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Quantitation of β -sitosterol from *Woodfordia fruticosa* (Linn.) Kurz and a polyherbal formulation used for treating female reproductive disorders

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ABSTRACT

Woodfordia fruticosa (Linn.) Kurz (syn. *W.floribunda* Salisb.); Family-Lythraceae, commonly known as Dhataki, is a woody shrub distributed throughout India. The flowers are commonly used in the management of female reproductive disorders. A sensitive, simple, and accurate high-performance thin-layer chromatographic method has been established for quantitation of β -sitosterol in plant powder of *Woodfordia fruticosa* (Linn.) Kurz (flowers) and in Wama tablets, a polyherbal formulation used against female reproductive disorder. The plant powder was extracted with methanol and used for quantitation. The concentration of β -sitosterol was found to be $0.18\mu\text{g mg}^{-1}$ in the plant powder of *Woodfordia fruticosa* (flowers) and $0.15\mu\text{g mg}^{-1}$ in the polyherbal formulation. Quantitation was carried out on HPTLC silica gel 60 F₂₅₄ pre-coated plates with the mobile phase toluene-ethyl acetate- methanol-glacial acetic acid, 8+1+0.5+0.3 (v/v/v/v). A TLC scanner set at 366nm in fluorescence/reflectance mode was used for quantitation. β -sitosterol response was linear over the range $10\mu\text{g mL}^{-1}$ to $70\mu\text{g mL}^{-1}$. The method was validated for linearity, precision, accuracy and robustness. © 2009 Trade Science Inc. - INDIA

1. INTRODUCTION

Woodfordia fruticosa (Linn.) Kurz (syn. *W.Floribunda* Salisb.), Family-Lythraceae, commonly known as Dhataki is a woody shrub distributed throughout India^[1,14]. The flowers are commonly used as an abortifacient^[9,14] and in the treatment of menorrhagia^[8,16]. Several herbal industries have been using Dhataki flowers in their herbal formulations which are used in managing menstrual disorders and Uterine disorders, e.g. Ashoka kalp, Jeevani, Femicare forte capsule, Gynin, Gynova liquid, Leucofre, Ashoka compound, Wama tablets. *Woodfordia fruticosa* (Linn.) Kurz has been reported to contain chemical constituents such as Woodfordin, Oenothin, Ellagic acid, Quercetin-3-rhamnoside, Kaempferol-3-glucoside, β -sitosterol^[3,4]. β -sitosterol has great potential in the treatment of diabetes, blood clotting, ulcers, cancer prevention, tumors, im-

munity, inflammation etc. It has also shown to have positive effects on the female reproductive system especially the uterus^[6]. The structure of β -sitosterol is shown in figure 1^[15].

The literature reveals that there is no high-performance thin-layer chromatographic method available for quantitation of β -sitosterol in flowers of *Woodfordia*

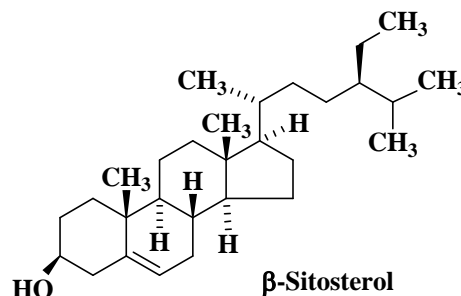


Figure 1: Structure of β -sitosterol standard

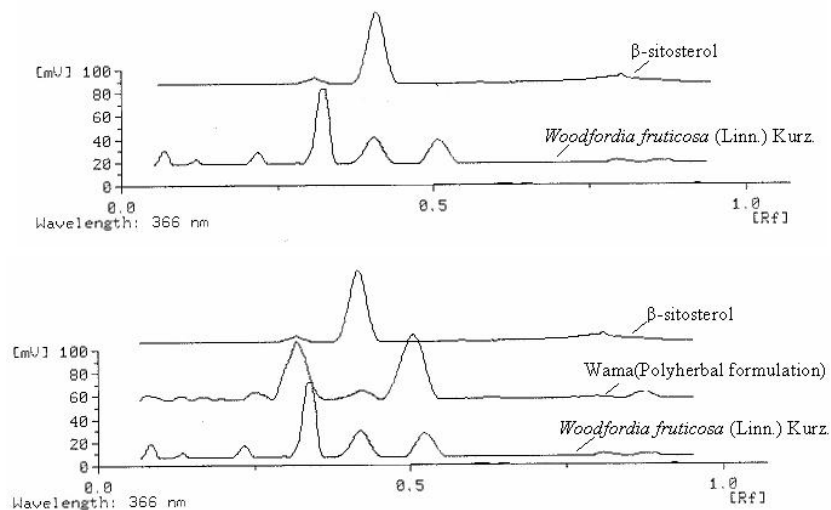


Figure 2 : (a) A typical HPTLC chromatogram of β -sitosterol standard, plant, (b) A typical HPTLC chromatogram of β -sitosterol standard, plant and formulation

fruticosa (Linn.) Kurz, however quantitation of β -sitosterol has been done from plants like *Cynodon dactylon* (Linn.) Pers^[2], *Leucas cephalotes* (Roth) Spreng^[11], *Capparis deciduas* (Forsk) Edgew^[12], *Leptadenia reticulata* (Retz.) Wight and Arn^[13] A sensitive, simple, and accurate high-performance thin-layer chromatographic method has been established for quantitation of β -sitosterol in plant powder of *Woodfordia fruticosa* (flowers) and a polyherbal formulation.

2. EXPERIMENTAL

2.1 Materials

Woodfordia fruticosa (Linn.) Kurz was collected from Karnala (Maharashtra) and was authenticated from National Botanical Research Institute PID (CSIR) R. and S. Cell No. 1894. Standard β -sitosterol (98% purity) was procured from Sigma Aldrich Chemie (Steinheim, Germany). The solvents toluene, ethyl acetate, methanol and glacial acetic acid were of analytical grade and were purchased from Qualigens Fine Chemicals, Mumbai, India, were used for the analysis.

2.2 Instruments

A TLC scanner with a computer system and Cats 3 Version Software (Camag, Muttenz, Switzerland) was used. The source of radiation was mercury lamp. Camag

Linomat IV was used as applicator. Separation was done on HPTLC silica gel 60 F₂₅₄ pre-coated plates procured from Merck (Darmstadt, Germany).

2.3 Standard and sample preparation

A stock solution of β -sitosterol ($1000\mu\text{g mL}^{-1}$) was prepared by dissolving 10.0 mg of accurately weighed β -sitosterol in methanol and diluting it to 10.0 mL with methanol. Aliquots (0.1 mL to 0.7 mL) of this stock solution were transferred to 10 mL standard volumetric flasks and the volume of each was adjusted to 10 mL with methanol, to obtain working standards containing $10\mu\text{g mL}^{-1}$ to $70\mu\text{g mL}^{-1}$.

Flowers of *Woodfordia fruticosa* (Linn.) Kurz were collected, washed, shade dried, powdered, sieved through an 80-mesh (BSS) sieve and stored in an airtight container at 25°C . 1.0 gm of the dried powder was accurately weighed, placed in a stoppered tube and 10 mL of methanol was added. The sample was vortexed for 1-2 min. and left to stand overnight at room temperature ($28 \pm 2^{\circ}\text{C}$). The contents of the tube were filtered through Whatmann filter paper No. 41 (E. Merck, Mumbai, India). The clear supernatant was collected and used for quantitation and validation.

Wama tablets were procured from local market. Five tablets were weighed accurately and crushed to obtain a fine powder. The weight equivalent to one tablet was determined, 1.0 g powder was transferred in a clean, dry, stoppered conical flask and 10 mL metha-

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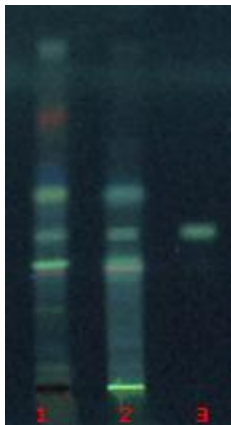


Plate 1 : The chromatographic plate of β -sitosterol standard and *Woodfordia fruticosa* (Linn.) Kurz. And Polyherbal formulation samples

TABLE 1 : Linearity data

Linearity range	10 to 70 $\mu\text{g mL}^{-1}$
Slope (m)	29.98
Intercept(c)	61.57
Correlation coefficient (R)	0.994
LOD	2 $\mu\text{g mL}^{-1}$
LOQ	4 $\mu\text{g mL}^{-1}$
Instrument Precision (n=5 RSD)	0.14
Intraday (precision)(n=3 RSD)	0.16
Interday (precision)(n=3 RSD)	0.17

$y = mx + c$, where, $y =$ peak area, $m =$ slope, $x =$ concentration, $c =$ intercept.

TABLE 2 : Results of accuracy analysis

Amount of β -sitosterol in preanalysed sample of <i>Woodfordia fruticosa</i> (Linn.) Kurz flowers(μg)	Amount of β -sitosterol standard added to preanalysed sample of <i>Woodfordia fruticosa</i> (Linn.) Kurz flowers (μg)	Total amount of β -sitosterol (μg)	SD	RSD (%) n=7	Recovery (%)
177	0	177.06	0.0150	0.0086	99.96
177	0.5	177.489	0.0180	0.0104	99.72
Mean	-	-	-	-	99.84

nol was added. The solution was vortexed for 1-2 min, allowed to stand overnight and filtered through Whatmann filter paper No. 41. This solution was used for the assay.

2.4 Chromatography

2.2.1 Procedure

Chromatography was performed on HPTLC silica gel 60 F₂₅₄ pre-coated plates^[4]. Samples (10 μL) were applied on the plates as bands of 7mm width with the help of a Camag Linomat IV sample applicator at the distance of 15mm from the edge of the plates. The mobile phase constituted of toluene-ethyl acetate-methanol-glacial acetic acid, 8.0+1.0+0.5+0.3 (v/vv/v). The plates were developed up to a distance of 85 mm in a Camag twin-trough chamber previously equilibrated with mobile phase for 30 min. The chromatographic conditions had previously been optimized to achieve the best resolution and peak shape. After development, plates were dried under current of air at room temperature, derivatised with freshly prepared Liebermann-Burchard reagent in a derivatisation chamber for 1.0 min, and dried at room temperature. After drying, plates were heated in oven at 105°C for 10 min before densitometric scanning^[6]. Densitometric evaluation of the plates was performed at 366 nm in fluorescence/reflectance mode using mercury lamp with a Camag Scanner II in conjunction with Cats 3 Version Software. A typical HPTLC chromatogram of β -sitosterol standard, plant, and formulation is shown in figure 2. The chromatographic plate of β -sitosterol and *Woodfordia fruticosa* (Linn.) Kurz. (flowers) and polyherbal formulation is shown in plate 1.

2.2.2. Linearity of detector response

Solutions containing β -sitosterol at seven different concentrations (10, 20, 30, 40, 50, 60, 70 $\mu\text{g mL}^{-1}$) were prepared in methanol. Each of these solutions (10 μL) was applied to a plate, the plate was developed and the detector response for different concentrations was measured. A graph was plotted using the peak area against concentration of β -sitosterol. The plot was linear in the range 10 to 70 $\mu\text{g mL}^{-1}$. The experiment was performed three times and the mean was used for the calculations. The linearity data is given in TABLE 1.

2.2.3 Accuracy

The accuracy of the method was established by performing recovery experiments by the standard addition method. Recovery of standard β -sitosterol added to the extract of plant powder (flowers) of *Woodfordia fruticosa* (Linn.) Kurz and powder of polyherbal formulation was studied at two different levels, each being

TABLE 3: Results of assay

Sample	Weight of sample in mg	Amount of β -sitosterol present in sample in $\mu\text{g mg}^{-1}$	RSD (%) n=7	Average % content of β -sitosterol
Plant powder (flowers) of <i>Woodfordia fruticosa</i> (Linn.) Kurz	1000	0.18 \pm 0.0011	0.645	0.018
Powder of Wama tablets (polyherbal formulation)	1000	0.15 \pm 0.0015	1.034	0.015

analysed in a manner similar to that described for the assay. The β -sitosterol content and the percent recovery was calculated. The results are given in TABLE 2.

2.2.4. Assay procedure

The standard solution of β -sitosterol ($50\mu\text{g mL}^{-1}$) and $10\mu\text{L}$ of sample solutions were spotted on a TLC silica gel 60 F₂₅₄ pre-coated plates. The amount of β -sitosterol present in this solution was calculated by comparison of area measured for the sample to that for the standard. The assay procedure described earlier was repeated seven times. The results of assay are given in TABLE 3.

The mean assay value of β -sitosterol was found to be 0.018 % in flowers of *Woodfordia fruticosa* (Linn.) Kurz and 0.015 % in polyherbal formulation.

3. RESULTS AND DISCUSSION

Woodfordin has been quantitated from the flowers of *Woodfordia fruticosa* (Linn.) Kurz^[14]. However, β -sitosterol from the flowers of *Woodfordia fruticosa* (Linn.) Kurz has not been reported. In the current study, β -sitosterol was detected and quantified using HPTLC silica gel 60 F₂₅₄ pre-coated plates with the mobile phase made of toluene-ethyl acetate-methanol- glacial acetic acid, 8.0+1.0+0.5+0.3 (v/v/v/v). The identity of the band of β -sitosterol in the plant extract was confirmed by overlaying the chromatogram of plant with that of the β -sitosterol standard and by comparing their R_F (0.41).

The detection of β -sitosterol was observed to be linear over a concentration range of 10 to 70 $\mu\text{g mL}^{-1}$ with correlation coefficient of 0.994. The concentration of β -sitosterol was found to be 0.018 $\mu\text{g mg}^{-1}$ in

flowers of *Woodfordia fruticosa* (Linn.) Kurz and 0.15 $\mu\text{g mg}^{-1}$ in Wama tablets (polyherbal formulation).

Instrument precision, intraday assay precision, interday assay precisions were measured to evaluate the precision of the method. The % RSD values were found to be less than 2%, indicating that the selected method is precise and reproducible.

The accuracy of the method was established by means of recovery experiment. The mean recovery was close to 100%, which indicates that the method is efficient. The mean recovery of β -sitosterol was 99.84 %.

The robustness of the method was studied, during method development, by determining the effects of small variation, of mobile phase composition ($\pm 2\%$), chamber saturation period, development distance and scanning time (10% variation of each). No significant change of R_F or response to β -sitosterol was observed, indicating the robustness of the method.

4. CONCLUSION

The proposed method is simple, rapid, selective, sensitive, and economical and can be used for routine quality-control analysis and quantitation of β -sitosterol from *Woodfordia fruticosa* (Linn.) Kurz (flowers) and the formulations containing *Woodfordia fruticosa* (Linn.) Kurz.

5. ACKNOWLEDGMENTS

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REFERENCES

- [1] API; The Ayurvedic pharmacopoeia of India, Ministry of Health and Family Welfare, Government of India, New Delhi, 1, (2001).
- [2] P.A.Champanerkar, V.V.Vaidya, Sunita Shailajan, G.R. Singh, Shah Willy; India Drugs, 44(1), (2007).
- [3] J.S.Chauhan, S.K.Srivastava, S.D.Shrivastava; J.Indian Chem.Soc., 56, 1041 (1979).
- [4] J.S.Chauhan, S.K.Srivastava, S.D.Shrivastava; Planta.Med., (1979): 183-184 (1979).
- [5] J.Sherma, B.Fried; 'Handbook of Thin Layer Chromatography', (1996).
- [6] Karl H.Pegel; South African Journal of Science,

Full Paper

- 93, 263-268 (1997).
- [7] K.M.Nadkarni; 'Indian Plants and Drugs (with their Medicinal Properties and uses)', 416-417.
- [8] A.Pakrashi, B.Basak, N.Mookerji; Indian J.Med. Res., **63**, 378-381 (1975).
- [9] P.C.Sharma, M.B.Yelna, T.J.Dennis; Database on Medicinal Plants used in Ayurveda, **3**, 206-210 (2001).
- [10] P.D.Sethi; Quality Analysis of Pharmaceutical Formulations, CBS, India, (1996).
- [11] Quality standards of Indian Medicinal Plants, Indian Council of Medical Research, New Delhi, **2**, 146-154 (2005).
- [12] Quality standards of Indian Medicinal Plants, Indian Council of Medical Research, New Delhi, **3**, 98-106 (2005).
- [13] Quality standards of Indian Medicinal Plants, Indian Council of Medical Research, New Delhi, **3**, 236-245 (2005).
- [14] Quality standards of Indian Medicinal Plants, Indian Council of Medical Research, New Delhi, **3**, 365-373 (2005).
- [15] The Merck Index, 11th edition, 8500 (1989).
- [16] The Wealth of India, A Dictionary of India Raw Material, Council of Scientific and Industrial Research, **10**, 586,587 (2003).