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Research Article

Preparation and Standardization of *Siddhalepa Ayur* Elixir 14 Blood Purifier (*Nimba arishta*), A Traditional *Ayurvedic* Formulation

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Abstract

Ayurveda consists of different types of formulations, including fermented varieties, arishtas and asavas. Because of their efficacy, consistency and attractive characteristics, arishtas are regarded as unique and valuable therapies. It was prepared using herbal drug decoction and contains self-generated alcohol. While these formulations are listed in traditional literature and their scientific research and reporting are used regularly, it is important to strengthen Ayurveda on the global market. this research attempt to plan and test *Siddhalepa Ayur* Elixir 14 Blood Purifier (*Nimba arishta*) was made. *Azadirachta indica* is a major component of *Nimba arishta* and It has been used to treat rashes, fever, rheumatic disorders and helminthiasis; it is also considered as blood-purifier. After fermentation of traditionally prepared *Nimba arishta* formulations, 8.32 percent w/w alcohol was made. Due to sweet taste combined with fine fragrance, which hides unpleasant taste and odour of added herbal ingredients, it is palatable to use and also has good shelf life. This study standardized a traditional ayurvedic arishata formulation *Siddhalepa Ayur* Elixir 14 Blood Purifier according to the international standards.

Keywords: Ayurveda; Fermented Formulation; Arishta; Siddhalepa Ayur Elixir

Abbreviations

PCA: Plate Count Agar; WHO: World Health Organisation; CFU: Colony Forming Units; ICH: International Council for Harmonisation; SLS: Sri Lanka Standards

Introduction

Ayurveda is one of the ancient medicine systems of the world and is being practised over 3000 years [1]. The term '*Ayurveda*' consisted of two epithets: *Ayu* (Life) and *Veda* (Knowledge). The source of this life science, which is hard to identify, was put somewhere around 6000 BC [2]. Ayurvedic remedies are considered as affordable, safe with no or reduced side effects [3]. This traditional system consists of different types of medicines, including fermented products which are known as *arishtas* and *asavas* (≈ medicinal wines). *Arishtas* are made with decoctions of various herbs; while asavas are prepared using fresh herbal juices [4]. They have been prepared by fermenting the herbal decoction or juices in airtight sealed vessels along with the flowers of *Woodfordia fruticosa*, sugar and occasionally with some other plant powders [5,6]. These are special self-alcohol generated liquid form of *ayurvedic* drugs [7] having longer shelf life, having wide array of therapeutic values [8] and generally consumed for their perceived prophylactic efficacy.

In Sri Lanka, *arishtas* one of the widely used *ayurvedic* form of drugs and they have been used for the treatment of a great variety of diseases [9]. According to *ayurvedic* philosophy, these alcoholic preparations possess a moderate potency and are therefore they can be useful to treat convalescent phase of a disease; while other forms are useful for the treatment of acute disorders [10,11]. As early as 320 BC Caraka, one of the sages of *ayurveda*, described 84 varieties of such alcoholic preparations. These preparations were considered to "strengthen the mind, body, the power of digestion, help in overcoming sleeplessness, grief and anorexia" [12].

Azadirachta indica A. Juss (Meliacae), commonly known as *Nimba* in Sanskrit is being used widely in *Ayurveda* to treat various illnesses in Sri Lanka [5]. The decoction of the *Azadirachta indica* barks (*Nimbadi quath*) is used to prepare *Nimba arishta*, one of the most commonly used arishtas in Sri Lanka. It has been used to treat rashes, fever, rheumatic disorders and helminthiosis; it is also con-

sidered as blood-purifier [13]. Siddhalepa is the flagship brand of the Hettigoda Group, Sri Lanka and it produces over 150 *ayurvedic* health care and cosmetic products. Siddhalepa Ayur Elixir 14 Blood Purifier (SAE-14) is the brand name of *nimba arishta* produced from Siddhalepa; which has been produced in accordance with *ayurvedic* pharmacopeia [14]. SAE-14 contains the barks of *Azadirachta indica* and the fruits of *Embelica ribes* as main ingredients. Previous reports found that *Nimba arishta* prepared from *Ayurveda* Municipal Manufacturing Company exhibited immunomodulatory [15] and anti-inflammatory [5] activities. In this communication, the quality parameters of SAE-14, a branded formulation of *Nimba arishta* was standardized using *Ayurvedic Pharmacopeia* and international standardization methods.

Methodology

Instrumentation and chemicals

All the analyses were done using following instrumentations. pH was analyzed on a pH meter Thermo-scientific (STAR A214). Refractive index and sugar content were analyzed on Rose Scientific Refractometer. Alcohol content was evaluated on ebulliometer. Specific gravity was estimated using specific gravity bottle. All the culture media were obtained from OXIOD and Lab M UK. All the reagents and chemicals obtained from Sigma at analytical grade.

Plant materials

Azadirachta indica barks, dried fruits of *Embelia ribes* and cane sugar were procured from local market, and their botanical identity was authenticated by R. A. S. W. Ransinghe, Botanist of the National Herbarium of Peradeniya, Sri Lanka. Voucher specimens of the plant materials were deposited in the herbarium at Department of Research and Development, Hettigoda Industries (Pvt) Ltd, 33/3, Sri Dharmarama Road, Ratmalana, Sri Lanka for future reference.

Preparation of SAE-14

SAE-14 was prepared in accordance with the method given in ayurveda pharmacopeia [14]. The list of ingredients is given in table 1. The raw drug materials were cleaned, washed and dried before their usage.

Ingredients	Quantity
Azadirachta indica	102 Kg
Embelia ribes	5.5 Kg
Cane sugar	310 Kg
Water	4014 l

Table 1: Composition of Siddhalepa Ayur Elixir 14 Blood Purifier (SAE-14).

Preparation of decoction

The barks of *Azadirachta indica* and fruits of *Embelia ribes* were pulverized and sieved with no. 44 sieve. This powder was mixed with required amount of potable water and boiled under mild heating till the final volume was reduced to one eight of its initial volume. The decoction was filtered through muslin cloth and filtrate was used for further processing [14].

Fermentation

Required quantity of cane sugar was dissolved in decoction by stirring and filtered through muslin cloth. Filtrate was collected in clean porcelain jar and the container was sealed with mud smeared cloth and kept in clean and dry room for fermentation. After fermentation the fermented material is filtered through muslin cloth. Filtrate was packed in air tight container and used for evaluation [14].

Physicochemical evaluations

Preliminary evaluation

Evaluation of organoleptic characteristics viz. odour, taste, and colour of SAE-14 was carried out using standard methods [16].

Estimation of alcohol content

50 mL of SAE-14 was taken for the ebulliometer estimations. After 1:1 dilution with distilled water, 50 mL of the working solution was kept into the boiling chamber. The temperature was read at the lowest constant boiling point. Distilled water was used as the blank and the percentage of alcohol was read from the scale [16,17].

Estimation of specific gravity

Specific gravity of SAE-14 was determined according to the method of British Pharmacopoeia [17]. The mass of empty specific gravity bottle was measured initially [(w1) g]; then the mass of specific gravity bottle with distilled water was measured [(w2) g]. The mass of SAE-14 in specific gravity bottle was then recorded [(w3) g] and the specific gravity of SAE-14 was calculated using the following formula:

$$\text{Specific Gravity} = \frac{(w3 - w1)g}{(w2 - w3)g}$$

Estimation of pH

Calibrated pH meter (Thermo-scientific STAR A214) was used to check the pH of SAE-14 [17].

Estimation of sugar content and refractive index

The refractive index and sugar content of SAE-14 were estimated by using refractometer [17].

Evaluation of microbiological limits

All the experiments were performed in accordance to the SLS standard methods [18-20].

Estimation of aerobic count

Using a sterile pipette, serial dilutions (10^{-2}, 10^{-3}, 10^{-4} and so on) of SAE-14 were prepared and one mL of each dilution was poured on the plate count agar (PCA) media. The media was allowed to solidify by inverting the dishes. The plates were incubated at 30 ± 1°C for 72 ± 3 h and the number of bacterial colonies were counted [19]. This experiment was done in triplicate.

Enumeration of yeasts and moulds

Using a sterile pipette, one mL of serially diluted SAE - 14 were poured on petri dishes containing 15 mL of Sabouraud Dextrose Agar (SDA). The media was allowed to solidify. The plates were kept inverted, incubated at 25 ± 1 °C for 3 - 5 days and the number of colonies were counted and recorded [19].

Detection of *Escherichia coli*

Ten mL of serially diluted SAE-14 were poured aseptically on sterile conical flasks containing 90 mL of Lactose broth. The contents were mixed, incubated at 37 ± 2 °C for 18 - 24 h. One mL of this broth was taken, aseptically transferred to 10 mL sterile MacConkey's broth tube having Durham tube and incubated at 36 ± 1 °C for 48 h. Presence of acid and gas in the MacConkey's broth tube indicated the presence of coliforms [18].

Confirmation of coliforms

A loopful of cultures from positive MacConkey's broth tubes were sub-cultured in tubes containing 10 mL of BGB broth. The tubes were incubated at 36 ± 1°C for 24 - 48 h and observed for gas production [18].

Examination of faecal coliforms

A loopful of culture from positive MacConkey's broth tubes were inoculated in 10 mL of BGB broth prewarmed to 44 ± 0.1°C. The tubes were incubated at 44 ± 0.1°C for 48 hours and observed for gas production [18].

Detection of *Salmonella*

One mL of lactose broth prepared at 2.4.6.3 was transferred aseptically in to 10 mL of Selenite-Cystine broth and incubated at 37 °C for 18 - 24 h. A loopful of this culture was streaked on the surface of Brilliant Green Agar plates. The plates were covered, inverted, incubated at 37 °C for 20 - 24 h and observed for the any noticeable change in the colour [20].

Test for stability

Stability test was carried out in accordance with the ICH guidelines [21]. SAE-14 containing bottles were kept in three different storage conditions (25 °C, 60% RH; 30 °C, 65% RH and 40 °C, 75% RH) The physico-chemical properties of SAE-14 was analyzed at three different time intervals (1st, 3rd and 6th month).

Results

Physicochemical evaluation

Organoleptic features of prepared SAE-14 are shown in table 2. It has characteristic color, odour and taste. Measured pH suggested that SAE-14 was acidic in nature (Table 3). Other physicochemical charters like sugar content, refractive index and specific gravity showed that the formulation was made in accordance with SLS.

Parameter	Description
Colour	Reddish brown
Odour	Aromatic
Taste	Astringent

Table 2: Organoleptic characteristics of *Siddhalepa Ayur* Elixir 14 Blood Purifier.

Parameter	Observed value
pH	3.29 ± 0.01
Specific gravity	1.03 ± 0.01
Alcohol (%w/w)	8.32
Sugar content %	27.21 ± 0.02
Refractive index	1.34 ± 0.03

Table 3: Physio-chemical properties of *Siddhalepa Ayur* Elixir 14 Blood Purifier.

Microbiological analysis

The microbial limits or lack of important microbial pathogens in SAE-14 is shown in table 4. As measures of microbial performance were used the total aerobic microbial count and the total yeast and mold count (presented as colony-forming units per ml CFU / ml) the absence of *Salmonellae, Escherichia coli* and Gram-negative bacterial species. It also confirmed the quality of raw materials.

Parameter	Microbial count
Aerobic plate count (cfu/mL)	3.7×10^3
Yeast/mold (cfu/mL)	3.1×10^2
Detection of coliform	Absent
Detection of *Salmonella*	Absent

Table 4: Average microbiological counts of *Siddhalepa Ayur* Elixir 14 Blood Purifier.

Effects in stability

Stability test results are shown in Table 5 and SAE-14 can be stored for six months at different selected storage.

Time	Description, organoleptic and physiochemical properties at different storage conditions																	
	Zone-I, 25 ^{0}C/60% RH						Zone-II, 30 ^{0}C/65% RH						Zone-III, 40 ^{0}C/75% RH					
	Colour	Ordur	Taste	pH	Alcohol content (%w/w)	Sugar content %	Colour	Odour	Taste	pH	Alcohol content (%w/w)	Sugar content %	Colour	Odour	Taste	pH	Alcohol content (%w/w)	Sugar content %
1st Month	Reddish brown	Aromatic	Astringent	4.00± 0.02	7.80	24.50± 0.02	Reddish brown	Aromatic	Astringent	3.90± 0.01	8.10	25.45± 0.01	Reddish brown	Aromatic	Astringent	4.00± 0.21	8.10	24.50± 0.41
3rd Month	Reddish brown	Aromatic	Astringent	3.72± 0.01	7.91	25.58± 0.04	Reddish brown	Aromatic	Astringent	4.00± 0.02	8.00	28.40± 0.11	Reddish brown	Aromatic	Astringent	4.50± 0.10	8.20	26.34± 0.50
6th Month	Reddish brown	Aromatic	Astringent	3.51± 0.01	7.70	26.45± 0.05	Reddish brown	Aromatic	Astringent	3.90± 0.10	8.21	29.40± 0.21	Reddish brown	Aromatic	Astringent	4.20± 0.11	8.10	28.45± 0.55

Table 5: Stability data of the Siddhalepa Ayur Elixir 14 Blood Purifier.

Discussion

For their potential application in the biomedical system, standardization of natural products is more important. By using existing methods and applying correct guidelines and principles, the WHO has emphasized the need to ensure quality control for herbal products [22]. In that contest this communication standardized the physicochemical parameters of SAE -14.

Organoleptic characteristics of SAE-14 revealed that it is palatable with fine aroma. The pH showed that SAE-14 had weak acidic properties. Since autogenic alcohol acts as a preservative and increases formulation stability; addition of preservatives is unnecessary.

Absence of specified microorganisms like *Salmonella, Escherichia coli* in SAE-14 suggested that SAE-14 is safe for human consumption. The stability of the SAE-14 results are reproducible, even on SAE-14 bottle that had been stored for six months at different storage conditions.

Formulation of decoction is the step of primary significance in the manufacturing of *arishta* formulations. Constant mild heating during the preparation of decoction facilitates extraction of water-soluble components of the herbal drug. But excessive heating results in charring of plant drug. Added *Embelia ribes* fruits and sugar are source of nutrient and also initiator of fermentation process. Specially *Embelica ribes* enhance the digestion in intestine [23]. Temperature at fermentation area can affect duration and extent of fermentation process hence season of fermentation is important for production of arishta by traditional method.

Previously Kroes and co-workers [5] showed the immunomodulatory effect of *Nimba arishta*. Anti-inflammatory and antimicrobial effect of *Azadirachta indica*, the main ingredient of this SAE-14 were also reported in the literature; it may contribute SAE-14's therapeutic value. Neem is considered as one of the most powerful blood purifier and antidote in *Ayurveda* [24]. It is an effective therapy for skin infections, rashes and pimples due to its powers of internal cleansing [25]. This study analyzed the quality and specifications of SAE-14 according the international standards. Biological activities and pathway studies need to be evaluated to get more knowledge on the application of SAE-14.

Conclusion

This study standardized a traditional *ayurvedic arishata* formulation SAE-14 according to the international standards. Traditional formulations are losing their value in international market. Alcohol percentage, pH, sugar content, refractive index, specific gravity and

microbial limits can be considered as the basic tools for the quality control measures of arishtas to improve their acceptance. Further, studies on their safety and efficacy will improve their acceptance.

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