Research Journal of

Medicinal Plant

ISSN 1819-3455

Academic Journals Inc.

www.academicjournals.com

Research Journal of Medicinal Plant 8 (3): 112-120, 2014
ISSN 1819-3455 / DOI: 10.3923/rjmp.2014.112.120
© 2014 Academic Journals Inc.

In vitro and *in vivo* GC-MS Profile and Antimicrobial Activity of Phytosterols of *Datura stramonium*

Richa Bhardwaj, Ankita Yadav, Pallavi Sharma and Ram Avatar Sharma
Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India

Corresponding Author: Ram Avatar Sharma, Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India Tel: +9783928587

ABSTRACT

The phytosterol composition in leaves and callus were studied using GC-MS. Phytosterols 3-phenyl lactic acid, β-sitosterol, cholesterol like compound, brassisterol, stigmasterol, fucosterol, 5-ergostenol, stigmasta 5.22-dien-3-ol, cholestane and campesterol were identified in leaf and callus but phytosterols friedelin, canophyllal and daturaolone were identified only in callus cultures. In present investigation beta-sitosterol concentration (10.31%) was found to be highest as compared to other phytosterols. In callus culture cholestane concentration (20.92%) was maximum. Canophyllal, friedelin and daturalone was not present in leaves but was present in callus. This is the first report on variation of phytosterols contents *in vitro* and *in vivo* condition in *Datura stramonium* identified by GC-MS. In future isolation of individual phytosterols and subjecting them to biological activity will definitely prove fruitful results in designing a novel drug. Antimicrobial activity of phytosterols crude extracts of plants has also shown to have maximum activity aginst *P. aeruginosa* (IZ 22.2±0.59 mm) and maximum activity aginst *A. niger* (14.5±0.25 mm).

Key words: *D. stramonium* , phytosterols , GC-MS, sitosterol, cholestane, canophyllal, friedelin, daturalone

INTRODUCTION

India has a great wealth of various naturally occurring plant drugs which have great potential pharmacological activities. *Datura stramonium* (*D. stramonium*) is one of the widely well known folklore medicinal herbs. Weed by nature, *D. stramonium*is a plant with both poisonous and medicinal properties and has been proven to have great pharmacological potential with a great utility and usage in folklore medicine. *D. stromonium* has been scientifically proven to contain alkaloids, tannins, carbohydrates and proteins. This plant has contributed various pharmacological actions in the scientific field of Indian systems of medicines like analgesic and antiasthmatic activities. The genus *Datura* is represented by many species, out of which *D. stramonium* is well known for its medicinal value. Phytosterols and phytostanols are a large group of compounds that are found exclusively in plants. They are structurally related to cholesterol but differ from cholesterol in the structure of the side chain. They consist of a steroid skeleton with a hydroxyl group attached to the C-3 atom of the A-ring and an aliphatic side chain attached to the C-17 atom of the D-ring. Sterols have a double bond, typically between C-5 and C-6 of the sterol moiety, whereas this bond is saturated in phytostanols. Phytosterols have always been a fascinating subject

of study because of their diversified physiological and pharmacological effects. Phytosterols are lipophilic, naturally occurring compounds. Over 40 natural plant sterols have been identified so far. The major phytosterols reported from plants are β-sitosterol, campesterol, stigmasterol and avenasterol. The predominant phytosterol is β-sitosterol and show hyperlipoproteinemic activity (Schlierf *et al.*, 1978). According to a recent market research phytosterols are the most heart health targeted and benefited from approved health claims in many markets (Hovenkamp *et al.*, 2008). Phytosterols have been used as blood cholesterol lowering agents for the last half century (Ostlund *et al.*, 2003; Kritchevsky and Chen, 2005) and protective effects on development of coronary heart disease (Fassbender *et al.*, 2008). Phytosterols have effects in the treatment of benign prostatic hyperplasia, rheumatoid arthritis, allergies and stress related illness and inhibit the development of colon cancer (Oomah and Mazza, 1999; Bradford and Awad, 2007). Aim of the present investigation was to identify and quantify the phytosterol present in leaf and in callus culture using GCMS technique.

MATERIALS AND METHODS

Plant material: The plant material was collected from Shyam nagar extension, Sodala, Jaipur. A voucher specimen no.RUBL15656 was deposited at the Herbarium of the Department of Botany, University of Rajasthan, Jaipur.

For call using, nodal segments were cultured on MS medium supplemented with 1 mg L^{-1} 2-4D+1.5 mg L^{-1} BAP.

Extraction procedure: Dried and powdered of *D. stramonium* and callus tissue were defatted individually with pet. ether (60-80°C) for 24 h on a water bath. Later, each defatted material was dried and re-extracted, with benzene for 24 h. Subsequently, the benzene extract was dried in vacuo, weighed and then analysed for chromatographic and GCMS analysis. For GCMS analysis, the sample was analysed in Jawahar Lal Nehru University, Advance Instrument Centre, New Delhi.

Chromatographic analysis: For this, silica Gel (G) coated plates were used, on which the test extracts reconstituted in benzene were applied along with reference sterols as marked and developed in a solvent system of benzene-ethyl acetate (85:75) (Heble *et al.*, 1968) and benzene-ethyl acetate (3:2) (Kaul and Staba, 1968) were used as this solvent system gave better separation of the compounds. Such chromatograms were air-dried, visualized under UV light and the fluorescence or the colours were noted. Later, each was sprayed with 50% H_2SO_4 (Bennett and Heftmann, 1962) or anisaldehyde reagent (prepared by mixing minimum 0.5 mL anisaldehyde + 1 mL H_2SO_4 + 50 mL glacial acetic acid; (Heftmann, 1965) separately and heated to 100°C for 5-10 min until characteristic colours developed. The reaction time required for initial appearance of the color in day light and after heating for 10 min was recorded (Table 1) and to locate the spots in unsprayed developed chromatograms on exposure to I_2 vapours also proved useful.

GC-MS conditions: GCMS-QP 2010 Plus was used for identification and quantification of alkaloids, using MS libraries previously compiled from purchased standards. For the acquisition of an electron ionization mass spectrum, an ion source temperature of 250°C was used. The GC was equipped with a SE-30 capillary column a split injection piece (270°C) and direct GC-MS coupling

(280°C). Helium (1.2 mL min^{-1}) was used as the carrier gas with a split ratio of 1:10. The oven temperature program for analyzing the extracts utilized an initial oven temperature of 100°C, maintained for 2 min, followed by a steady climb to 200°C at a rate of 7°C min^{-1} allowed to increase to 190°C at a rate of 30°C min^{-1}. This oven temperature was again maintained at 190°C for 5 min and then allowed to increase to 300°C at a rate of 7°C min^{-1}. This oven temperature was maintained for 2 min and finally ramped to 300°C at a rate of 10°C min^{-1} and maintained for a further 22 min. Injection temperature and volume was 250°C and 1 μL, respectively. The total GC running time was about 43.28 min. The MS operating conditions were as follows, Interference temperature of 260°C, Ion source temperature of 250°C, mass scan (m/z)-40-450, solvent cut time 7 min, scan speed 2000 amu/s total MS running time-50.28 min and threshold-1000.

Identification of components: GCMS is a valuable aid for identifying unknown peak as well as for confirming the identification of identified phytoconstituents. Identification of components was based on directs comparison of the retention times and mass spectral data with those for standard compounds and computer matching with the library (Wiley library, NIST data bank, database NIST 98) as well as by comparison of the retention time those reported in the literature (Song *et al*., 2000; Holser *et al*., 2004; Lembcke *et al*., 2005; Phuruengrat and Phaisanserthicol, 2006; Chen *et al*., 2007; Delazar *et al*., 2010; Winkler-Moser, 2011).

Antibacterial and antifungal activity

Sources of test organisms

Bacteria: The bacterial strains *Escherichia coli* MTCC 1652, *Staphylococcus aureus* MTCC 3160 (Gram+ve), *Pseudomonas aeruginosa* MTCC 847 (Gram+ve) are procured from the microbial type culture collection (Institute of Microbial Technology, Chandigarh, India).

Fungi: The fungal strains *Aspergillus flavus* MTCC 2456, *Aspergillus niger* MTCC 282, *Fusarium culmorum* MTCC 349 and *Rhizopus stolonifer* MTCC 2591 are procured from the microbial type culture collection (Institute of Microbial Technology, Chandigarh, India).

Culture of test microbes: For the cultivation of bacteria, Nutrient Broth Medium (NBM) was prepared using 8% nutrient broth (Difco) in distilled water and agar-agar and sterilized at 15 Ibs psi for 25-30 min. Agar test plates were prepared by pouring ~15 mL of NBM into the petri dishes (10 mm) under aseptic conditions. A peptone saline solution was prepared (by mixing 3.56 g KH_2PO_4 + 7.23 g NaH_2PO_4 + 4.30 g, NaCl + 1 g peptone in 1000 mL of distilled water, followed by autoclaving) and the bacterial cultures were maintained on this medium by regular sub-culturing and incubation at 37°C for 24 h. However, for the cultivation of fungi, potato dextrose agar medium was prepared by mixing 100 mL potato infusion + 20 g agar + 20 g glucose, followed by autoclaving) and the test fungi were incubated at 27°C for 48 h and the cultures were maintained on same medium by regular subculturings.

To prepare the test plates, in both bacteria and fungi, 10 to 15 mL of the respective medium was poured into the petri dishes and used for screening.

For assessing the bactericidal efficacy, a fresh suspension bacteria was prepared in saline solution from a freshly grown agar slant while for fungicidal efficacy, a uniform spread of the test fungi was made using sterile swab.

RESULTS AND DISCUSSION

Four spots (A-D; R_f 0.75, 0.68, 0.73, 0.23) were observed uniform in the extracts of *D. stramonium in vitro* and *in vivo* and out of these, four major spots, which were the same in their position (R_f 0.75, grey pink; D-R_f 0.68, pink; E-R_f 0.73 pink; F-R_f 0.23, pink, sprayed with 50% H_2SO_4 coinciding to **β**-sitosterol, stigmasterol, lanosterol, campesterol respectively were observed and identified. These spots also gave colour reactions comparable to the markers with anisaldehyde reagent. Three such replicates in each case were run and their average R_f values were calculated.

Four phytosterols compounds were identified on the basis of chromatography and colour (Table 1). Phytosterols chromatogram revealed the presence of four spots which were identified as **β**-Sitosterol (Rf = 0.75), Stigmasterol (Rf = 0.68), Lanosterol (Rf = 0.73), Campesterol reaction (Rf = 0.23) *in vitro* and *in vivo* (Table 1).

GC-MS analysis revealed the presence of phytosterols in callus and leaves. The GC-MS chromatograms of phytosterols of leaves and callus are shown in Fig. 1 and 2. The leaves and callus extracts appeared to have 10 and 13 phytosterols respectively as shown in Table 2. Out of 13 sterols identified in *D. stramonium*, three of them i.e., **β**-sitosterol, stigmasterol and campesterol are the most common plant sterols (Weihrauch and Gardner, 1978). Sitosterol is present more frequently in plants than any other phytosterols (Pollak, 1953). In present investigation sitosterol

Table 1: Chromatographic data of phytosterol (*in vitro* and *in vivo)* and colour reaction of phytosterol present in *D. stramonium* (*in vivo*)

Phytosterols				Colour after heating 50% sulphuric acid in day light			
Compound	*In vitro*	*In vivo*	R_f value×100 in solvent system	Initial	Final	UV	Anisaldehyde reagent
β-sitosterol	Identified	Identified	75	GY-PK	PU-BN	DL-RD	PU
Stigmasterol	Identified	Identified	68	PK	GY	DK-BU	PU
Lanosterol	Identified	Identified	73	PK	PK-BN	DK-BN	PK
Campesterol	Identified	Identified	23	PK	GY	DL-BN	BU

Solvent system, Benzene: Ethyl acetate (3:2), BU: Blue, PK: Pink, DK: Dark, PU: Purple, DL: Dull, RD: Red, BN: Brown, GY: Grey

Table 2: Retention time, molecular weight and percentage area by setting the total peak area to 100% of phytosterols identified by GC-MS of *D. stramonium* in leaf and callus

Compounds	Leaf (%)*	Callus (%)*	R.T (min)	Mol. wt.	Mol. formula	References
3-Phenyl lactic acid	Present	Present		166	$C_9H_{10}O_3$	
Sitosterol	10.31	1.56	22.834	414	$C_{29}H_{50}O$	Phuruengrat and Phaisansuthichol (2006)
Cholesterol like compound	1.85	1.24	20.662	386	$C_{27}H_{46}O$	Phuruengrat and Phaisansuthichol (2006)
Brassisterol	1.03	2.89	24.177	398	$C_{28}H_{46}O$	Phuruengrat and Phaisansuthichol (2006)
Stigma sterol	1.51	0.32	21.868	412	$C_{29}H_{48}O$	Phuruengrat and Phaisansuthichol (2006)
Fucosterol	0.86	0.54	24.233	412	$C_{29}H_{48}O$	Delazar *et al*. (2010)
5-ergostenol	Present	Present		400	$C_{28}H_{48}O$	Delazar *et al*. (2010)
Friedelin	Absent	0.63	24.974	426	$C_{30}H_{50}O$	Phuruengrat and Phaisansuthichol (2006)
Stigmasta-5, 22, dien-3-ol	4.32	0.85	22.541	412	$C_{29}H_{48}O$	Phuruengrat and Phaisansuthichol (2006)
Canophyllal	Absent	Present		440	$C_{30}H_{48}O_2$	
Cholestane	1	20.92	10.152	372	$C_{27}H_{48}$	Phuruengrat and Phaisansuthichol (2006)
Campesterol	13.85	0.47	19.748	400	$C_{28}H_{48}O$	
Daturaolone	Absent	Present		440	$C_{30}H_{48}O_2$	

*Data given in the table is not a true quantification

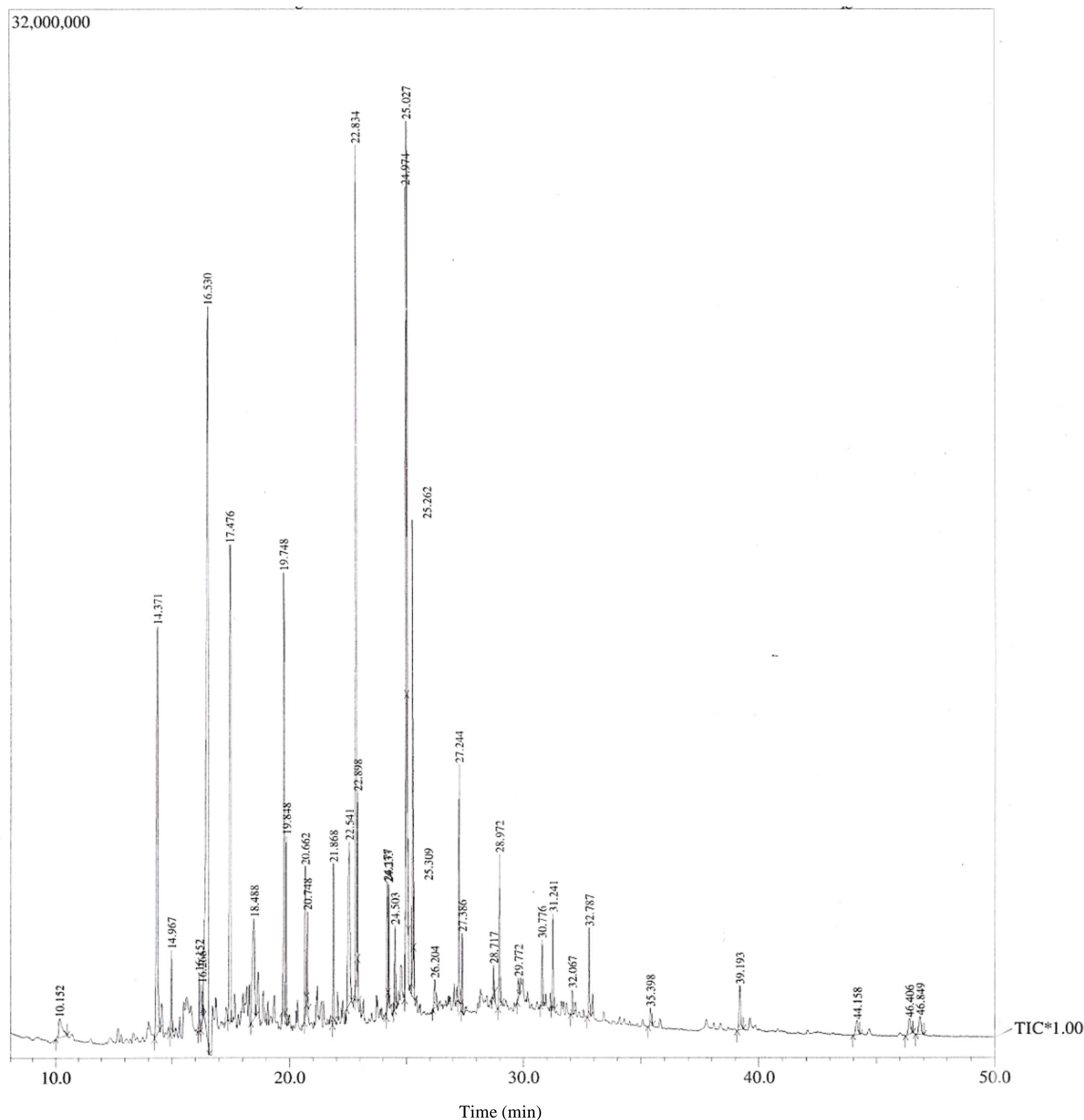

Fig. 1: GC-MS chromatogram of phytosterols present in *D. stramonium* (leaf)

concentration (10.31%) was found to be highest as compared to other phytosterols. In callus culture cholestane concentration (20.92%) was maximum. Canophyllal, friedelin and daturalone was not present in leaves but was present in callus. Phytosterol like friedelin, daturaolone and canophyllal were not present in leaves but present in callus. For the first time large number of phytosterols were reported in callus as compared to *D. stramonium* leaves. This showed that conditions provided during tissue culture studies were favourable for maximum accumulation of phytosterols. According to the non adaptive hypothesis, the distribution of secondary metabolites within organs may be roughly equivalent to the distribution of the primary metabolic pathways responsible for the creation of the secondary metabolite (as a byproduct) and thus they do not necessarily have an adaptive function in each organ (Eriksson and Ehrlen, 1998).

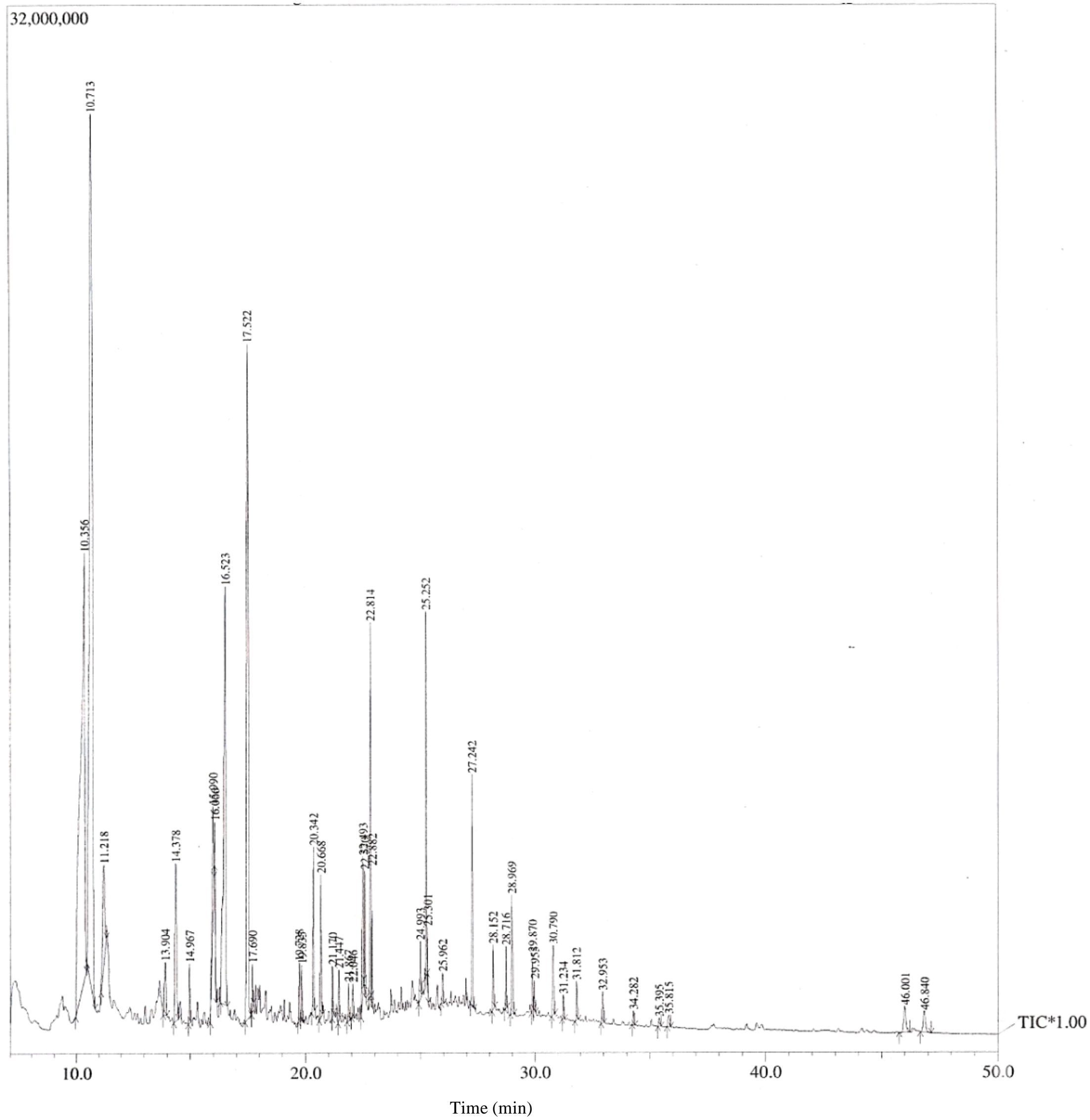

Fig. 2: GC-MS chromatogram of phytosterols present in *D. stramonium* (callus)

Composition was estimated on the basic of calculation of the GC peak areas in percent by setting the total peak areas to 100%. Table 2 demonstrates compositions of phytosterols its molecular formula, molecular weight, retention time and percentage area.

The Table 3 shows that the phytosterols were also effective against all test bacteria and fungi and showed highest activity against *P. aeruginosa* (IZ = 22.20±0.59 mm) of all bacterial strains and maximum activity was recorded against *A. niger* (IZ = 14.5±0.25 mm) amongst all fungal strains tested. Table 4 shows the MIC (μg μL^{-1}). MIC indicates the potential of each extract to inhibit the microbial growth at lowest concentration for isolated phytosterols against test microorganisms recorded in mg $disc^{-1}$ of the diametrical sections of the respective zones of inhibition for each metabolite. Phytosterols against tested bacterial strains show highest MIC value aginst *S. aureus*

Table 3: Bactericidal and fungicidal efficacy of phytosterols of crude extracts of *D. stramonium* (leaf)

Microorganisms	Phytosterols	Standard
Bacteria		
S. aureus	IZ	19.5±0.52
MTCC 3160	AI	0.937
E. coli	IZ	13.2±0.31
MTCC 1652	AI	0.68
P. aeruginosa	IZ	22.2±0.59
MTCC 647	AI	0.932
Fungi		
R. stolonifer	IZ	10.40±0.56
MTCC 2591	AI	0.684
A. niger	IZ	14.5±0.25
MTCC 282	AI	1.169
F. culmorum	IZ	10.4±0.21
MTCC 349	AI	0.584
A. flavus	IZ	10.3±0.63
MTCC 2456	AI	0.5

IZ: Inhibition zone (mm) including the diameter of disc (6 mm), Activity index = Inhibition area of the test sample/Inhibition area of the standard, Results are Mean±SD from atleast three experiments, $S.E(\sigma_x) = \frac{\sigma}{\sqrt{n}}$, σ = Standard deviation, n = No. of set

Table 4: MIC of phytosterols crude extracts of *D. stramonium* (leaf)

Organisms	MIC ($\mu g\ \mu L^{-1}$)	Phytosterols	Standard
Bacteria			
S. aureus MTCC 3160	MIC	30.50±0.35	23.5
E. coli MTCC 1652	MIC	44.90±0.50	33.2
P. aeruginosa MTCC 647	MIC	31.10±0.27	20.6
Fungus			
R. stolonifer MTCC 2591	MIC	51.60±0.29	9.3
A. niger MTCC 282	MIC	27.10±0.21	10.0
F. culmorum MTCC 349	MIC	34.90±0.27	30.7
A. flavus MTCC 2456	MIC	44.70±0.39	19.0

MIC: Minimum inhibitory concentration, Results are mean value SD from atleast three experiments, $S.E(\sigma_x) = \frac{\sigma}{\sqrt{n}}$, σ = Standard deviation, n = No. of set

(30.5±0.35) while lowest against *E. coli* (44.90±0.50). While against all tested fungal strains show highest MIC value aginst *A. niger* (27.10±0.21) lowest against *R. stolonifer* (51.60±0.29). antimicrobial activity of the phytosterols extracted from other plant source leaves of *Annona squamosa*, *Adenocalymna alliceum* and *Amaranthus tricolor* yielded n-alkanes, n-alkanols, 16-hentriacontanone and sterols. These were purified, characterized and evaluated for their antibacterial activity against *Staphylococcus aureus*, *Staphylococcus albus* and *Streptocorus viridans* (all gram-positive bacteria) and *Escherichia coli*, *Pseudomonas pvocyanea* and *Klebsiella* (all gram-negative bacteria) (Sharma, 1993). Potential antimicrobial activity of phytosterols in milk have also been investigated (Monu *et al*., 2008). The bacteriostatic experiment of phytosterols in pumpkin seed indicates that it has strong inhibitory effects against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Salmonella* and the growth of bacteria with the concentration of 3.0 mg mL^{-1} can be completely inhibited (Xu *et al*., 2012).

CONCLUSION

The extracts of *D. stramonium in vitro* and *in vivo* showed the presence of phytosterols majorly **β**-sitosterol, stigmasterol, lanosterol, campesterol, respectively. The antimicrobial activities of the

crude compounds also showed to have broad spectrum action against range of bacterial and fungal strains. The ability of these compounds to inhibit the growth of tested microbes has confirmed the effectiveness of *D. stramonium* for the treatment of various human diseases caused due to these pathogenic strains.

REFERENCES

Bennett, R.D. and E. Heftmann, 1962. Thin-layer chromatography of steroidal sapogenins. J. Chromatogr. A, 9: 353-358.

Bradford, P.G. and A.B. Awad, 2007. Phytosterols as anticancer compounds. Mol. Nutr. Food Res., 51: 161-170.

Chen, Q., L. Steinhauer, J. Hammerlindl, W. Keller and J. Zou, 2007. Biosynthesis of phytosterol esters: Identification of a sterol O-acyltransferase in arabidopsis. Plant Physiol., 145: 974-984.

Delazar, A., E. Nazifi, A. Movafeghi, H. Nazemiyeh, S. Hemmati, L. Nahar and S.D. Sarker, 2010. Analysis of phytosterols and free radical scavengers in the bulbs of *Ornithogalum cuspidatum* Bertol. Boletin Latinoamericanodel Caribe De Plantae Medicinales Aromaticas, 9: 87-92.

Eriksson, O. and J. Ehrlen, 1998. Secondary metabolites in fleshy fruits: Are adaptive explanations needed? Am. Naturalist, 152: 905-907.

Fassbender, K., D. Lutjohann, M.G. Dik, M. Bremmer and J. Konig *et al*., 2008. Moderately elevated plant sterol levels are associated with reduced cardiovascular risk-The LASA study. Atherosclerosis, 196: 283-288.

Heble, M.R., S. Narayanaswami and M.S. Chadha, 1968. Diosgenin and ß-sitosterol: Isolation from *Solanum xanthocarpum* tissue cultures. Science, 161: 1145-1145.

Heftmann, E., 1965. Thin-layer chromatography of steroids. Chromatographic Rev., 7: 179-195.

Holser, R.A., G. Bost and M. van Boven, 2004. Phytosterol composition of hybrid *Hibiscus* seed oils. J. Agric. Food Chem., 52: 2546-2548.

Hovenkamp, E., I. Demonty, J. Plat, D. Lutjohann, R.P. Mensink and E.A. Trautwein, 2008. Biological effects of oxidized phytosterols: A review of the current knowledge. Prog. Lipid Res., 47: 37-49.

Kaul, B. and E.J. Staba, 1968. *Dioscorea* tissue cultures. I. Biosynthesis and isolation of diosgenin from *Dioscorea deltoidea* callus and suspension cultures. Lloydia, 31: 171-179.

Kritchevsky, D. and S.C. Chen, 2005. Phytosterols-health benefits and potential concerns: A review. Nutr. Res., 25: 413-428.

Lembcke, J., U. Ceglarek, G.M. Fiedler, S. Baumann, A. Leichtle and J. Thiery, 2005. Rapid quantification of free and esterified phytosterols in human serum using APPI-LC-MS/MS. J. Lipid Res., 46: 21-26.

Monu, E., G. Blank, R. Holley and J. Zawistowski, 2008. Phytosterol effects on milk and yogurt microflora. J. Food Sci., 73: M121-M126.

Oomah, B.D. and G. Mazza, 1999. Health benefits of phytochemicals from selected Canadian crops. Trends Food Sci. Technol., 10: 193-198.

Ostlund Jr., R.E., S.B. Racette and W.F. Stenson, 2003. Inhibition of cholesterol absorption by phytosterol-replete wheat germ compared with phytosterol-depleted wheat germ. Am. J. Clin. Nutr., 77: 1385-1389.

Phuruengrat, A. and S. Phaisansuthichol, 2006. Preliminary study of steroids in *Sericocalyx schomburgkii* (craib) Bremek by GC-MS. Songklanakarin J. Sci. Technol., 28: 39-44.

Pollak, O.J., 1953. Reduction of blood cholesterol in man. Circulation, 7: 702-706.

Schlierf, G., P. Oster, C.C. Heuck, H. Raetzer and B. Schellenberg, 1978. Sitosterol in juvenile type II hyperlipoproteinemia. Atherosclerosis, 30: 245-248.

Sharma, R.K., 1993. Phytosterols: Wide-spectrum antibacterial agents. Bioorganic Chem., 21: 49-60.

Song, Y.S., C. Jin and E.H. Park, 2000. Identification of metabolites of phytosterols in rat feces using GC/MS. Arch. Pharm. Res., 23: 599-604.

Weihrauch, J.L. and J.M. Gardner, 1978. Sterol content of foods of plant origin. J. Am. Diet. Assoc., 73: 39-47.

Winkler-Moser, J., 2011. Gas chromatographic analysis of plant sterols. The AOAC Lipid Library, pp: 1-18. http://lipidlibrary.aocs.org/topics/phytosterols/index.htm.

Xu, Y.Q., L.P. Pang, H.J. Qi, Y. Yang and Y.L. Yang, 2012. Antioxidant activity and antibacterial effect of phytosterol from pumpkin seeds. Acad. Periodical Farm Prod. Process., 8: 14-16, 26.