-	-
8.	**Incubation temperature and period for Yeast and Mold Count:**	20-25 ℃ for 5-7 days	-	-	-	-
9.	**Method Used:**	By Pour Plate	-	-	-	-

CONCLUSION

A remarkable effect on microbial growth can be observed in the herbal extracts through use of combination of preservatives and treatment for 30 minutes at 80°C temperature. However, sufficient effect could not be seen after preservatives addition alone or either evaporation before and after the treatment process. It is therefore recommended to use a combined approach for better microbial control.

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