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Research Article

ANTI MICRO-BIOLOGICAL ANALYSIS OF ARJUNARISTA

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ABSTRACT

Arjunarista, an imperative Ayurvedic formulation used for treatment of cardiovascular disorders and prepared by fermenting the decoction of specified plant materials i.e. *Arjuna twak* (*Terminalia arjuna*), *Draksha phala* (*Vitis vinifera*), *Madhuk puspa* (*Madhuka indica*), *Dhataki puspa* (*Woodfordia fruticosa*) and *Jaggery* (*Saccharum officinarum*). **Aim:** To assess anti-microbial analysis of *Arjunarista*. **Setting and Design:** *Arjunarista* was prepared as per process of *Shandhan kalpana* mentioned in manuscripts of *Ayurveda* with consideration of two variations. These variations were at the level of pot like earthen pot and porcelain pot and addition/deletion of flowers of *Dhataki* (*Woodfordia fruticosa* (L.) Kurz). **Methods and Materials:** Micro-biological analysis was done for total bacterial count, total fungal count and specific pathogens. Total bacterial count, total fungal count was done via plate count agar method and potato dextrose agar respectively. Test for specific pathogens like *E. coli*, *Salmonella sp.*, *Staphylococcus aureus* & *Pseudomonas aeruginosa* was done by Violet red bile agar, Salmonella Agar and Mueller Hinton Agar (for last two bacteria). **Results:** Total bacterial count (TBC) was absent in all batches except one sample of batch II and batch IV respectively. Total fungal count (TFC) was not present in all batches except one sample of batch I. Specific pathogens count were not present at all in any batches. **Conclusion:** *Arjunarista* showed anti micro-biological activity irrespective of variations taken in study.

KEY WORDS: *Arjunarista*, Total bacterial count, Micro-biological, Specific pathogens.

INTRODUCTION

Herbal medicinal preparations have been used since ancient times to treat a wide range of diseases [1]. They are promoted as natural and safe, therefore preferred as a choice [2] for treatment of cardiovascular disorders and World Health Organization (WHO) survey indicates that about 70-80% of world population rely on complementary and alternative medicines [3]. WHO also developed the technical guidelines for the assessment of microbiological quality of herbal medicines [3]. Use of herbal preparations by patients is increasing, there is an urgent need for pharmacists and physicians to have conscious about the safety & efficacy of these preparations [5].

The quality assessment of herbal formulations is very important in order to justify their acceptance in current scenario. It is thus

mandatory that micro-biological limit tests of herbal medicinal preparations be done to ensure that the product is free from microbes. In India, many formulations are handmade or made by physicians directly and are not subjected to aseptic conditions during various stages of preparation like packaging, storage, transport etc, as required by regulatory norms. Plant materials carry a huge number of bacteria and fungi, mainly originating in soil, aerobic sporulating bacteria frequently predominate in this, to which additional contamination and microbial growth occur during harvesting, handling and production [4].

Arjunarista was prepared by *Arjuna* (*Terminalia arjuna*), *Draksha* (*Vitis vinifera*), *Madhuka* (*Madhuka indica*), *Dhataki* (*Woodfordia fruticosa*), & *Jaggery* (*Saccharum officinarum*) by

the process of *Sandhan kalpana*. Among this grape juice consist of 79% water, 20% carbohydrate, 1% organic acid and nitrogenous compound [6]. *Madhuka* flowers have yeast strain *sacharomyces cerevisiae*-3090 was obtained from "national collection of industrial microorganism" [7]. Besides this, nine morphologically different strains of yeast were isolated from fresh, fermenting and fermented juice of *Madhuka* flowers, identified by morphological and biochemical characteristic [8]. Micro-biological flora from nectarines of *W.fruticosa* flowers remain as valuable source of sugar tolerant yeast that drives the fermentation [9]. It was observed that above mentioned ingredients contain specific micro-organism in crude form. Thus it is mandatory to analyse micro-biological limit tests of *Arjunarista* preparations to ensure that the product is free from hazardous effect.

SUBJECTS AND METHODS

Pharmaceutical Study

Arjunarista was prepared with *Arjuna twak* (*Terminalia arjuna*), *Draksha phala* (*Vitis vinifera*), *Madhuk puspa* (*Madhuka indica*), *Dhataki puspa* (*Woodfordia fruticosa*) and *Jaggery* (*Saccharum officinarum*) by process of *Sandhan kalpana* [10].

Preparation of Arjunarista

In the present study, two variations were taken in to consideration i.e. pot and *Dhataki puspa*. In pot variation two types of pot were used i.e. earthen pot and porcelain pot, and in *Dhataki puspa*, addition and deletion of *Dhataki puspa* was considered. In this way four batches of *Arjunarista* were prepared and in each three sample were prepared, thus total no. of sample were twelve.

Procurement & Identification

Fresh *Arjuna* (*Terminalia arjuna*) stem bark was collected from BHU campus nearby Dhanwantari Bhawana, Faculty of Ayurveda, IMS, BHU, and Nagarjuna Doctors Hostel BHU. *Jaggery* (*Saccharum officinarum*) was taken from the home made by sugar-cane, and rest of the drugs i.e. *Draksha* (*Vitis vinifera*), *Madhuka* (*Madhuka indica*), *Dhataki* (*Woodfodia fruticosa*) purchase from the local market of Varanasi publically known as Dina Nath Gola market. Identification of all raw materials was done in Dept. of Dravyaguna, Faculty of Ayurveda, IMS, BHU.

Pot Preparation

Washing of Porcelain pot with detergent and rinsing it then after dried in sunlight for 12 hrs, earthen pot was newly purchase from the market and soaked overnight. *Lepana* (coating) of Honey and Cow ghee was applied on internal surface of pots. *Dhoopana* (fumigation) was done along with *Guggulu*, *Karpura*, *Raal*, *Sarsapa*, *Guda*. Duration of fumigation was 20 min. in all pots. *Arjuna* stem bark was washed properly with tap water to avoiding foreign matter as a impurity like dust, sand etc.

Decoction [11]

Freshly *Arjuna* stem bark (20.7 kg) was dried on indirect sunlight for 3-4 days, and then after weight had been taken from electronic weighing machine it was noted 10.2 kg. Loss on drying: 50% (Approx). Powdering of dried *Arjuna* bark was done in *Ayurvedic Pharmacy*, IMS, BHU. Sieve applied for this purpose was 40 no. within the range of coarse powder. Rest of the drug used after insuring its genuineness & free from impurity. *Yavakuta churna* (coarse powder) of *Arjuna* (*Terminalia arjuna*) stem bark *Draksha* (*Vitis vinifera*), & *Madhuka* (*Madhuka indica*) used as a whole in preparation of decoction at mild temperature i.e. (150-250 °C). When *kwatha* reduced to ¼ of initial amount then *kwatha* was strained with double layered cotton cloth and measured it.

Wort Formation

After straining of *Kwatha* with double folded cotton cloth in another cleaned vessel. Now *jaggery* and *Dhataki puspa* was added and dissolved it thoroughly by proper mixing with steel ladle. Finally wort was filled in earthen as well as porcelain pots and placed at proper place. Colour of wort was dark brown. Smell felted of wort was very peculiar sweet & fruity. Viscosity was increased than *Kwatha drava*. Proper mixing was done to make a homogenous mixture. *Jaggery* and *Dhataki puspa* was added in decoction after half an hour later when getting temp of 40-50°C.

Fermentation Process

Prepared wort was filled in earthen as well as porcelain pot. These pots were placed in husk and temperature of husk as well as room temp. were recorded during procedure, it was 36°C and 35°C - 37°C respectively. After starting the fermentation process, mouth of pot was sealed with cotton cloth smeared with clay. Whole arrangement was kept and observations

were recorded during process. After acquiring the *Siddhi lakashan* (completion test), prepared

Arjunarista was filtered with cotton cloth and stored in containers.

Table 1: Ingredients with quantity of ingredients [11]

S.No	Ingredients	Quantity in B-I	Quantity in B-II	Quantity in B-III	Quantity in B-IV
1.	<i>Arjuna twak</i>	1Tula(4.8 Kg.)	1Tula(4.8 Kg.)	1Tula(4.8 Kg.)	1Tula(4.8 Kg.)
2.	<i>Draksha phala</i>	½Tula(2.4 Kg.)	½Tula(2.4 Kg.)	½Tula(2.4 Kg.)	½Tula(2.4 Kg.)
3.	<i>Madhuk puspa</i>	20Pal(960 Gm.)	20Pal(960 Gm.)	20Pal(960 Gm.)	20Pal(960 Gm.)
4.	<i>Dhataki puspa</i>				
5.	<i>Jaggery</i>	1Tula(4.8 Kg.)	1Tula(4.8 Kg.)	1Tula(4.8 Kg.)	1Tula(4.8 Kg.)
6.	Quantity of Obtained <i>Arjunarista</i>	2.505 L (35.05%)	1.712 L (24.45%)	5.006 L (63.29%)	4.287 L (62.49%)

Micro-biological Contamination

Arjunarista was subjected to micro-biological study including total bacterial, fungal count and specific test for pathogen to ensure its contamination. This test has been done in Center of Food Science & Technology, Institute of Agricultural Sciences, Banaras Hindu University.

Total Bacterial Count/Total Viable Count [12]

Plate count agar (HIMEDIA, India) was used to determine the total viable count in the *Arjunarista* samples. The media used for the TVC was composed of 5 gm casein enzyme hydrolyses, 2.5 gm yeast extract, 1 gm dextrose and 9 gm agar per liter and the pH was adjusted to 7.0 ± 0.2 . 8.75 gm of the dry media was suspended in 500 ml distilled water. The mixture was then boiled to dissolve the medium completely. It was then filled in conical flask and the mouths of the conical flask closed with cotton plugs. The conical flask was then sterilized by autoclaving at 15 psi pressure (121°C) for 15 min. Total viable counts were obtained with the help of standard procedure given by Indian standard (SP: 18 (part XI) – 1981). The plates were incubated at 37°C in inverted position for 48 hours and the colony count expressed as cfu/g of the product.

Total Fungal Count [12]

All the procedure, materials, equipment, precaution were the same only differ in culture media which is potato dextrose agar (PDA) (HIMEDIA, India) was used to enumerate yeast and mould counts in the *Arjunarista* samples. The media contained 300 g potatoes, 20 g dextrose and 15 g agar per liter, and the pH was adjusted to 5.6 ± 0.2 . 19.5 gm of PDA powder was suspended in 500 ml distilled water and then boiled to dissolve the medium completely. It was then transferred to conical flasks which

were then sterilized by autoclaving at 15 psi pressure (121°C) and the pH 5.6 of the media was lowered by using 10 % sterile tartaric acid solution. Yeast and mould were enumerated following the standard protocol delineated in Indian Standards (SP: 18 part XI-1981). The plates were incubated at 25°C for 3-5 days and counts were expressed as cfu/g.

Test for Specific Pathogen [12]

This test was done for specific pathogens like *E. coli*, *Salmonella sp.*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.

Violet Red Bile Agar [12]

All the procedure, materials, equipment, precaution were the same only differ in culture media which is violet red bile agar (VRBA) (HIMEDIA, India) was used to enumerate *E. coli* counts in the *Arjunarista* samples. The media contained 7 gm/dl peptic digest, yeast 3.0, bile salt, sodium chloride and the pH was adjusted to 7.4 ± 0.2 . 20.75 gm of VRBA powder was suspended in 500 ml distilled water and then boiled to dissolve the medium completely. It was then transferred to conical flasks which were then sterilized by autoclaving at 15 psi pressure (121°C) and the pH 5.6 of the media was lowered by using 10 % sterile tartaric acid solution. *E. coli* were enumerated following the standard protocol delineated in Indian Standards (SP: 18 part XI-1981). The plates were incubated at 25°C for 48hrs and counts were expressed as cfu/g.

Salmonella Agar [12]

Salmonella Agar (HIMEDIA, India) was used to enumerate *Salmonella* counts in the *Arjunarista* samples. The media contained yeast 3.0gm, meat 6.0, lactose 11.50g, sucrose 13g bile salt 3.8g, ferric citrate, and phenylalanine. 38g powder was suspended in 500 ml distilled water

and then boiled to dissolve the medium completely. It was then transferred to plate for stabilizing. No autoclaving required. Salmonella were enumerated following the standard protocol delineated in Indian Standards (SP: 18 part XI-1981). The plates were incubated at 25°C for 48hrs and counts were expressed as cfu/g.

Mueller Hinton Agar ^[12]

Used for both the bacteria *Pseudomonas* & *Staphylococcus*. Mueller Hinton Agar (MHA) (HIMEDIA, India) was used to enumerate *Pseudomonas* as well as *Staphylococcus* counts in the *Arjunarista* samples. The media contains beef 300g, starch 1.50g, casscinated hydrolyses 17.50g and the pH was adjusted to 7.3 ± 0.1. 19 gm of MHA powder was suspended in 500 ml

distilled water and then boiled to dissolve the medium completely. It was then transferred to conical flasks which were then sterilized by autoclaving at 15 psi pressure (121°C). *Pseudomonas* and *Staphylococcus* were enumerated following the standard protocol delineated in Indian Standards (SP: 18 part XI-1981). The plates were incubated at 25°C for 48hrs and counts were expressed as cfu/g.

RESULTS

Total bacterial count (TBC) were not present in all batches except two samples, total fungal count (TFC) not present in all batches except one sample and no specific pathogens were present in any samples. (Details were summarised in table no.2)

Table 2: Showing micro-biological analysis of all samples of Arjunarista.

Parameters	B-I			B-II			B-III			B-IV		
	E1 (D-)	E2 (D-)	E3 (D-)	E1 (D+)	E2 (D+)	E3 (D+)	P1 (D-)	P2 (D-)	P3 (D-)	P1 (D+)	P2 (D+)	P3 (D+)
TBC cfu/ml	Nil	Nil	Nil	1x10 ⁷	Nil	Nil	Nil	Nil	Nil	Nil	39x10 ⁷	Nil
TFC cfu/ml	1x10 ⁵	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
E. coli	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Salmo. sp.	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Staph. aur.	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Pseud. aeru.	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

TBC- total bacterial count, TFC- total fungal count, Staph. aur.- *staphylococcus aureus*, Pseud. aeru.- *pseudomonas aeruginosa*, Salmo- *salmonella sp.*, E - earthen pot, P - porcelain pot, (D-) = without *dhataki puspa*, (D+) = with *dhataki puspa*, B- I = batch 1, B- II = batch 2, B - III = batch 3, B - IV = batch 4.

DISCUSSION

Water is universal solvent, used to extract plant products with antimicrobial activity. Though traditional healers use primarily water but plant extracts from organic solvents have been found to give more consistent antimicrobial activity compared to water extract. During the preparation of *Arjunarista*, decoction was prepared with *Arjuna twaka* (*Terminalia arjuna*), *Draksha phala* (*Vitis vinifera*), and *Madhuka puspa* (*Madhuka indica*) at temperature at 1000 C. At that temperature micro-organism present in these ingredients are destroyed due presence of higher temperature. Thus the chance of micro-organism present in above ingredients to move in to further process is negligible. Next to this process Jaggery and *Dhataki* flowers (*Woodfordia fruticosa*) were added to this decoction. The chance of micro-

organism present in Jaggery and *Dhataki puspa* may possible to move in to final product, but during the process of *Sandhan kalpana* (fermentative process) alcohol was observed which was present here by self generation. Concentration of ethyl alcohol which was present in our final product of *Arjunarista* was 2-5%, and low concentrations of ethanol (2-4%) have a specific anti-microbial effect ^[11]. Thus absence of microbes in *Arjunarista* may be due to presence of low concentration of ethyl alcohol which is self generated during pharmaceutical process. Although several percentage of alcohols have been shown to be effective anti-microbial, ethyl alcohol (ethanol, alcohol), isopropyl alcohol (isopropanol, propan-2-ol) and n - propanol (particular in Europe) are the most widely used ^[12]. Alcohols exhibit rapid broad-

spectrum anti-microbial activity against vegetative bacteria (including mycobacteria), viruses, and fungi but are not sporicidal activity. Lower concentrations may also be used as preservatives and to potentiate the activity of other biocides. Generally, the anti-microbial activity of alcohols is significantly lower at concentrations below 50% and is optimal in the 60 to 90% range [13]. Little is known about the specific mode of action of alcohols, but based on the increased efficacy in the presence of water; it is generally believed that they cause membrane damage and rapid denaturation of proteins, with subsequent interference with metabolism and cell lysis [14].

This is supported by specific reports of denaturation of *Escherichia coli* dehydrogenases [15] and an increased lag phase in *Enterobacter aerogenes*, speculated to be due to inhibition of metabolism required for rapid cell division [16]. The most feasible explanation for the anti-microbial action of alcohol is denaturation of proteins. This mechanism is supported by the observation that absolute ethyl alcohol, a dehydrating agent, is less bactericidal than mixtures of alcohol and water because proteins are denatured more quickly in the presence of water [17].

Protein denaturation also is consistent with observations that alcohol destroys the dehydrogenases of *Escherichia coli* [18] and that ethyl alcohol increases the lag phase of *Enterobacter aerogenes* [16], and that the lag phase effect could be reversed by adding certain amino acids. The bacteriostatic action was believed caused by inhibition of the production of metabolites essential for rapid cell division. *Madhuca longifolia* extracts have broad inhibitory activities to pathogenic micro-organism and to act as potential anti-microbial agent from natural sources [19]. *Dhataki puspa* Gram-negative bacteria were more susceptible to the plant extract than Gram-positive bacteria [20]. Organic extract obtained from the *T. arjuna* bark and leaves may be used to treat the bacterial ear pathogens especially *S. aureus*, which has shown greater inhibition zones than the herbal drops [21]. Also water soluble flavonoids (mostly anthocyanins) have anti-microbial significance and water soluble phenolics only important as antioxidant compound [22].

The higher activity of the ethanolic extracts as compared to the aqueous extract can

be attributed to the presence of higher amounts of polyphenols as compared to aqueous extracts. It means that they are more efficient in cell walls and seeds degradation which have unipolar character and cause polyphenols to be released from cells. More useful explanation for the decrease in activity of aqueous extract can be ascribed to the enzyme polyphenol oxidase, which degrade polyphenols in water extracts, whereas in methanol and ethanol they are inactive. Moreover, water is a better medium for the occurrence of the micro-organisms as compared to ethanol [23]. The microorganism present were 1×10^7 and 39×10^7 cfu/ml total bacteria count present in sample E1 (D+) of batch II and sample P2 (D+) of batch IV respectively, total fungal count present in sample E1 (D-) of batch I of pharmaceutically prepared *Arjunarista*. But these bacteria are not pathogenic in nature so these are not harmful to human.

CONCLUSION

It was observed that water used for extraction of active substances of herbs and self generated organic solvent (alcohol) was appear due to pharmaceutical process. This shows that our formulation has anti-microbial activity and no specific pathogens were present in *Arjunarista*.

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