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BAMBUSA BALCOOA ROXB. A NEW SOURCE FOR PHYTOSTEROLS

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Assessment of the level of phytosterols in the parts of the plant *Bambusa balcooa* Roxb. grown in Manipur was carried out which ranges from 0.001 to 0.34% wt. The succulent bamboo shoot slices were subjected to fermentation which resulted in an enrichment of phytosterols from 0.3 to 0.6% dry wt. as compared to that of the fresh one (0.18% dry wt). Further, purification of crude phytosterols was done to isolate different phytosterols by TLC and further UV spectral analysis was done as compared to the authentic samples procured from Sigma. Stigmasterol, ß-sitosterol and campesterol were isolated from the crude samples procured by the petroleum ether, benzene, KOH extract of the fermented samples.

Key Words: *Bambusa balcooa*/phytosterols/fermentation.

Phytosterols are secondary plant products found in high quantity in many plants (Jain *et al.*, 1980, Srivastava *et al.*, 1983, 1985; Jain and Agarwal 1988, Srivastava 1990). Some of the phytosterols are precursors of many pharmaceutically important steroidal drugs including corticosteroids, sex hormones and oral contraceptives. The increasing demand for steroidal drugs has resulted in the depletion of many natural resources (Jain *et al.*, 1980; Srivastava *et al.*, 1985; Srivastava, 1990). Hence an alternative source for a starting material is imperative. In this context phytosterols which are used for the production of steroidal drugs, are of importance. In the present paper, succulent bamboo shoots of *Bambusa balcooa* are proposed as an alternative source of phytosterols. *Bambusa balcooa* is cultivated in many tropical countries. In Manipur, the fresh succulent bamboo shoot and the fermented preparation of bamboo shoot slices, locally called 'soibum' is a highly prized vegetable item.

MATERIALS AND METHODS

In order to screen out whether all parts of the plant material contain phytosterols, each of the *Bambusa balcooa* plant material (Rhizome, stem sheath, bristles on the stem sheath, mature stem scraped, leaves, succulent bamboo shoot, inflorescence) were collected from different localities of Imphal and were oven dried at 60°C for 12h. The dried samples were powdered and the concentration of total phytosterols were determined colorimetrically using the Leibermann-Burchard reagent (Katayama, 1974).

For enrichment of sterols during anaerobic digestion, fermentation of the fresh succulent bamboo were done by inoculating thin slices with the exudate obtained from already fermented slices of bamboo shoots (Traditional way of fermentation) sold in the local market in the name of "soibum". After inoculation, the sample were kept in an incubator at 30°C±2°C for a period of 60 days. Weekly interval analysis on the changes in the level of total phytosterols was carried out during fermentation using Liebermann Burchard reaction (Katayama, 1974). To purify phytosterols, the first step was to extract pure phytosterols in large quantity from the fermented samples. For this, 100 g of powdered fermented material (oven dried at 60°C) was refluxed with a solvent mixture of benzene, petroleum ether and 2N ethanolic KOH (10:5:1) for 12 h in a 1 litre soxhlet extraction flask. The extract was decanted and concentrated to 20 ml slurry. The slurry was transferred to a pre-weighed petri-plate and the solvent was dried off at 80°C in an oven followed by cooling at room temperature which resulted in formation of soft cake. The soft cake was refluxed with 180 ml of methylcyanide for 30 min and the hot extract was decanted into a pre-weight petri-plate and then allowed to cool .at room temperature. A cream coloured crystalline precipitate was obtained which contained mixture of phytosterols.

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Table 1. Level of phytosterols in different plant parts of *Bambusa balcooa.*

Part of the plants	Percentage of phytosterol (dry wt.)
Rhizome	0.001 ± 0.02*
Mature stem scarp	0.121 ± 0.09
Leaves	0.124 ± 0.02
Inflorescence	0.339 ± 0.08
Stem sheath	0.025 ± 0.05
Bristles on the stem sheath	0.016 ± 0.07

*Standard error of the mean (n=3)

A little quantity of this precipitate was subjected to thin layer chromatography (Stahl, 1965) using solvent pair Hexane and ethylacetate (3:1). Crystals of each spot obtained from preparative thin layer chromatography were then subjected to UV spectral studies. For UV spectral studies the absorbance of the compounds was measured from 225 nm to 400 nm on a Beckman DU-64-Spectro-photometer. The UV absorption spectra in UV-range of the authentic sample (Sigma Chemical St. Louis, U.S.A) which was also taken simultaneously in DU-64-Spectrophotometer. And for further confirmation of the different phytosterols, the partially purified samples were sent to CDRI, Lucknow for IR and Mass Spectral analysis.

RESULTS AND DISCUSSION

The result showed that the level of phytosterol varies in different parts of *Bambusa balcooa*. The rhizomes an the bristles contained the lowest percentage of phytosterols (0.001 to 0.017% dry wt.), followed by the stem sheath (0.025%). The stem scraps contain 0.121% and leaves contain 0.124% dry wt, while the delicate succulent bamboo shoot contains 0.18% and the highest concentration was noticed in the inflorescence.

Since the rhizome, mature stem scraps, stem sheath and bristles on stem sheath possess less quantity of phytosterols, these source cannot be preferred for phytosterol extraction. The Inflorescence, on the other hand, contains very high content of phytosterols but it is rarely available. The result showed that succulent shoots of bamboo possessed a considerable amount of phytosterols as shown in Table-1. Therefore, it seems essential to proceed for a detailed study on extraction and eenrichment of phytosterols through biochemical means.

Table 2. Changes in the level of total phytosterols at different stages of fermentation in *Bambusa balcooa.*

Name of the species	Concentration of total phytosterol (%dry wt.)						
	Fermentation period (days)						
	0	7	14	21	28	35	60
B balcooa	0.18 ±0.01*	0.34 ±0.00	0.35 ±0.02	0.38 ±0.01	0.4 ±0.04	0.45 ±0.05	0.6 ±0.05

Standard error of the mean (n=3)

Table 3 Rf value of different spots on TLC plate separated with Hexane and Ethylacetate solvent pairs. The chromatogram was run at 28°C for 90 min and for development of sports the plates were sprayed with Lieberman-Burchard reagent followed by heating in oven at 80°C for 30 Min.

Solvent pair	Spots position	Rf value	Possible phytosterols using standard samples on co-chromatography
Hexane:Ethylacetate (3:1)	Ist (lowered)	0.0428	unidentified
	2nd	0.320	ß-sitosterol
	3rd	0.732	Stigmasterol
	4th	0.814	Campesterol

The concentration of phytosterols in fermented bamboo shoots was maximum (ranging from 0.30 to 0.60% dry wt.) as compared to that of the fresh one (0.18% dry wt). Thus, there was an increasing trend in the concentration of total phytosterols from the initial stage of fermentation (0-day) till day 60 days (Table-2). The increase in the level of phytosterols in the fermented samples was due to anaerobic digestion by microorganisms that cause degradation of the organic matter and resulted in the enrichment of phytosterols (Sarangthem & Srivastava, 1997).

The TLC plate developed with Liebermann-Burchard reagent showed four spots. One of these four spots was light green colour just above the spotting point and the remaining three were violet coloured and clearly visible on the plate in the heated condition and become light coloured at room temperaturre. The co-chromatography with standard samples revealed presence of campesterol, stigmasterol and ß-Sitosterol. The Rf value of each spot was calculated as shown in table 3. The TLC study showed presence of at least four phytosterols tentatively identified as campesterol, ß-Sitosterol, stigmasterol and one unidentified.

The UV spectra of the peaks for the authentic samples were found at 247 nm for ß-sitosterol, 244 nm for stigmasterol and 243 nm for campesterol. The purified samples of phytosterols extracted from fermented bamboo shoots of *Bambusa balcooa* also showed similar peaks. The IR spectral peak of the samples corresponds to that of the authentic samples (Sigma Chemicals, U.S.A). The molecular weight of the samples was found to be 412 and 417 Mass Spectra for the first two compounds which corresponds to that of stigmasterol and sitosterol. The molecular weight of the third compound could not be identified perfectly due to contamination of fatty acids with sterols. This third compound will be further purified and confirmed at CDRI, Lucknow.

The conclusion which may be drawn after assessing the present work is that both fresh and fermented succulent shoots of bamboos may be used as an alternative and potent source for production of phytosterols.

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