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Original Article

SAFETY EVALUATION OF *GYMNEMA SYLVESTRE* AND *TERMINALIA BELERICA*

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Abstract

Herbal drugs are not safe always. They may be contaminated with pathogen microbes, aflatoxins, pesticide residues and heavy metals. In the present work two herbal drugs Gymnema sylvestre and Terminalia belerica, which are commonly used as ayurvedic medicines are evaluated for the presence of these contaminants. Pathogenic microbes, aflatoxins and pesticide residues are found to be absent. Metals and heavy metals are found to be present but in small concentrations.

Key words: *Gymnema sylvestre, Terminalia belerica*, Aflatoxin, Pesticide residues.

INTRODUCTION

The statement that the herbal drugs are safer than the synthetic drugs may not be true always. The current methods of cultivation, harvesting and processing, environmental conditions and social conditions have the great impact on the toxicity of the medicinal plants and their products (WHO, 1998). Hence the WHO has been fixed the std. Protocol, which says that the medicinal plants must be evaluated for various safety and toxicological parameters like microbial count, metal determination, aflatoxin detection and pesticide residue detection etc. The pathogens like *Staphylococcus aureus, Pseudomonas aeroginosa, E. Coli,* other bacteria, molds and yeast may contaminate the herbal drug and may cause serious effects (WHO, 1991 & 1993).

Gymnema sylvestre (whole plant) and *Terminalia belerica* (fruit) are the herbs used widely in traditional system of medicines for different purposes since long time. *Gymnema sylvestre* (Gurmar in Hindi) Family Asclepiadaceae leaves are commonly used in diabetes (Sivaprakasam *et al*, 1984). *Terminalia belerica* (Bahera in Hindi) Family Combretaceae fruit is official in Ayurvedic Pharmacopoeia of India and is ingredient of important Ayurevedic formulation Triphala (Lalla *et al*, 2001)

In the present study we carried out microbial count, test for aflatoxins, test for pesticide residue and metal analysis in the samples of *Gymnema sylvestre* (whole plant) and *Terminalia belerica* (fruit).

MATERIALS AND METHOD

The samples of *Gymnema sylvestre* (whole plant) and *Terminalia belerica* (fruit) used for study were purchased from the local market and authenticated by National Bureau of Plant Genetic Resources (Indian Council of Agricultural Research) Pusa campus, New Delhi. The herbal drugs were dried at 1500°C to a constant weight; the dried plant material was then ground to the fine powder and used for study.

Microbial count

Microbial count i.e. test for staphylococcus aureus, Pseudomons aeruginosa *E. coli*, total bacterial count and total combined molds and yeasts count was done as per USP - 2004 protocol.

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Detection of Aflatoxins (Tropical Products, 1972)

Sample preparation

About 50 gm of the powdered drug was weighed into a mixing jar, added 25 ml of saturated sodium chloride solution and 250 ml of Methylene chloride. Blended for 3 min. at high speed. Filtered through high porosity folded paper into 50 ml graduated cylinder. Transferred 50 ml of filtrate to 250 ml glass stoppered Erlenmeyer flask. Evaporated the extract to near dryness on steam bath and added methanol: 5% sodium chloride: hexane (50:50:50); shaken for 10 min on wrist action shaker and then transferred to 250 ml separating funnel. Allowed to stand for 5-10 min., drained lower aqueous layer into another 250 ml separating funnel. Added 50 ml of carbon tetrachloride to aqueous layers, shaken vigorously for 1 min. and allowed to separate the layer. Added 50 ml of Methylene chloride to retain aqueous layer, shaken for 1 min., drained methylene chloride layer into 250 ml Erlenmeyer flask and extracted aqueous layer with additional 25 ml Methylene chloride. Combined the methylene chloride extracts. Evaporated the combined methylene chloride extracts to near dryness on steam bath.

Column chromatography

A bed of glass wool in the bottom of chromatographic column was placed and 1 cm high anhydrous sodium sulphate was added to give base for silica gel. Methylene chloride was added to settle silica, there was 3 cm high methylene chloride. Slowly added 2 cm bed of anhydrous sodium sulphate. Dissolved the extract in 5 ml, methylene chloride, charged the extract solution to the column. Eluted sequentially at maximum flow rate with 40 ml methylene chloride, 40 ml benzene acetic acid (9:1), 40 ml hexane and 40 ml anhydrous ether, discarded the elutes. Chloroform acetone (80:20) was used as an eluting system for aflatoxins. The elute was collected and evaporated to dryness on steam bath under nitrogen. Reserved this dry extract for thin layer chromatography. Aflatoxins reference sample Different aflatoxin reference samples were prepared in benzene-acetonitrile (98:2)

Particulars of TLC

Thin layer plates Pre coated silica gel G F2 54 plates (10 X 20 cm) of uniform thickness (0.2 mm)
Chromatographic chamber Glass tank with a lid

Solvent system Chloroform-acetone-isopropanol (85:10:05)
Detection Under UV chamber fitted with 15-watt long wave ultraviolet lamp
Detection of Pesticide Residues (Pluta, 1988)

Test sample

20-50 gm of the powder was taken, added acetonitrile water mixture (650:350) and blended for 5 min. at high speed and filtered. Transferred the filtrate into one liter separating funnel and added 100 ml of light petroleum ether. The contents were shaken for one to two minutes and added 10 ml of sodium chloride (400 gm/lit) and 600 ml of distilled water. Shaken the separating funnel vigorously for 30-45 seconds and allowed the solvent layer to get separated. Collected the petroleum ether layer, washed with water (thrice). Then treated with anhydrous sodium sulphate. The extract was subjected to column chromatography. The column was packed with activated florosil and the column was eluted with petroleum ether. Collected three fractions of 200 ml each.

The first elute contains chlorinated pesticides like aldren, benzene hexachloride, DDT, etc. while second elute contains dieldrin, the third elute contains malathion.

The elutes were concentrated to 10 ml and was used for the thin layer chromatography.

Standard samples

All the reference samples were prepared in petroleum ether.

Adsorbent

Pre coated silica gel G F 254 plate (10X20 cm) of uniform thickness (0.2 mm)

Solvent system

n-hexane: acetone (7:3)
Detection

1. Under iodine treatment
2. Under UV chamber

Metal Analysis (Neera *et al*, 1992)
Precleaned silica crucible was heated at 600^{0}C until the weight of the crucible was constant. Powdered plant material (5 gm) was taken in the silica crucible and heated in a muffle furnace at 400^{0}C till there was no evaluation of smoke. The crucible was then taken out, cooled at room temp by keeping it in a desiccator and the ash was moistened with conc. Sulphuric acid (0.5 ml). It was than heated on a heating mantel till the fumes of sulphuric acid ceased. The crucible with sulphated ash was then heated in a muffle furnace at 600^{0}C till the weight of the contents was constant (2 to 3 hrs).
The sulphated ash obtained above was then dissolved in 100 ml 5% HCl solution (5 ml conc. HCl + 95 ml. Double distilled water). These solutions were used for the determination of various elements by using Atomic Absorption spectrophotometer (AA, 640-13) Shimadzu Japan. Standard solutions of different elements were prepared according to IP/BP procedures.

RESULTS AND DISCUSSION

The pathogens like *Staphylococcus aurous, Pseudomonas aeroginosa, E. Coli*, other bacteria, molds and yeast may contaminate the herbal drugs and cause serious health hazard. Staphylococcus aurous, Pseudomonas aeroginosa, *E. Coli* found absent, total bacterial, molds and yeast count is found to be very low in *Gymnema sylvestre* and *Termianalia belerica* (Table 1). The aflatoxins i.e. the metabolic end products of bacteria are also harmful to the body and should not be present in the drugs. *Aflatoxins* were also found to be absent in the samples of *Gymnema sylvestre* and *Terminalia belerica* (Table 2).

The presence of pesticide residues has been commonly observed in the herbal drugs. The pesticide residues if present in significant amount are dangerous to human health. Therefore their presence has been detected. The results (Table 3) show the absence of Alderein, Dieldrein and Malatein in the given sample of G*ymnema sylvester* and *Terminalia belerica.* In literature information is available regarding the presence of metals and heavy metals in herbal drugs. If these are present beyond certain limit may causes toxic effects. The Indian pharmacopoeia has prescribed the limits for various heavy metals. Table 4 shows the presence of metals and heavy metals in the samples of *Gymnema sylvestre* and *Terminalia belerica*, but in small concentration.

Table 1

Sample	S.aureus	P.aeruginosa	E.coli	Total bacterial count	Total combined Molds and Yeasts count
Gymnema sylvestre	-ve	-ve	-ve	6600 µ gm/gm	1100 µ gm/gm
Terminalia belerica	-ve	-ve	-ve	1300 µ gm/gm	1005 µ gm/gm

Microbial count of Gymnema sylvestre and Terminalia belerica

Table 2

Sample	Rf value	Observation under U. V.	lampInference *n=3
Aflatoxin B^1 (Ref)	0.59	Blue fluorescence	The Aflatoxins were found to be absent in both the drug samples
Aflatoxin B^2 (Ref)	o.51	Blue fluorescence	
Aflatoxin G^1 (Ref)	0.47	Green fluorescence	
Aflatoxin G^2 (Ref)	0.44	Green fluorescence	
Test sample 1 *Gymnema sylvestre*	Nil	No spot	
Test sample 2 *Terminalia belerica*	Nil	No spot	

Detection of Aflatoxins in *Gymnema sylvestre* and *Terminalia belerica*

Table 3

Particulars	Rf value	Observation under		Inference (n = 3)
		Iodine	UV lamp	
Aldrein	0.40	Yellowish-light brown	Greenish blue	The Pesticide residues were found to be absent in the drug samples.
Dieldrein	0.36	Yellowish-light brown	Greenish blue	
Malathien	0.41	Yellowish-light brown	Greenish blue	
DDT	0.36	Yellowish-light brown	Greenish blue	
Sample 1 *Gymnema sylvestre*	Nil	No Spot	No Spot	
Sample 2 *Terminalia belerica*	Nil	No spot	No Spot	

Detection of Pesticide Residues in *Symnema sylvestre* and *Terminalia belerica*

Table 4

S.No	Element	% Conc. of various elements in PPM *n = 3	
		(Gymnema sylvester)	*(Terminalia belerica)*
1.	Cu	1.34	0.52
2.	Ca	0.97	1.48
3.	Fe	0.62	0.36
4.	Zn	1.18	1.02
5.	Ba	0.23	0.48
6.	Al	0.08	0.33
7.	Ni	0.38	0.17
8.	Ag	0.12	0.44
9.	Pb	0.08	0.12
10.	Mg	0.73	1.33
11.	Na	0.08	0.04
12.	K	0.128	0.102
13.	Cr	0.67	0.49

Metal analysis of *Gymnema sylvestre* and *Terminalia belerica*

CONCLUSION

The study shows the absence of pathogens, aflatoxins and pesticide residues in the samples of *Gymnema sylvestre* and *Terminalia belerica*. The metals and heavy metals are found to present but in small concentrations. Therefore the herbal drugs *Gymnema sylvestre* and *Terminalia belerica* may be used safely.

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