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HPTLC Based Screening of β-Sitosterol from *Andrographis paniculata*

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ABSTRACT

Sterols are secondary metabolites of plants known for their inhibitory effects on cancer cell growth, lower cholesterol and enhance immunity. β-sitosterol belongs to phytosterol which enhances antioxidant enzymes and thus reduces the oxidative stress. In present experiment, β-sitosterol was detected from *Andrographis paniculata* belonging to family Acanthaceae by using HPTLC. The plant is popularly known as Kalmegha having multiple pharmacological properties used for treatment of several diseases. Leaf, stem and root extracts prepared in chloroform, methanol and petroleum ether were used for detection of β-sitosterol from the plant. The chromatography was performed on TLC plates coated with Al silica gel 60 F254 and solvents used for mobile phase was toluene:ethyl acetate:formic acid (15:4.5:1.5). After development, the plates were derivatized with 10% methanolic sulphuric acid, scanned and quantified at 510 nm. The results showed the presence of β-sitosterol in all the parts with Rf value 0.62. Calibration curve was prepared and the amount of β-sitosterol was quantified in the extracts by comparing the respective peak areas with that of the standard. The correlation coefficient for β-sitosterol against reference sample was found significant (r= 99.769057%) for the concentration range of 0.5 to 4.0 µg. Leaf methanol extract showed the highest concentration of β-sitosterol, i.e., 147.6 µg/mL. The extraction efficiency of β-sitosterol was found higher in methanol followed by petroleum ether and chloroform extract. Thus, our finding shows that *Andrographis paniculata* has a significant concentration of β-sitosterol, which may be useful for pharmacological application against cancer, hypercholesterolemia, inflammation and for angiogenesis process.

INTRODUCTION

β-sitosterol is a known plant sterol and characterized by cyclopenta perhydro phenanthrene structure and exist in free or in esterified form (Kurvinen et al. 2012). β-sitosterol helps in reducing absorption of cholesterol in plasma and body. Lijia et al. (2012) reported reduction of 10-15% of LDL-cholesterol and total cholesterol with the daily intake of 1-3 gram of phytosterol in the diet. Choudhary & Tran (2011) and De Smet et al. (2012) reported biological properties of phytosterol based on experimental data (animal xerograft models and epidemiological studies, *in vitro* tests) and found effective inhibitory effect on various forms of cancer such as breast, ovarian, stomach, lungs and others. Woyengo et al. (2009) studied the role of sterols in enhancing the oxidative enzymes and reducing the oxidative stress. Phenolic compounds are well known for their antioxidant property, but in recent years, on the basis of molecular structure this action was also found in phytosterol. β-sitosterol is an imortant class of bio-organic molecules present in plants, animals, fungi, and is structurally similar to cholesterol and biologically synthesized from both mevalonate and deoxyxylulose pathways (Weihrauch & Gardner 1978). The three predominant phytosterol in human diet herbal nutrition contain 65%, 3% and 30% of β-sitosterol, stigmasterol and campeseterol respectively. Phytosterols have been recognized as safe (GRAS) with no undesired side effects. Sudhop (2002) reported that access of pytosterol may cause phytosterolaemia a genetic disease, which is related to some kind of mutation in ABCG5/G8 proteins which play the role of protein pump to enter the sterols into enterocytes and hepatocytes. In 1982, Anonymous (1982) reported that the two main factors responsible for hypercholesterolemic effect is the high concentration of β-sitosterol and linoleic acid. Various authors have established a positive role of β-sitosterol, viz. hepatoprotective property (Shailajan et al. 2005), anti-inflammatory (Gupta et al. 1980), immunomodulatory (Karl 1997), colon cancer (Awad et al. 2000a), rheumatoid arthritis (Bouic et al. 1996), antioxidant and antipyretic (Ahmed et al. 2001 and Ali 1967), prostatic hypertrophy (Awad et al. 2001 and 2000b), and breast cancer (Awad et al. 2000c). Rahuman et al. (2008) reported the moderate larvicidal activity in petroleum ether extracts of *Abutilon indicum* and identified that β-sitosterol exhibits novel mosquito larvicidal activity. In 2007, Gomes et al. (2007) reported β-sitosterol in methanol root extract of *Pluchea indica* (Asteraceae), which was studied on experimental ani-

mals and found that the compound acts as a neutralizing agent on viper and cobra venom. Based on above literature it has been proved that that β-sitosterol is a pharmacologically significant, ubiquitous in nature and found throughout the plant kingdom. *Andrographis paniculata* (Acanthaceae family) popularly known as Kalmegha is a significant plant used in traditional Sidha and Ayurvedic medicine and also in tribal medicine for treatment of several diseases. Whole parts of the plant are bitter in taste which is due to the presence of andrographolide. The plant is known for multiple pharmacological properties such as analgesic, antibacterial, antipyretic, anti-inflammatory, anti-hepatotoxic, antidiabetic and antifertility activities (Mishra et al. 2010). Andrographolide is the major active ingredient of the plant having antidiabetic, antimalarial, antiallergic, and neoandrographolide possess anti-inflammatory property (Madav et al. 1996). Other bioactive molecules of the plant include andrographolide, neoandrographolide, deoxyandrographolide, β-sitosterol, bisandrographolide, ninandrographolide, andropanolide, isoandrographolide, panicalin and apigenin. Sethi (1996) introduced HPTLC, a chromatography technique for estimation of active biomolecules. HPTLC is more advanced and sophisticated technique due to its easy sample preparation, less time requirement, and results of high accuracy, precision and reproducibility. WHO introduced chromatography for standardization of plant products and accepted for evaluation and identification of quality of plant derived products (Dhalwal et al. 2010). In the present study, β-sitosterol was detected from leaf stem and root in methanol, chloroform and petroleum ether extracts of *Andrographis paniculata* by HPTLC method.

MATERIALS AND METHODS

Preparation of sample extract: *Andrographis paniculata* was collected from Chhattisgarh, India and authenticated from Botanical Survey of India, Allahabad. Chloroform, methanol and petroleum ether extract of leaf, stem and root part of plant was prepared by using Soxhlet apparatus. Extracts were kept in tight bottles and stored at -20°C.

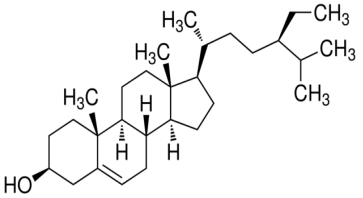

Fig 1: Structure of β-sitosterol adapted from Saraf and Samant (2015).

Synonyms: α-phytosterol, Cupreol, Cinchol, Quebrachol, Rhamnol.
Molecular formula: $C_{29}H_{50}O$
Molecular weight: 414.71
Chemical class/group: Terpenes (Subclass: Triterpenes)

Preparation of standard solution: Standard β- sitosterol of 1 mg/mL was prepared in methanol. Reference standard solution of 0.5 to 4 µL concentration was applied on chromatography plates.

Chromatography: Chromatography was performed on Al silica gel precoated (20×10 cm) TLC plates 60F 254 (Merck), and VisionCAT software version 2.5.18072.1 (CAMAG - Switzerland) was used for data analysis. Linomat 5 sample applicator fitted with 100 µL of Hamilton syringe was used for spotting samples on TLC plate with 8 mm band and distance of solvent front was 70 mm. Before chromatography development tank, TTC 20×10 was saturated for 20 min and the mobile phase used contain a mixture of toluene: ethyl acetate: formic acid (15:4.5:1.5). After development, the plates were dried at room temperature for 5 min and derivatized with 2 mL of 10% methanolic sulphuric acid in a derivatization chamber. After derivatization, the plates were dried in air and bands were observed in visualizing chamber and CAMAG scanner.

RESULTS AND DISCUSSION

The fringerprint analysis of β-sitosterol of *Andrographis paniculata* was visualized by CAMAG TLC Scanner IV (Fig. 2 & 3). TLC plates confirmed the presence of β-sitosterol in all the extracts of *Andrographis paniculata.* The Rf value of standard β-sitosterol was 0.62 and found similar to Rf values found in all the extracts of *Andrographis paniculata* which confirms presence of the compound. TLC plates were scanned at 510 nm in scanner 4 and a sharp peak of β-sitosterol was obtained for the standard and samples (Fig. 4 & 5). Graph of peak area and concentration of β-sitosterol in extracts of *Andrographis paniculata* showed linear relationship when plotted. The amount of β-sitosterol per 10.0 µL sample was calculated. The correlation coefficient for β-sitosterol against reference sample was found significant (r= 99.769057%) for the concentration range of 0.5 to 4 µg. The calibration curve (Fig. 6) of β-sitosterol was in the range of 0.5 to 4 µL and the linear regression equation obtained was $Y = 3.062 \times 10^{-9} x + 4.077 \times 10^{-3}$ and coefficient of variation was 1.99%. The quantitative analysis of β-sitosterol in sample was obtained automatically via graph (Fig. 7). The highest concentration of β-sitosterol was found in leaf methanol extracts (147.6 µg/mL) of *Andrographis paniculata*. Stem and root methanol extracts contain 145.9 and 119.9 µg/mL of β-sitosterol respectively. Methanol extracts showed greater extraction efficiency for β-sitosterol than petroleum ether and chloroform extracts. Rahmana et

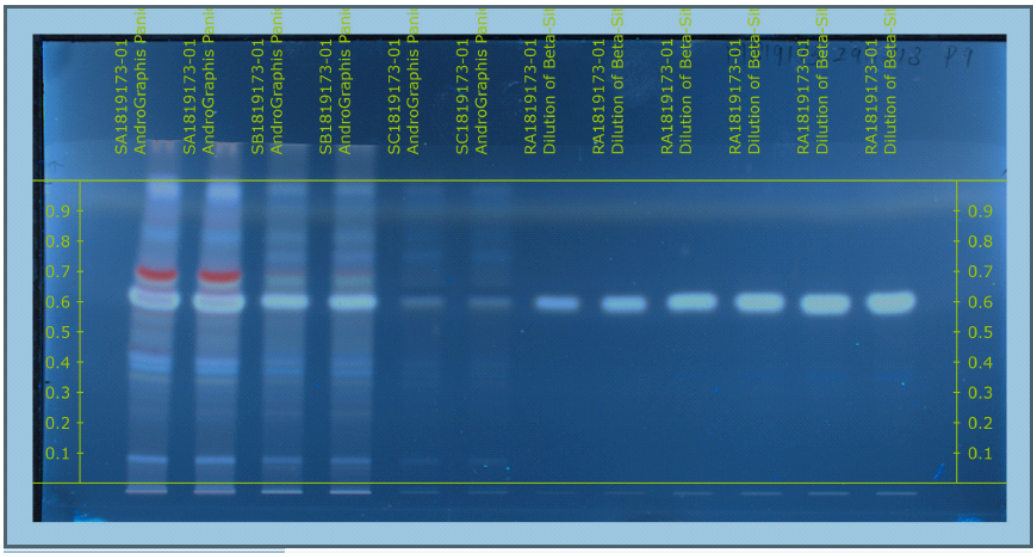

Fig. 2: Chromatoplate at R366nm after derivatization with 10% methanolic sulphuric acid showing bands of β-sitosterol in petroleum ether extracts of *Andrographis paniculata.*
SA= Leaf extract in petroleum ether, SB= Stem extract in Petroleum ether, SC= Root extract in petroleum ether, RA= Reference standard β-sitosterol.

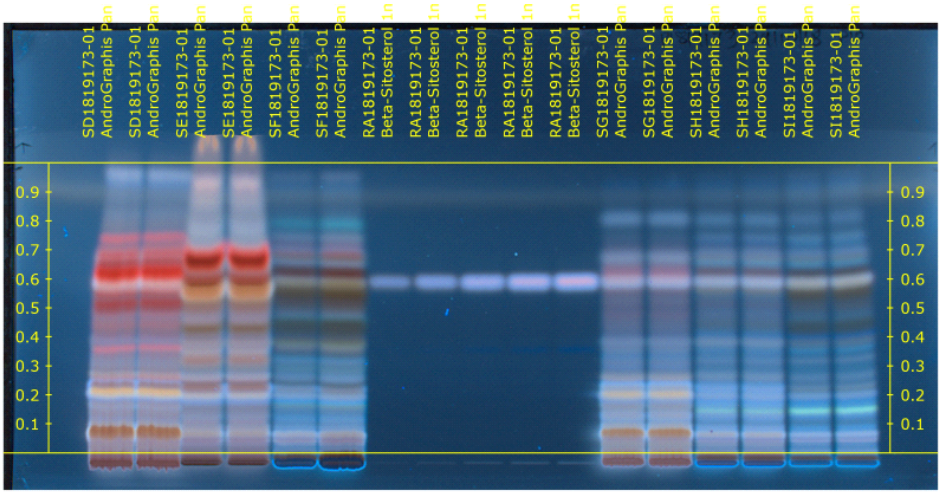

Fig. 3: Chromatoplate at R366nm after derivatization with 10% methanolic sulphuric acid showing bands of β-sitosterol in chloroform and methanol extracts of *Andrographis paniculata.*
SD= Leaf extract in chloroform, SE= Stem extract in chloroform, SF=Root extract in chloroform, SG= Leaf extract in methanol, SH= Stem extract in methanol, SI= Root extract in methanol, RA= Reference standard β-sitosterol.

al. (2009) isolated β-sitosterol-D-glycoside from petroleum ether extract of the leaves of *Ocimum sanctum.* Saraf & Samant (2015) reported quantification of β-sitosterol in the roots of *Achyranthes asper* by HPTLC method and found 1.277 ng/µg. Soybean oil contains β-sitosterol which helps to reduce the cholesterol level (Cicero et al. 2002). β-sitosterol from vegetable oils was analysed by GC-MS method by Zhang *et al.* (2005). High pressure liquid chromatography atmospheric pressure chemical ionization mass spectroscopy (HPLC-APCI-MS) method was used to analyse sterol in plant by Mezine (2003). Similarly, in the present study β-sitosterol was found higher in methanol followed by petroleum ether and chloroform extracts of leaf, stem and root. Considering the wide therapeutic application of β-sitosterol, the HPTLC method is sensitive, specific and reproducible for quantification and to ensure quantity and quality from different parts of *Andrographis paniculata* in different seasons and different regions.

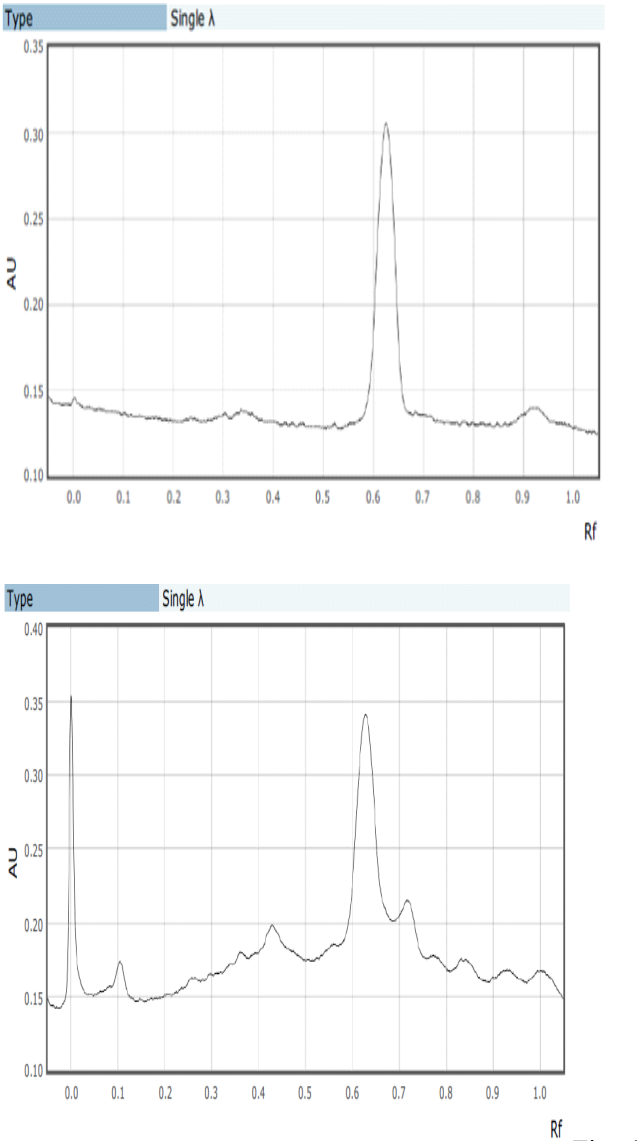

Fig 4: Spectra of β-sitosterol at 510 nm for (A) standard and (B) methanol based leaf sample.

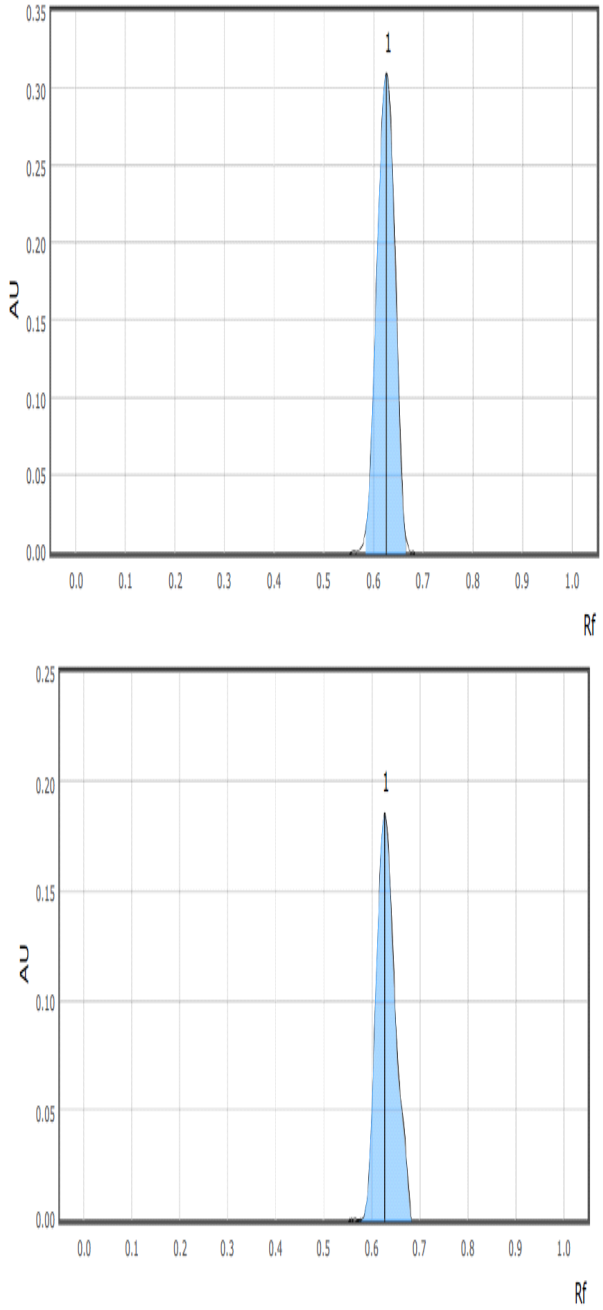

Fig. 5: HPTLC chromatogram of (A) standard β-sitosterol (B) methanol based leaf sample.

CONCLUSION

β-sitosterol is a well known phytosterol found in many plants, having various biological properties such as anti-oxidant, hepatoprotective, anti-diabetic, antipyretic, anti inflammatory, anticancer, trypanocidal and mosquito larvicidal. In the present work, β-sitosterol was detected in petroleum ether, chloroform and methanol extracts of *Andrographis paniculata.* Methanol extracts showed high concentration of β-sitosterol. This study should be beneficial for isolation of pharmacologically significant β-sitosterol compound from *Andrographis paniculata* and used for formulation of drugs for treatment of several diseases such as cancer, malaria, hypercholesterolemia, inflammation and for angiogenesis process.

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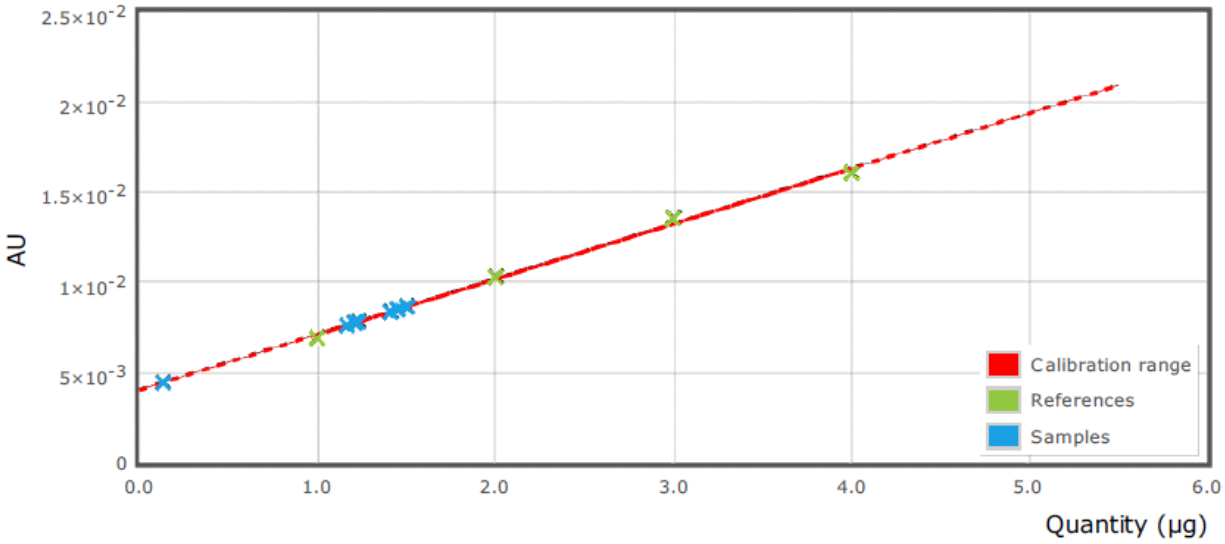

Fig. 6: Calibration area for standard β-sitosterol at 510 nm.

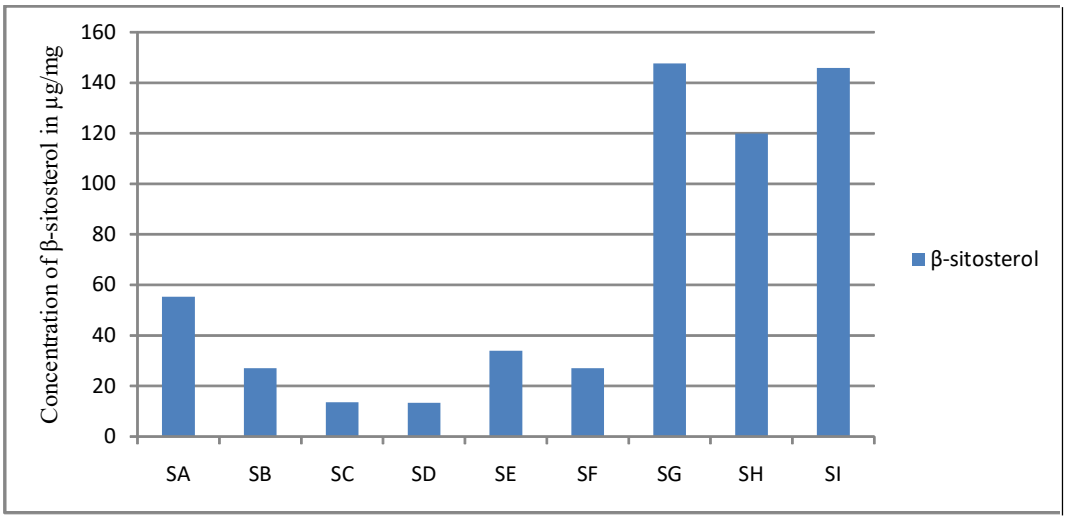

Fig. 7: Concentration of β-sitosterol in µg/mL of *Andrographis paniculata* extracts.
SA= Leaf extract in Petroleum ether, SB= Stem extract in Petroleum ether, SC= Root extract in petroleum ether, SD= Leaf extract in chloroform, SE= Stem extract in chloroform, SF=Root extract in chloroform, SG= Leaf extract in methanol, SH= Stem extract in methanol, SI= Root extract in methanol, RA= Reference standard β-sitosterol.

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