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RESEARCH ARTICLE

Microbial Analysis of *Barleria prionitis* Linn.

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ABSTRACT

Meicinal plant materials carry a great number of bacteria and moulds from soil. The current practices of harvesting, handling and production of herbs often cause additional microbial contamination. The pathogenic bacteria normally seen in the plant materials are *Escherichia coli, Salmonella* sp., *Pseudomonas aeruginosa*, and *Staphylococcus aureus* (Indian Herbal Pharmacopoeia). The Indian Herbal Pharmacopoeia also specifies limit for *E. coli*, and *Salmonella* sp. for medicinal plant material (Trease and Evans, 1985). Before coming to the market, the herbal drug should be made free from any pathogens, bacteria, moulds or fungi. If heating is not involved in manufacturing process, herbs should be properly sterilized by ethylene oxide or gamma radiation. The WHO has also emphasized for the control of microbial contamination in plant materials and prescribed guidelines for testing total viable count, *E. coli*, and *Salmonella* sp. in the plant materials (Kokate, 1991). In the present study culture media were prepared as per the formula and procedure given in Indian Pharmacopoeia 1996 Vol-II. Total aerobic microbial count of the sample of leaf and root of *Barleria prionitis* were determined. Total Viable Count, Fungal Count and tests for specific microorganisms in sample of leaf and root of *B. prionitis* was determined. In the sample of leaf, *E. coli, Salmonella,* and *Staphylococcus* were found negative, but *Pseudomonas* was found positive. In the sample of root, *E. coli* and, *Staphylococcus* were negative, but *Salmonella* and *Pseudomonas* were found positive. The absence of *Staphylococci* could be ascribed to the absence of human and animal's contamination. The present study and its findings would serve as an important source of information helpful in establishing the purity and efficacy of the plant.

Keywords: *Barleria prionitis* Linn, *Escherichia coli*, microbial analysis, *Pseudomonas, Salmonella, Staphylococcus*

INTRODUCTION

Meicinal plant materials carry a great number of bacteria and moulds from soil. A large range of microorganisms from the naturally occurring microflora of herbs and aerobic spore forming bacteria are also present.[1] The current practices of harvesting, handling and production of herbs often cause additional microbial contamination.[2] The pathogenic bacteria normally seen in the plant materials are *Escherichia coli, Salmonella* sp., *Pseudomonas aeruginosa*, and *Staphylococcus aureus* (Indian Herbal Pharmacopoeia). Before coming to the market, the herbal drug should be made free from any pathogens, bacteria, molds, or fungi.[3] If heating is not involved in manufacturing process, herbs should be properly sterilized by ethylene oxide or gamma radiation. The WHO has also emphasized for the control of microbial contamination in plant materials and prescribed guidelines for testing total viable count, *E. coli*, and *Salmonella* sp. in the plant materials (Kokate, 1991). The Indian Herbal Pharmacopoeia also specifies limit for *E. coli* and *Salmonella* sp. for medicinal plant material (Trease and Evans, 1985). In the present studies, the microbial contamination in the formulations was tested by procedure prescribed by Indian Pharmacopoeia 1996 Vol-II.[4]

EXPERIMENTAL WORK

Preparation of culture media

The following culture media were prepared as per the formula and procedure given in Indian Pharmacopoeia 1996 Vol-II.

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- Buffered sodium chloride-peptone solution
- Casein soyabean digest agar medium
- Sabouraud dextrose agar medium with antibiotics
- Lactose broth medium
- Nutrient broth medium
- MacConkey broth medium
- Selenite F. Broth medium
- Tetrathionate bile brillant green broth medium
- Bismuth sulfite agar medium
- Xylose-lysine desoxycholate agar medium
- Urea broth medium
- Triple sugar iron agar medium
- Cetrimide agar medium
- Mannitol salt agar medium
- Peptone water.

Total aerobic microbial count

Pretreatment

About 1 g sample of leaf and root of *Barleria prionitis* was accurately weighed, ground and suspended in 100 ml buffered sodium chloride-peptone solution (pH 7.0). The suspension was mechanically homogenized and 0.1% w/v of polysorbate 80 was added as wetting agent. The pH of the suspension was adjusted to 7.0 (Indian Pharmacopoeia 1996 Vol-II).

Total viable count

(Indian Pharmacopoeia 1996 Vol-II) about 1 ml of above-pretreated suspension was diluted to 25 ml with buffered sodium chloride-peptone solution. Aliquots measuring 0.5, 1.0, and 2.0 ml of above preparation were spread on the surface of solidified casein soya bean digest agar medium in three different petri dishes. The dishes were incubated at 35°C for 5 days. The number of colonies formed was counted and results were calculated on the basis of 1 g of the sample and given in Table 1. The photomicrograph given overleaf shows the presence of total viable colonies in herb sample of leaf and root.

Fungal count

(Indian Pharmacopoeia 1996 Vol-II) Aliquots measuring 0.5, 1.0, and 2.0 ml of pretreated suspension were spread on the surface of sabouraud dextrose agar with antibiotics (containing 0.1 g of benzyl penicillin sodium and 0.1 g of tetracycline in 1000 ml of media) in three different dishes. The dishes were incubated at 25°C for 5 days. The number of colonies formed was counted and results were calculated on the basis of 1 g of the sample and result given in Table 2. The photomicrograph overleaf shows the presence of fungal count in sample of leaf and root.[5]

Table 1: Total aerobic microbial count of leaf and root of *Barleria prionitis*

Sample	Total viable count(/g)	Fungal count(/g)
Leaf	8136	3552
Root	20192	4419

Test for specific micro organisms

About 10 g of sample of the leaf and root of *B. prionitis* was weighed accurately, ground, and suspended in 100 ml lactose broth media. The suspension was mechanically homogenized and 0.1% w/v of polysorbate 80 was added as wetting agent; the pH of the suspension was adjusted to 6.9 (Indian Pharmacopoeia 1996 Vol-II) [Figure 1].

E. coli

About 2 ml of above-pretreated sample preparation was transferred in sterile screw-capped tube containing 50 ml nutrient broth. The tubes were shaken thoroughly and allowed to stand for 1 h and again shaken and then incubated at 37°C for 24 h after loosening the caps of the tubes.[6]

Primary test

About 1 ml of the above enrichment culture was added to a tube containing 5 ml of MacConkey broth. The tubes were incubated at 37°C for 48 h.

Secondary test

The secondary test was performed when the primary test was showing the presence of acid and

Table 2: Test for specific microorganisms of leaf and root of *Barleria prionitis*

Sample	*Escherichia coli* sp.	*Salmonella* sp.	*Pseudomonas aeruginosa* sp.	*Staphylococcus aureus*
Leaf	Negative	Negative	Positive	Negative
Root	Negative	Positive	Positive	Negative

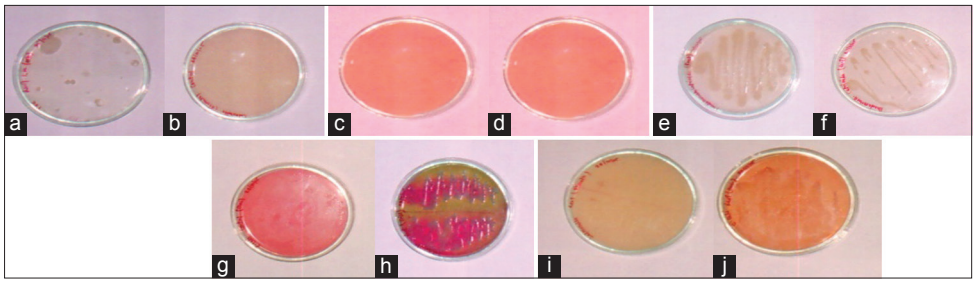

Figure 1: (a) Total fungal count, (b) total microbial count, (c) *Staphyloccous* leaf, (d) *Staphyloccous* root, (e) *Pseudomonas* leaf, (f) *Pseudomonas* root, (g) *Escherichia coli* leaf, (h) *E. coli* root, (i) *Salmonella* leaf, (j) *Salmonella* root

gas in the tubes. Culture 0.1 ml obtained in primary test was mixed with 5 ml of MacConkey broth and 5 ml of peptone water separately. Both the tubes were incubated at 44°C for 24 h and examined for the presence of acid, gas, and indole. For testing the indole, 0.5 ml of Kovac's reagent was added in the tube containing culture in peptone water and shaken and allowed to stand for 1 min. A red color produced in the reagent layer showed the presence of indole.[7] The presence of acid and gas and indole in the secondary test confirmed the presence of *E. coli*. The results are given in Table 3 repeating the primary and secondary tests; a control test was carried out. Then, 1.0 ml of enrichment culture and *E. coli* (NCTC 9002) organism containing broth was added to 5.0 ml of MacConkey broth. The result indicated that the control contained *E. coli.*[8]

Genus *Salmonella*

About 10 ml of pretreated preparation was transferred to 100 ml of nutrient broth in a sterile screw-capped tube. It was thoroughly shaken and allowed to stand for 4 h and shaken again. The cap of the tube was loosened and the tube was incubated at 37°C for 24 h.

Primary test

About 1 ml of enrichment culture was added to each of the two tubes containing (a) 10 ml of selenite F broth, and (b) 10 ml of tetrathionate bile brilliant green broth and incubated at 37°C for 48 h. From each of these two tubes, cultures were subcultured on bismuth sulfite agar and xylose lysine desoxycholate agar, respectively, and incubated at 37°C for 24 h.[9]

Secondary test

The secondary test was performed in those cases, where colonies confirmed the description of genus *Salmonella*. The colonies showing the characterstic of genus *Salmonella* were subcultured in triple sugar iron agar by first inoculating the surface of the slope and then making the stab cultured with the same inoculating needle.[10] The tube of urea broth was also inoculated at the same time. The tubes were incubated at 37°C for 24 h.[11] The presence of *Salmonella* was confirmed by the formation of acid and gas in the stab culture and the absence of acidity from the surface growth in the triple sugar iron agar and also with the absence of red color in the urea broth. The results are given in Table 3. Repeating the primary and secondary tests, a control test was carried out. 1 ml of enrichment culture and *Salmonella* Abony (NCTC 6017) organism containing nutrient broth was added and inoculated the tubes (a) and (b) the results indicated that the control contains *Salmonella*.[12]

Table 3: Total aerobic microbial count of dried leaf of *Barleria prionitis*

Sample	Total viable count(/g)	Fungal count(/g)
Dried Leaf	7895	2654

P. aeruginosa

Pretreated sample preparation containing 1.0 g of the sample was inoculated in 100 ml of fluid soya bean casein digest medium. It was mixed and incubated at 37°C for 48 h. The samples, which showed the growth of organism, were taken for further examination. The growth sample was streaked on the surface of centrimide agar medium on petri dish and incubated at 37°C for 24 h.[13]

S. aureus

Pretreated sample preparation containing 1.0 g of the sample was inoculated in 100 ml of fluid soya bean casein digest medium. It was mixed

and incubated at 37°C for 48 h. The samples, which showed the growth of organism, were taken for further examination. The growth sample was streaked on the surface of manitol salt agar medium on petri dish and inoculated at 37° for 24 h. The results are given in Table 3.[14]

RESULTS AND DISCUSSION

The samples were tested for microbial contamination as per the procedures of the Indian Pharmacopoeia 1996 Vol-II. The results are shown in Tables 1 and 3. The samples of the root showed higher microbial count than the prescribed limits. The fungal count was also high of the root. In the sample of leaf, *E. coli, Salmonella,* and *Staphylococcus* were found negative, but *Pseudomonas* was found positive. The Morphological and anatomical structure of leaves seems to be more important for bacterial charge. Those rich in covering trichomes or glandular hairs with revolute margings or with hairs at the pit entrances are more heavily contaminated. Irrigation with polluted water can be attributed to the presence of *Pseudomonas*. In the sample of root, *E. coli* and *Staphylococcus* were negative, but *Salmonella* and *Pseudomonas* were found positive. The presence of spore formers in the sample of root can be attributed to soil contamination, irrgation with polluted water. The absence of *Staphylococci* could be ascribed to the absence of human and animal's contamination. Vegetable matter is easily contaminated by several microorganisms (Salunke, 1974). According to Andrews *et al*. (1980), there are four main factors which can affect the contamination level:

a. Physiological leaf condition
b. Vector activity
c. Micro climate
d. Accessibility of leaves to microorganisms.

Other factors to be considered include the contamination due to human beings or animals (Frazier et Westhoff, 1976, Romond, 1979), the height of leaves from soil (Grahan *et al*., 1977; Weeb et mundt, 1978), the micro biological quality of the irrigation water, the kind of manure (Robinson et Adams, 1978), and possibility of diffusion of spores, particularly those of mycetes, through the air (Flannigar et Hue, 1976; Andrewes *et al*., 1980). The use when possible of a mechanized system of harvesting could reduce the contamination by humans (Lenoble *et al*., 1980). As per the WHO guidelines and Indian Herbal Pharmacopoeia, the drugs or formulations used for internal purposes should not contain genus *Salmonella.* Hence, the sample of leaf was found to be in compliance with the microbiological requirements for drugs and formulations used for internal administration as per the Indian Herbal Pharmacopoeia and the WHO guideline.

CONCLUSION

The fungal count was also high of the root. In the sample of leaf, *E. coli, Salmonella,* and *Staphylococcus* were found negative, but *Pseudomonas* was found positive. In the sample of root, *E. coli* and *Staphylococcus* were negative, but *Salmonella* and *Pseudomonas* were found positive. The absence of *Staphylococci* could be ascribed to the absence of human and animals contamination. As per the WHO guidelines and Indian Herbal Pharmacopoeia, the drugs or formulations used for internal purposes should not contain genus *Salmonella.* Hence, the sample of leaf was found to be in compliance with the microbiological requirements for drugs and formulations used for internal administration.

In the microbial study of the leaf and root sample of the drug in the present study, the sample of the root exhibited a higher microbial and fungal count. *E. coli* and *staphylococcus* were negative, but *Salmonella* and *Pseudomonas* were found positive. This may be attributed to soil contamination or irrigation with polluted water. In the leaf sample, *E. coli, Salmonella,* and *Staphylococcus* were found negative, but *Pseudomonas* was found positive. Irrigation with polluted water and accessibility of leaves to organisms may be the reason for the presence of *Pseudomonas*. As per the WHO guidelines and Indian Herbal Pharmacopoeia the drugs or formulations used for internal purposes should not contain genus *Salmonella*. Hence, leaf sample was in compliance with the WHO guidelines and Indian Herbal Pharmacopoeia.

REFERENCES

1. Lotlikar MM, Rao MR/Pharmacology of a hypoglycaemic principle isolated from the fruits of *Momordica charantia*. Indian J Pharm 1986;28:129.
2. Malaisse WJ. Alloxan toxicity to the pancreatic beta-cell. A new hypothesis. Biochem Pharm 1982;31:3527-34.
3. Mangle MS, Jolly CL. Saponins in *Tribulus terrestris* chemistry and bioactivity. Indian Drugs 1998;35:189.
4. Marles RJ, Framsworth NR. Antidiabetic plants and their active constituents. Phytomedicine 1995;2:137-89.
5. Mathew PT, Augusti KT. Hypoglycemic effect of onion, *Allium cepa* Linn on diabetes mellitus a preliminary report. Ind J Physiol Pharm 1973;19:213.
6. Modak AT, Rao MR. Hypoglycemic activity of a non-nitrogenous principle from the leaves of *Adhatoda vasica* nees. Ind J Pharm 1966;28:105.
7. Mukherjee PK, Kumar MR, Saha K, Giri SN, Pal M, Saha BP. Preparation and evaluation of tincture of *Gymnema sylvestre* by physico-chemical, TLC and spectroscopic characteristics. J Sci Ind Res 1990;55:178.
8. Munday R. Dialuric acid autoxidation. Effect of transition metals on the reaction rate and on the generation of "active species" oxygen species. Biochem Pharm 1988;37:409-13.
9. Nagarajan S, Jain HC, Aulkah GS. Indigenous plants used in the contid of diabetes. In: Atal CK, Kapoor BM, editors. Cultivation and Utilization of Medicinal Plants. Jammu-Tawi: Council of Scientific and Industrial Research; 1982. p. 584-604.
10. Nair KV, Yoganarasimhan SN, Keshava Murthy KR, Shantha TR. Studies on some South Indian market samples of ayurvedic drugs-II. Anc ci Life 1983;3:60-6.
11. Noor H, Ashcroft SJ. Pharmacological characterisation of the antihyperglycaemic properties of *Tinospora crispa* extract. J Ethnopharmacol 1998;62:7-13.
12. Oleszek WA. Chromatographic determination of plant saponins. J Chromatogr A 2002;967:147-62.
13. Oomachan MM. Ethno-botanical and conservation aspects of medicinal plants of Madhya Pradesh. Indian J Pure Appl Sci 1991;6:39.
14. Ong KC, Khoo HE. Biological effects of myricetin. Gen Pharm 1997;29:121-6.