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Microbial Quality and Antibacterial Activity of Herbal Medicines

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Abstract

Use of herbal medicine faces constraint particularly imparting knowledge in identifying whether a product is microbiologically fit for health or not. There has been relatively less research on microbial quality of the herbal medicines in Nepal. In this context, this research has focused on microbial quality of different herbal medicines. A total of twenty one herbal medicines were collected from different sales outlet of Kathmandu. The microbial load in herbal medicine was determined by aerobic plate count method and bacterial isolates were identified based on morphological, cultural and biochemical tests. Out of twenty one herbal medicines analyzed, all were found free from pathogenic bacteria and indicator organism of fecal contamination. However , *Bacillus* spp. were isolated from ten herbal medicines. The microbial load on Nutrient Agar was found within the range of $1.20x10^{3}$- 6.06õ10^{5} cfu/ml (or g). Altogether six different *Bacillus* spp were identified and the most predominant was *Bacillus subtilis*.

In vitro antibacterial activity of the herbal medicines, from which microorganisms were not detected, were determined against six test bacteria by cup plate method. Out of eleven different herbal medicines, five showed the zone of inhibition against all test bacteria and at least two test bacteria were inhibited by each of the herbal medicines. The highest zone of inhibition was 30 mm shown by *Chitrakaharitaki Churna* of concentration 100mg/ml against *Pseudomonas aeruginosa*.

Key words: Herbal medicines, antibacterial activity

Introduction

The earliest recorded evidence of the use of herbal medicines in Indian, Chinese, Egyptian, Greek, Roman and Syrian texts dates back to about 5000 years. Plants had been used for medicinal purposes long before recorded history (Zhang *et al.*2007). In Nepal, use of herbal medicine is a common practice since time immemorial. Modern medicines though available are expensive and sometime non- approachable to the poor people in remote areas ,who depend upon the native therapy for their primary health care (Manandhar 1998). Although small in size, Nepal possesses distinct ecological zones and diversity of plants. The uniqueness in the climate due to altitudinal variation allow the country to experience from tropical to alpine climate(despite of the less variation on geographical position). Hence, Nepal boasts the diversity of plants. It is estimated that about 1,400 plants are used for manufacturing of herbal medicine (Nepal & Sapkota 2005).

Herbal medicines are the major components in all traditional medicine systems, and a common element in Ayurvedic, homeopathic, naturopathic, traditional Chinese medicine, and native American medicine (Sofowora 1994). World Health Organization (WHO) survey indicates that about 70-80% of the world population particularly in the developing countries rely mainly on herbal medicines for their primary healthcare (WHO 1998). Recognition of values of herbal medicine remained confined to the developing countries for long but recently its popularity has been spread throughout the world. Herbal medicines are also in great demand in the developed world for primary health care because of their efficacy, safety and lesser side effects (Kamboj,V.P. 2000).

In Nepal, Singhadarbar Vaidya Khana produces most of herbal medicines. It produces nearly 160 types of drugs using 400 different types of herbs, minerals and animal parts by following the principles of Ayurveda (www.mohp.gov.np). Beside this, many pharmaceuticals like, Herbs Production and Processing Co. Ltd; Dabur, Ban, Himalaya, Basu, etc are working in Nepal to produce herbal medicines. Their products are getting good market in Nepal and abroad. (Ayurnepal Groups 2005 A).

In herbal medicines microbes might come along with the raw materials or due to contaminated processing unit (Esimone *et al*. 2002). Microbes might contaminate herbal medicines during storage and improper handling as well. Although herbal remedies are often perceived as being natural and therefore safe, they are not free from adverse effect. And this might be due to factors such as adulteration, substitution, contamination, misidentification, and lack of standardization, incorrect preparation, dosage, inappropriate labeling and advertisement (Okunlola *et al*. 2007). With increasing popularity, the safety, efficacy and quality of these medicines have become an important issue for health authorities and health professionals.

Adulteration and substitution of herbal ingredients due to either genuine error or unscrupulous practices can be detected by means of reliable quality control methods. The quality control of the herbal medicinal product through the application of standards is one of them (Leung *et al*.2006). Counting bacterial colonies on agar plates is a simple and effective method for determining the number of viable bacteria in a sample (WHO 2005).

In this study a total of 21 herbal medicines were collected from different sales outlets of Kathmandu. The microbial load in herbal medicine was determined. *In vitro* antibacterial activity of the herbal medicines was determined against six bacteria. A study was carried out at Microbiology Laboratory of National College (NACOL), Lainchaur, Kathmandu, Nepal.

Methodology

Collection of samples

Arjunarista, Ashwagandha oil, Avipattikara Churna, Chandraprabha Vati, Chitrakaharitaki Churna, Gokshuradi Guggulu, Kustadi Dantamanjan, Mimiya, Mritunjaya Rasa, Navarasa, Peedahar, Rasaparpati, Sankha Bhasma, Saptamrita Lauha, Silajit, Sitopaladi Churna, Suddha Gandhaka, Tamrachudadi oil, Trayodashanga Guggulu, Tulsi Tea and Yogaraja Guggulu manufactured by Singhadarbar Vaidya Khana were collected from different sales outlet of Kathmandu.

Microbial quality determination

Microbial quality was determined by aerobic plate count (APC) method using standard protocol given by Okunlola *et al*.(2007). One gram powder in case of solid preparation and one ml in case of liquid preparation were added in 9ml of sterile distilled water to get ten folds dilution of original sample. Similarly, ten folds serial dilutions were carried out up to the concentration 10^{-6} of the original sample. One ml from each dilution was poured into petri- plate and molten. Nutrient agar (NA) having temperature around 45^0C was poured and the petri -plates were rotated to distribute the sample uniformly in the media. Media on the petri-plates were allowed to solidify and then the plates were incubated at 37^0C for 24 hrs.

Identification of bacterial isolates

Bacterial isolates during APC were identified based on cultural, morphological and biochemical tests. All types of colonies obtained on NA plates were sub-cultured on separate NA plates to obtain pure cultures. Colony characteristics on NA plates were studied. Gram staining was performed for morphological study. Different biochemical tests such as carbohydrate fermentation, starch hydrolysis, gelatin hydrolysis, growth in nutrient broth with 6% NaCl , Triple Sugar-Iron Agar, Voges - Proskauer, Nitrate reduction, indole and growth in urea agar were performed.

Test bacterial cultures for antibacterial activity

Standard hospital isolates of *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus* and *Staphylococcus epidermidis* were provided by Tribhuvan University, Teaching Hospital. One to two isolated colonies from fresh culture of test bacteria were transferred into test tubes containing 1ml of nutrient broth (NB) and incubated at 37°C for 3-4 hrs till the development of turbidity equivalent to 0.5 McFarland's Nephelometer Standard .This standard is estimated to have bacterial suspension of $1.5X10^8$ CFU_s(CFU/ml).

Antibacterial activity

One gram of herbal medicine in case of solid sample and one ml of herbal medicine in case of liquid sample were transferred into clean, sterile screw capped test tubes aseptically and then ten ml of methanol was added in each of the test tube to make 100 mg/ml working suspension/solution of herbal drug. The antibacterial study was conducted by agar well diffusion method as described by *Brooks et al.* (2004).

Results and Discussion

Out of twenty-one herbal medicines, bacterial growth was observed from ten herbal medicines in NA.

Table 1. Herbal medicines ,their uses and bacterial growth on NA plates

SN	Herbal medicines	Uses	Microbial growth (pour plate technique on NA plate)	Microbial load
1	Arjunarista	Tonic for heart disease	No	-
2	Ashwagandha tail	Stress ,fatigue and arthritis	No	-
3	Avipattikara Churna	Urine infection	Yes	$1.20x10^3$
4	Chandraprabha Vati	Urine infection	Yes	$4x10^5$
5	Chitrakaharitaki Churna	Rhinitis and respiratory problem	No	-
6	Gokshuradi Guggulu	Releif of pain during urination	Yes	$6.06x10^5$
7	Kustadi Dantamanjan	Dental problem	Yes	$3.6x10^3$
8	Mimiya	Pain and wound	No	-
9	Mritunjaya Rasa	High fever	Yes	$2.70x10^3$
10	Navarasa	Cold and high fever	Yes	$2x10^3$
11	Peedahar	Pain	No	-
12	Rasaparpati	Indigestion	No	-
13	Sankha Bhasma	Indigestion,jaundice and dysentry	No	-
14	Saptamrita Lauha	Eye infection and jaundice	No	-
15	Silajit	Tonic	No	-
16	Sitopaladi Churna	Cough and respiratory problem	Yes	$1.8x10^4$
17	Suddha Gandhaka	Skin and blood diseases	No	-
18	Tamrachudadi tel	Pain and paralysis	No	-
19	Trayodashanga Guggulu	Body and joint pain	Yes	$1.44x10^5$
20	Tulsi Tea	Respiratory problem	Yes	$5.60x10^4$
21	Yogaraja Guggulu	Cold and cough.	Yes	$2.43x10^4$

In NA plate highest count detected was 6.06×10^5 cfu / g and that was from Gokshuradi Guggulu. The microbial load by pour plate (APC) method on NA plate was found within the range of 1.20×10^3 - $6.06\tilde{o}10^5$cfu / ml (or g) (Table 1)

Table 2 . Microbial profile of herbal medicines

S.N.	Herbal medicines	Organisms isolated and identified
1.	Avipattikara Churna	*Bacillus cereus , Bacillus subtilis*
2.	Chandraprabha Vati	*Bacillus licheniformis, Bacillus pumilus, Bacillus subtilis*
3.	Gokshuradi Guggulu	*Bacillus subtilis, Bacillus cereus, Bacillus polymyxa*
4.	Kustadi Dantamanjan	*Bacillus subtilis*
5.	Mritunjaya Rasa	*Bacillus subtilis, Bacillus licheniformis*
6.	Navarasa	*Bacillus pumilus, Bacillus subtilis*
7.	Sitopaladi Churna	*Bacillus subtilis, Bacillus laterosporus*
8.	Trayodashanga Guggulu	*Bacillus cereus, Bacillus subtilis, Bacillus licheniformis*
9.	Tulsi Tea	*Bacillus subtilis*
10.	Yogaraja Guggulu	*Bacillus licheniformis, Bacillus cereus*

Based on colony morphology and different biochemical tests such as catalase, nitrate reduction, indole, TSI, urease, VP, carbohydrate fermentation, starch hydrolysis, gelatin hydrolysis, growth in NB with 6% NaCl, growth at 40 ^{o}C all isolates were identified to be *Bacillus* spp. (Table 2)

Table 3 . Antibacterial property of herbal medicines

S.N	Herbal Medicine	Zone of inhibition (mm) produced against (diameter of well = 6mm) test bacteria					
		Staphylococcus epidermidis	*Staphylococcus aureus*	*Proteus mirabilis*	*Pseudomonas aeruginosa*	*Salmonella typhi*	*Escherichia coli*
1	Arjunarista	-	7	-	-	-	7
2	Ashwagandha oil	-	7	8	-	-	-
3	Chitrakaharitaki Churna	9	13	12	30	7	20
4	Mimiya	7	8	7	7	7	9
5	Peedahar	10	8	-	7	7	14
6	Rasaparpati	7	9	8	-	-	15
7	Sankha Bhasma	8	7	-	-	17	15
8	Saptamrita Lauha	11	18	12	15	10	12
9	Silajit	6	8	6	10	12	14
10	Suddha Gandhaka	7	9	12	7	10	12
11	Tamrachudadi oil	-	-	7	8	9	7

Table 3 summarizes the antibacterial property of herbal medicines in terms of zone of inhibition produced against the test bacteria. All eleven herbal medicines showed antibacterial activity to at least two test bacteria. Herbal medicines like Chitrakaharitaki Churna, Mimiya, Saptamrita Lauha, Silajit, Suddha Gandhaka showed antibacterial activity against all the test bacteria. Peedahar showed antibacterial activity against all bacteria except *Proteus mirabilis*. The largest zone of inhibition was 30 mm produced by Chitrakaharitaki Churna against *Pseudomonas aeruginosa*.

This study revealed that *Bacillus* spp. were the predominant bacteria in the herbal medicines. This result is comparable with the results obtained by Oyetayo (2008). He assessed Nigerian herbal medicines and reported almost all herbal medicines tested were contaminated with *Bacillus* spp which are commonly found in soil, air, dust ,etc. Many aerobic species of *Bacillus* produce endospore that helps them not only to resist environmental stress but also to ensure their long-term survival under adverse conditions.

All herbal medicines, from which microorganisms were not isolated during APC showed antibacterial property. The antimicrobial components of herbal medicines might be one of the reasons for not getting any bacteria except *Bacillus* spp from all herbal medicines.

Among the *Bacillus* spp, the most predominant was *Bacillus subtilis.* The result is comparable with the result obtained by Esimone (2007). He had also reported *Bacillus subtilis* as the most predominant one in herbal medicines. Similarly, Okunlola *et al.* (2007) reported that *E. coli, Salmonella* spp. and *Staphylococcus aureus* in herbal medicines. However, in this research such microorganisms were not detected. Presence of *Bacillus subtilis* and *Bacillus cereus* in herbal medicines also matches with similar study carried by Adeleye *et al*. (2005).

Microbial contamination usually occurs because of improper drying or storage of the plant material which eventually results in degradation of the plant constituents. Microbial contamination can also render plant material toxic, either by transforming the chemicals in the plant material or through the production of toxic compounds by the microbes. Therefore, microbial quality tests should be applied to starting plant materials, intermediate and finished products where necessary. During the quality analysis, precautions must be taken to ensure that conditions do not adversely affect any microorganisms that are to be measured.

The good antibacterial activity of herbal medicines implies that the antimicrobial compounds present in herbal medicines is possibly controlling the microbial activity .Herbal medicines showed varying degrees of invitro antibacterial activity against test bacteria .Both Gram positive and Gram negative bacteria *E.coli* and *Staphylococcus aureus* were found to be sensitive to 90.9% herbal medicines .Similarly *Proteus mirabilis, Staphylococcus epidermidis* and *Salmonella typhi* were sensitive to 72.72% of herbal medicines. *Pseudomonas aeruginosa* was sensitive to 63.6% of herbal medicines.

Most of the herbal medicines were found free from pathogenic bacteria. Only *Bacillus* spp were isolated as a predominant bacteria from herbal medicines .The herbal medicines showed significant antibacterial properties which justify the usefulness of the herbal medicines to control different infectious diseases.

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