



DETECTION OF BIOACTIVE COMPOUNDS BY USING HPTLC FROM WRIGHTIATINCTORIA (R.BR.) AND AMORPHOPHALLUS CAMPANULATUS (ROXB.)

BHAGWAN M. WAGHMARE AND RAHUL K. DHABALE

Botany Research Centre, Department of Botany, Maharashtra Mahavidyalaya, Nilanga Dist. Latur (M.S.) India.

ABSTRACT

A plant always has been used as sources of medicine and play a vital role in health care system not only in India also abroad. The *W. tinctoria* (R.Br) and *A. campanulatus* (Roxb.) highly medicinal and has been reported the presence of bioactive compounds. This paper concentrates on an important of ethno-medico point of view and due to their phytochemical activities of fruit of *W. tinctoria* (R.Br) and corm of *A. campanulatus* (Roxb.). It has been regarded to the presence of Bioactive compounds which were determined by using HPTLC techniques for detection with methanol, petroleum ether and ethyl acetate fruit extracts of *W. tinctoria* (R.Br) and corm of *A. campanulatus* (Roxb.). The results are notable, different separation pattern was observed. The Bioactive compound separated at Rf values 0.37 (± 0.02) and 0.51 (± 0.02) were found with characteristic colour reaction by anisaldehyde sulphuric acid reaction. The isolated compounds are determined and identified as beta-sitosterol, lupeol from fruit and corm extracts of *W. tinctoria* (R.Br) and *A. campanulatus* (Roxb.) respectively.

KEYWORDS: *W. tinctoria* (R.Br), *A. campanulatus* (Roxb.), Bioactive compounds, beta-sitosterol, lupeol.



BHAGWAN M. WAGHMARE

Botany Research Centre, Department of Botany, Maharashtra Mahavidyalaya, Nilanga. Dist. Latur (M.S.) India.

*Corresponding author

Received on: 27.10.2016

Revised and Accepted on :

DOI: <http://dx.doi.org/10.22376/ijpbs.2017.8.2.p56-64>

INTRODUCTION

W. tinctoria (R.Br) and *A. campanulatus* (Roxb.) are medicinal plants which have phytochemicals⁹ and are ethno-medico-botanical significance¹⁴. The phytochemicals like flavonoids, alkaloids, steroids, phenol and tannins and are multi-potentially used for the treatment of various diseases¹⁵. The extracts of fruit and corm extracts of *W. tinctoria* (R.Br.) and *A. campanulatus* (Roxb.) respectively, play an important role in antimicrobial activity¹, hepatoprotective activity^{15,19}, antitumor activity⁹. The study emphasizes on isolation of phytochemicals which are biologically active and multi-dimensional in their significance. *A. campanulatus* (Roxb.) belongs to the family *Araceae* as a corm or tuber producing plant largely cultivated through the Indian plain parts and commonly known as Suran⁴. It is a tuberous, stout, indigenous herb having 1.5-1.5 m in length. The corm of *A. campanulatus* (Roxb.) contains an active digestive enzyme amylase, betulinic acid, β-sitosterol, stigmasterol, β-sitosterol palmitate, lupeol, triacontane, amino acid, carbohydrates, saponin, thiamine, riboflavin, niacin and carotene and is beneficial for the treatment of common ailments¹². *W. tinctoria* (R.Br)

belongs to the family *Apocynaceae*. It is well known by its common name as Indrajav. It is very important traditionally for healing of different ailments. *W. tinctoria* (R.Br) is considered as a therapeutically very effective jaundice plant in the indigenous system of medicine. Fruit extracts of *W. tinctoria* (R.Br.) have qualitative and quantitative presence of various bioactive compounds which have been reported in literature. The present work is on the estimation of bioactive compounds from the corm of *A. campanulatus* (Roxb.) and fruit of *W. tinctoria* (R.Br) and recorded the results.

MATERIALS AND METHOD

High performance thin layer chromatography (HPTLC)

Solvent systems and visualizing reagents were finalized from a depth of thin layer chromatography (TLC) study and the results were confirmed by subjecting the extracts to high performance thin layer chromatography (HPTLC) analysis using the finalized set of solvent systems and visualizing reagents as follows.

Wrightia tinctoria R.Br.- fruit

| | | |
|---------------------|---|--|
| Plate | : | Silica gel 60 GF ₂₅₄ for HPTLC |
| Solvent system | : | Acetone : Toluene : Methanol (0.9 : 8.9 : 0.2, v/v/v) |
| Sample preparation | : | 100 mg of extract was dissolved in solvent and filtered. 10 µl of filtrate was applied on HPTLC plate as a band of 6 mm width. |
| Detection | : | UV light at 254 nm, 366 nm and daylight |
| Visualizing reagent | : | Iodine vapours (Reagent 1), anisaldehyde-sulphuric acid reagent (Reagent 2). |

Amorphophallus campanulatus corm

| | | |
|-----------------------|---|---|
| Plate | : | Silica gel 60 GF ₂₅₄ for HPTLC |
| Solvent system | : | Acetone : Toluene : Methanol (0.9: 8.9: 0.2, v/v/v) |
| Sample preparation | : | 100 mg of extracts was dissolved in solvent and filtered. 10 µl of filtrate was applied on HPTLC plate as a band of 6 mm width. |
| Detection daylight | : | UV light at 254 nm, 366 nm and |
| Visualizing reagent | : | Iodine vapours (Reagent 1), Anisaldehyde - Sulphuric acid reagent (Reagent 2) |

High performance thin layer chromatography (HPTLC) analysis

The High performance thin layer chromatography (HPTLC) analysis was carried out using "CAMAG® Linomat V" sample applicator, CAMAG® developing chambers (20 x 10 cm and 10 x 10 cm), "CAMAG® TLC 3" densitometric scanner and "CAMAG® WinCATs" software (CAMAG, Switzerland, Version 1.2.3) on pre-coated HPTLC plates (Merck KGaA, Germany).

HPTLC Fingerprinting

Considering the results obtained from preliminary phytochemical tests, antimicrobial, analgesic and anti-inflammatory activities of the extracts, further investigated for High performance thin layer chromatography (HPTLC). All extracts of *W. tinctoria* (R.Br) and *A. campanulatus* (Roxb.) were further selected for high performance thin layer chromatography (HPTLC) fingerprinting. Lupeol and β-sitosterol were used as standard triterpenoids.

RESULTS

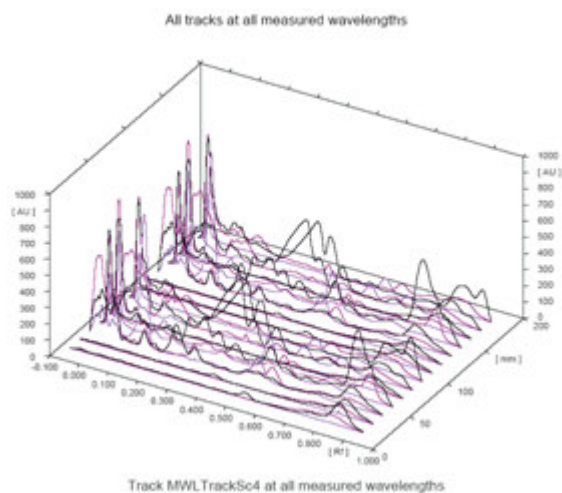


Figure 1
3-D view of HPTLC Plate scanned at multi-wavelength (200 nm – 400 nm) for Standard drugs (Lupeol and Beta-Sitosterol) and test drugs (Wrightia tinctoria R.Br. and Armophophallus campanulatus Roxb. extracts).

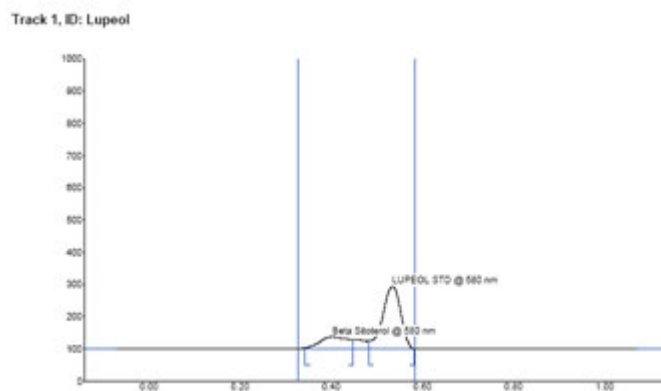


Figure 2
HPTLC spectrum of Lupeol scanned 580 nm

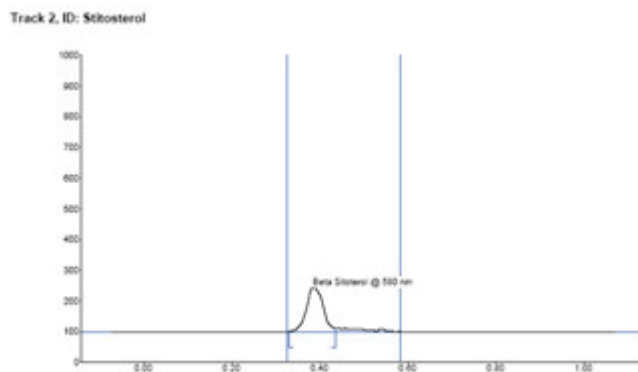


Figure 3
HPTLC spectrum of Beta-Sitosterol scanned 580 nm

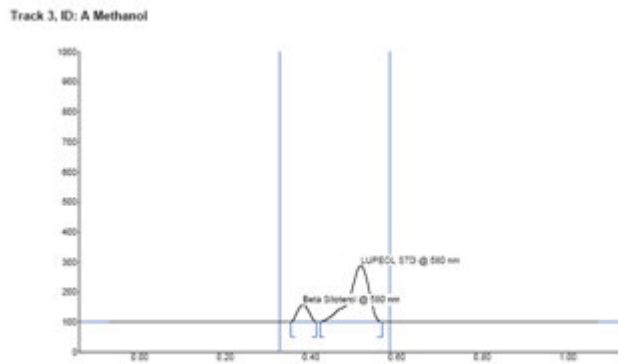


Figure 4
HPTLC spectrum of Methanol extract of *Wrightia tinctoria* R.Br. scanned 580 nm

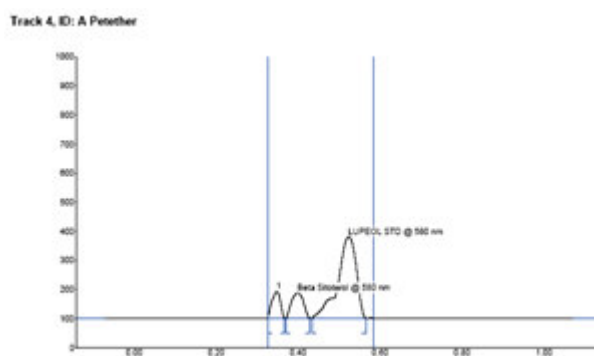


Figure 5
HPTLC spectrum of Pet ether extract of *Wrightia tinctoria* R.Br. scanned 580 nm

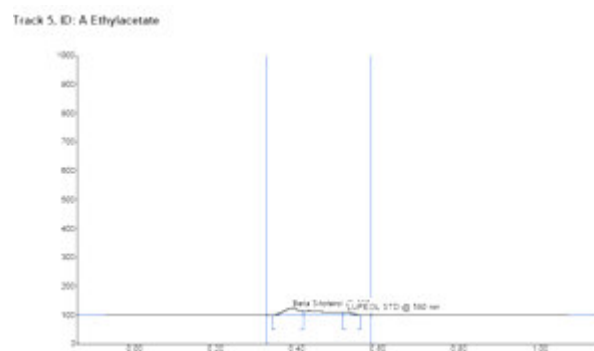


Figure 6
HPTLC spectrum of Ethyl acetate extract of *Wrightia tinctoria* R.Br. scanned 580 nm

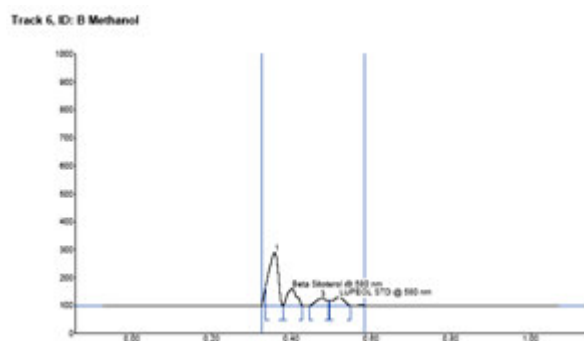


Figure 7
HPTLC spectrum of Methanol extract of *Amorphophallus campanulatus* Roxb. scanned 580 nm

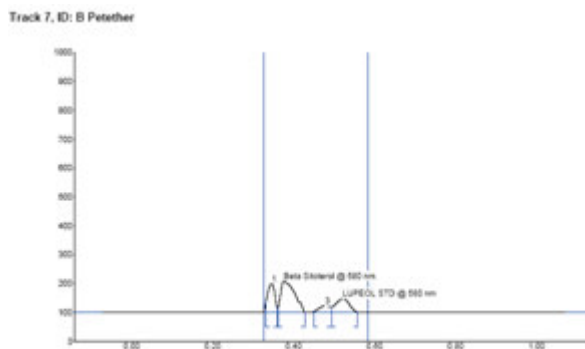


Figure 8
HPTLC spectrum of Pet Ether extract of *Amorphophallus campanulatus* Roxb. scanned 580 nm

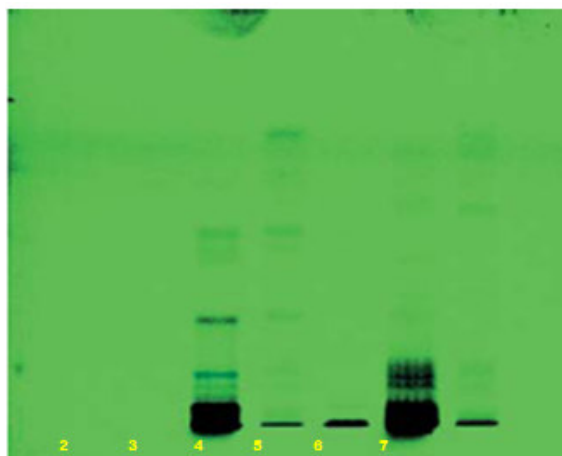


Figure 9
HPTLC plate for *Wrightia tinctoria* R.Br. and *Amorphophallus campanulatus* Roxb. extracts scanned at 254 nm before derivatization.

1 = Standard Drug, Luepol, 2 = Standard Drug, Beta-Sitosterol, 3 = Methanol extract of *Wrightia tinctoria* R.Br.
 4 = Pet. Ether extract of *Wrightia tinctoria* R.Br., 5 = Ethyl Acetate extract of *Wrightia tinctoria* R.Br.
 6 = Methanol extract of *Amorphophallus campanulatus* Roxb., 7 = Pet Ether extract of *Amorphophallus campanulatus* Roxb.

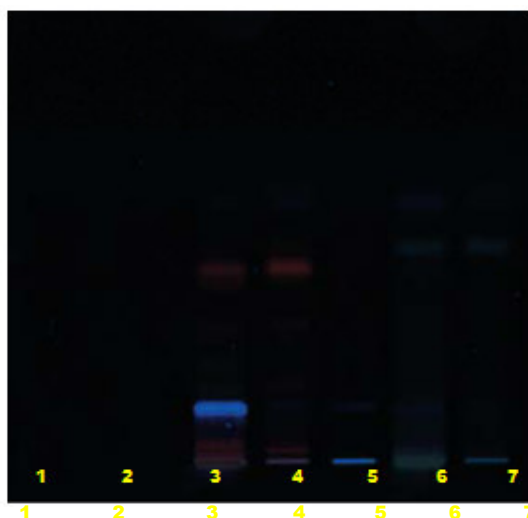


Figure 10
HPTLC plate for *Wrightia tinctoria* R.Br. and *Amorphophallus campanulatus* Roxb. extracts scanned at 366 nm before derivatization.

1 = Standard Drug, Luepol, 2 = Standard Drug, Beta-Sitosterol, 3 = Methanol extract of *Wrightia tinctoria* R.Br., 4 = Pet. Ether extract of *Wrightia tinctoria* R.Br., 5 = Ethyl Acetate extract of *Wrightia tinctoria* R.Br., 6 = Methanol extract of *Amorphophallus campanulatus* Roxb.
 7 = Pet Ether extract of *Amorphophallus campanulatus* Roxb.

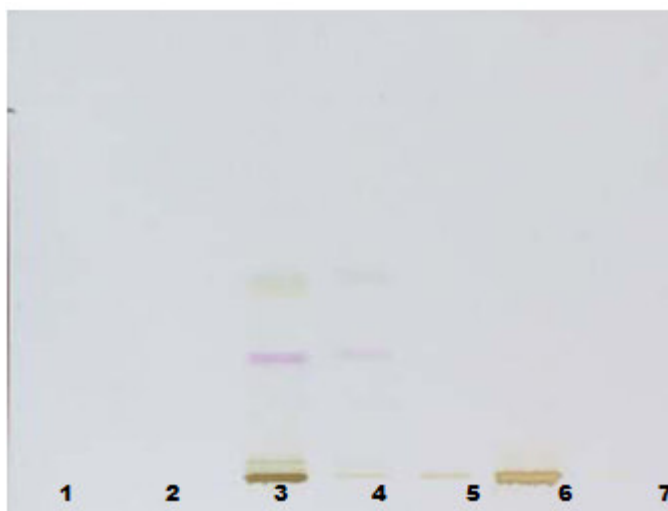


Figure 11
HPTLC plate for *Wrightia tinctoria* R.Br. and *Amorphophallus campanulatus* Roxb. extracts scanned at 400 nm (in daylight) before derivatization.

1 = Standard Drug, Lupeol, 2 = Standard Drug, Beta-Sitosterol, 3 = Methanol extract of *Wrightia tinctoria* R.Br., 4 = Pet. Ether extract of *Wrightia tinctoria* R.Br., 5 = Ethyl Acetate extract of *Wrightia tinctoria* R.Br., 6 = Methanol extract of *Amorphophallus campanulatus* Roxb, 7 = Pet Ether extract of *Amorphophallus campanulatus* Roxb.

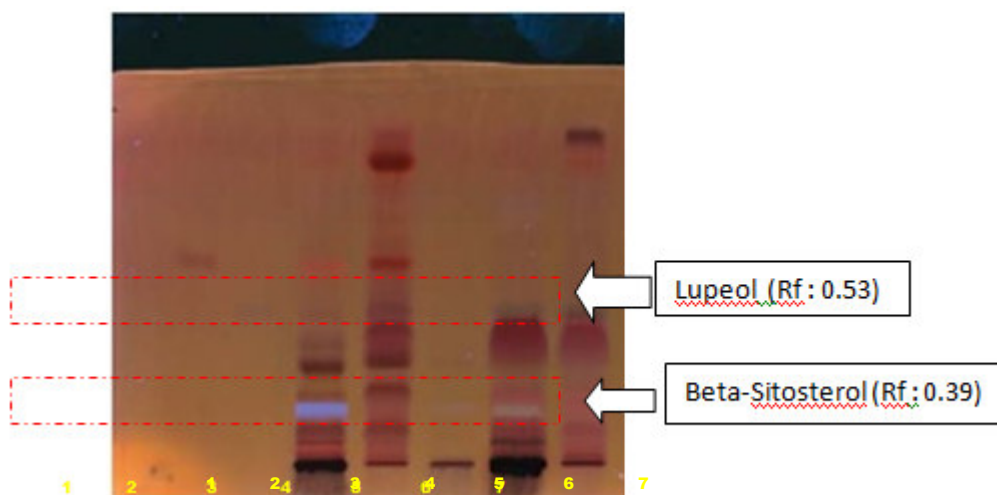
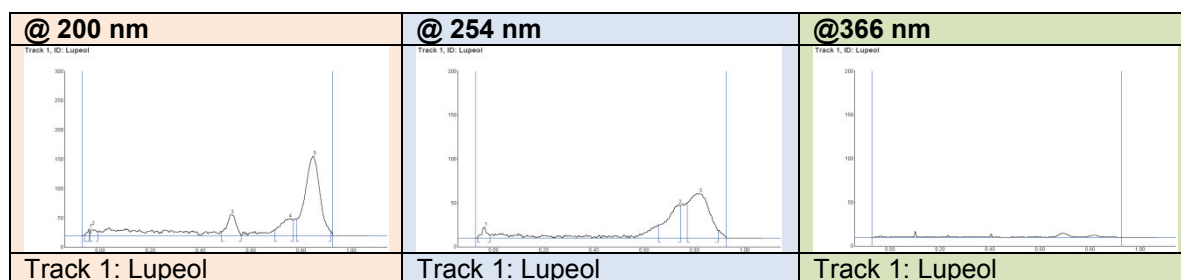
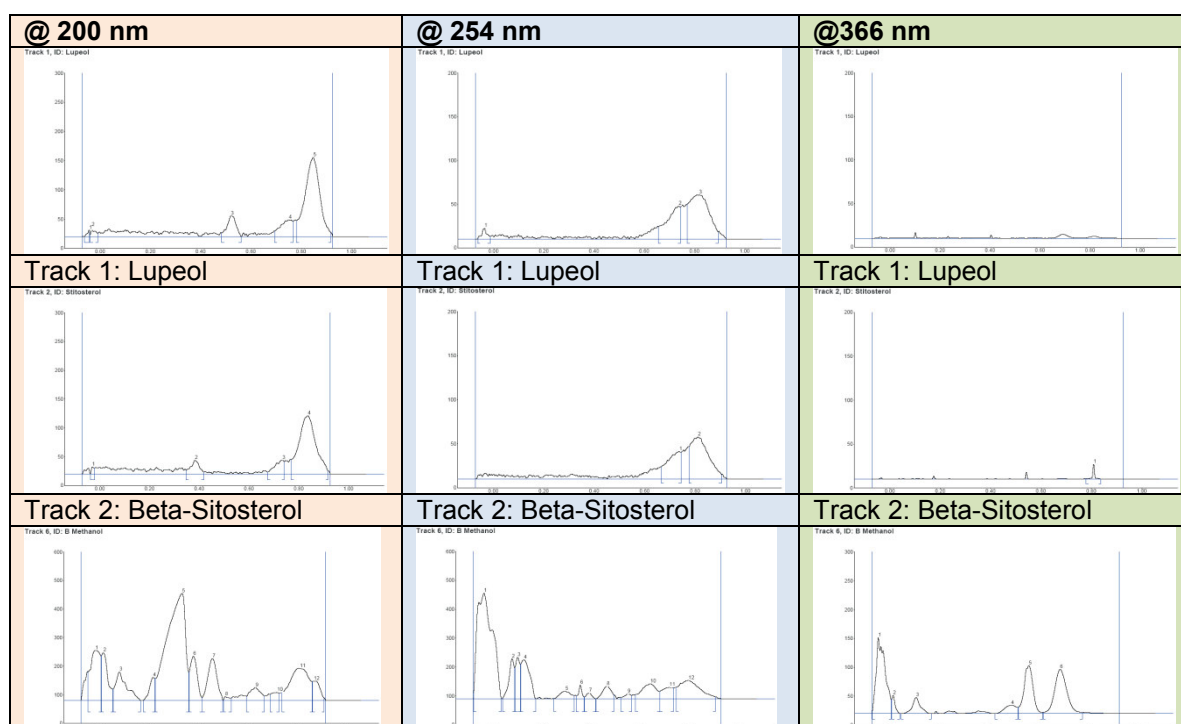
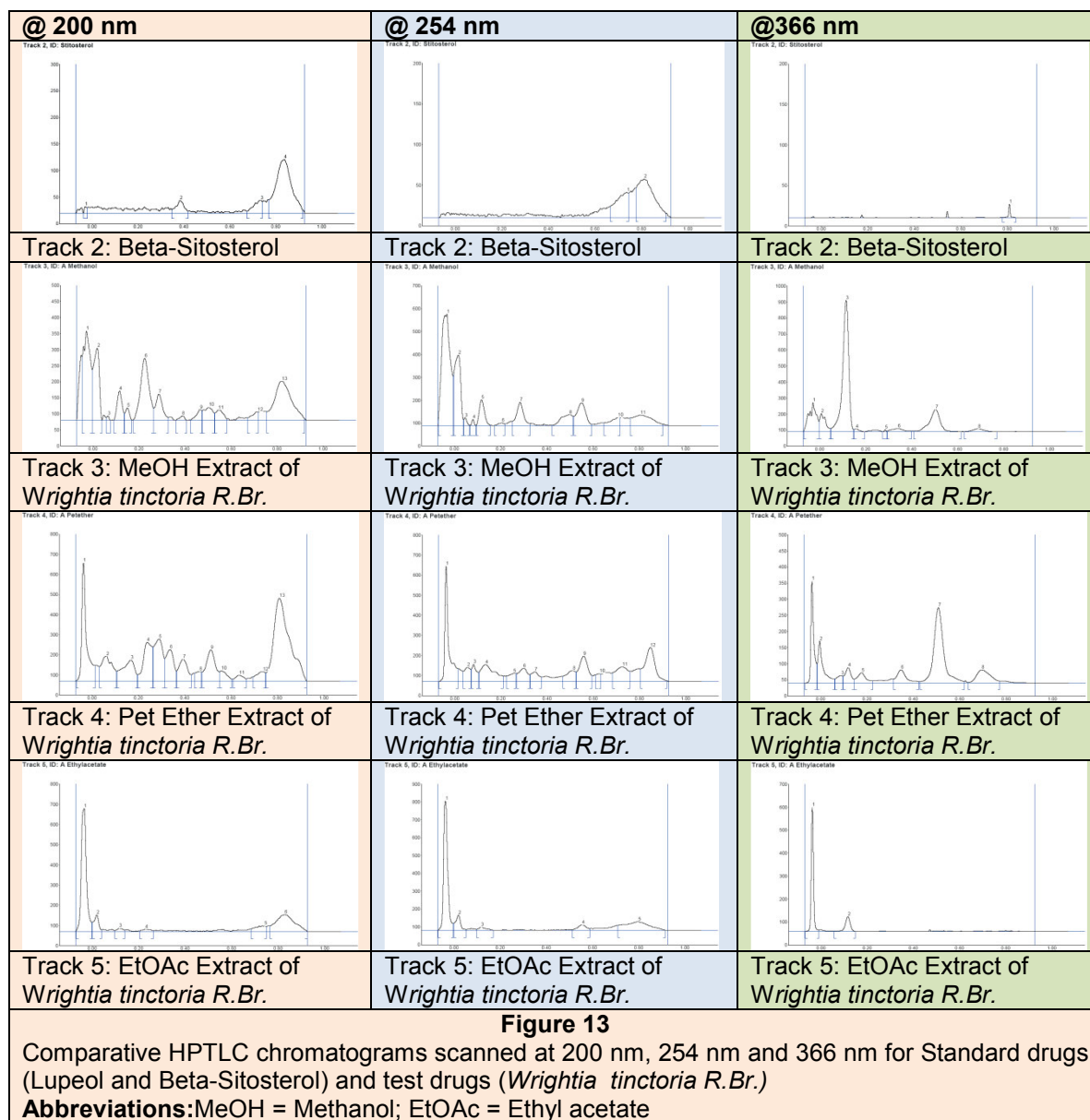


Figure 12
Photo of HPTLC plate for *W. tinctoria* and *Amorphophallus campanulatus* Roxb. extracts scanned at 580 nm after derivatization with Anisaldehyde-sulphuric acid reagent.

[Note: Compounds separated at Rf value 0.39 (± 0.02) and 0.53 (± 0.02) were identified as beta-Sitosterol and Lupeol, respectively, by comparison with standard drugs used (Track 1 and 2, respectively).]

1 = Standard Drug, Lupeol, 2 = Standard Drug, Beta-Sitosterol, 3 = Methanol extract of *Wrightia tinctoria* R.Br., 4 = Pet. Ether extract of *Wrightia tinctoria* R.Br., 5 = Ethyl Acetate extract of *Wrightia tinctoria* R.Br., 6 = Methanol extract of *Amorphophallus campanulatus* Roxb, 7 = Pet Ether extract of *Amorphophallus campanulatus* Roxb.





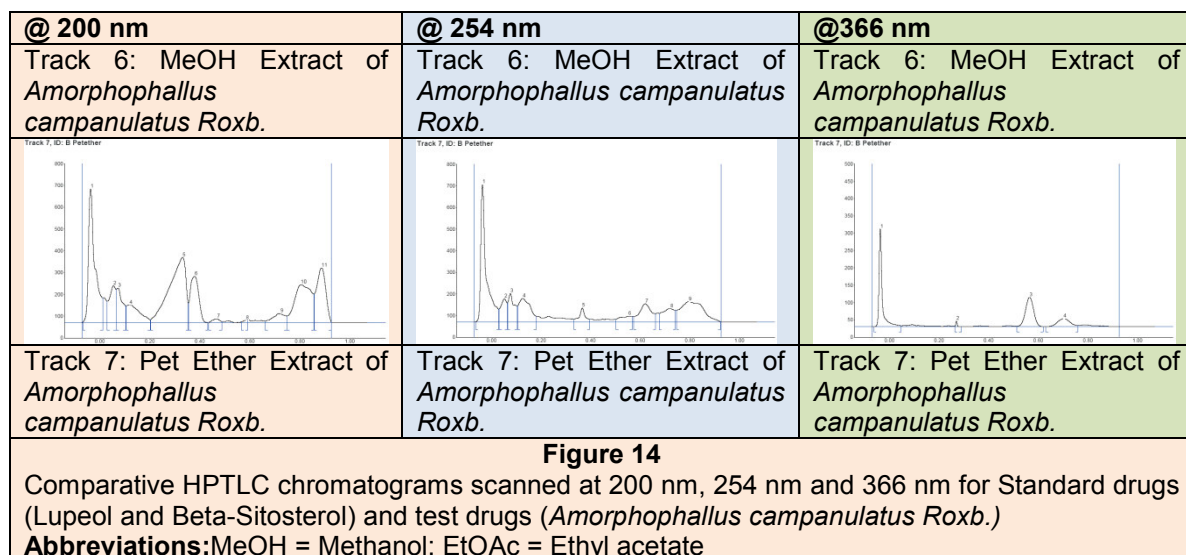


Table 2
Table of high performance thin layer chromatography (HPTLC) chromatograms scanned at 200 nm, 254 nm and 366 nm before derivatization

| Track | Peak | Initial Rf | Initial height | Max Rf | Max height | Max % | Final Rf | Final height | Area | Area % |
|----------------------------------|------|------------|----------------|-------------|------------|-------|----------|--------------|--------|--------|
| Peak data table at 200 nm | | | | | | | | | | |
| 1 | 3 | 0.48 | 6.2 | 0.52 | 35.5 | 15.66 | 0.56 | 0.2 | 1029.2 | 11.57 |
| 2 | 2 | 0.35 | 6.4 | 0.38 | 23.8 | 14.80 | 0.42 | 2.9 | 657.5 | 9.30 |
| 3 | 8 | 0.36 | 0.2 | 0.39 | 13.7 | 1.15 | 0.41 | 0.4 | 232.5 | 0.71 |
| 3 | 11 | 0.53 | 23.0 | 0.55 | 32.1 | 2.70 | 0.58 | 4.0 | 862.2 | 2.64 |
| 4 | 7 | 0.37 | 50.4 | 0.39 | 108.8 | 4.89 | 0.43 | 31.4 | 3336.3 | 4.68 |
| 4 | 9 | 0.48 | 44.9 | 0.51 | 155.2 | 6.98 | 0.55 | 46.1 | 4968.7 | 6.96 |
| 6 | 6 | 0.37 | 100.3 | 0.39 | 155.2 | 10.58 | 0.42 | 10.4 | 3639.9 | 6.47 |
| 6 | 8 | 0.51 | 10.3 | 0.52 | 13.7 | 0.93 | 0.54 | 7.8 | 214.7 | 0.38 |
| 7 | 6 | 0.36 | 90.2 | 0.38 | 213.1 | 10.47 | 0.43 | 0.1 | 5879.1 | 9.04 |
| Peak data table at 254 nm | | | | | | | | | | |
| 3 | 8 | 0.42 | 0.9 | 0.50 | 47.4 | 3.63 | 0.51 | 41.0 | 1879.9 | 5.18 |
| 4 | 8 | 0.47 | 26.6 | 0.51 | 54.9 | 3.82 | 0.53 | 49.8 | 1776.1 | 5.02 |
| 6 | 7 | 0.38 | 6.6 | 0.40 | 19.7 | 1.80 | 0.42 | 0.1 | 354.2 | 0.93 |
| Peak data table at 366 nm | | | | | | | | | | |
| 3 | 7 | 0.41 | 3.6 | 0.50 | 138.4 | 10.46 | 0.62 | 0.7 | 5134.3 | 16.02 |
| 4 | 7 | 0.43 | 5.2 | 0.51 | 234.2 | 27.04 | 0.62 | 4.6 | 8058.9 | 41.21 |

Table 3
Table of high performance thin layer chromatography (HPTLC) chromatograms scanned at 580 nm after derivatization

| Track | Peak | Initial Rf | Initial height | Max Rf | Max height | Max % | Final Rf | Final height | Area | Area % |
|-------|------|------------|----------------|-------------|------------|--------|----------|--------------|---------|--------|
| 1 | 1 | 0.34 | 3.5 | 0.40 | 39.9 | 16.95 | 0.45 | 29.2 | 1948.3 | 23.59 |
| 1 | 2 | 0.48 | 25.2 | 0.53 | 195.5 | 83.05 | 0.58 | 0.3 | 6312.4 | 76.41 |
| 2 | 1 | 0.33 | 0.7 | 0.39 | 143.6 | 100.00 | 0.44 | 11.7 | 4457.1 | 100.00 |
| 3 | 1 | 0.35 | 0.3 | 0.38 | 57.7 | 23.46 | 0.41 | 0.2 | 1260.9 | 15.27 |
| 3 | 2 | 0.42 | 1.5 | 0.52 | 188.3 | 76.54 | 0.57 | 0.0 | 6997.0 | 84.73 |
| 4 | 2 | 0.37 | 0.0 | 0.40 | 88.5 | 19.05 | 0.43 | 0.8 | 2130.4 | 15.13 |
| 4 | 3 | 0.43 | 0.9 | 0.52 | 281.9 | 60.63 | 0.57 | 0.3 | 10377.6 | 73.70 |
| 5 | 1 | 0.34 | 0.0 | 0.39 | 23.9 | 68.25 | 0.42 | 13.2 | 755.2 | 79.83 |
| 5 | 2 | 0.51 | 9.0 | 0.52 | 11.1 | 31.75 | 0.56 | 0.2 | 190.8 | 20.17 |
| 6 | 2 | 0.38 | 0.9 | 0.40 | 61.1 | 19.89 | 0.43 | 1.0 | 1133.8 | 18.91 |
| 6 | 4 | 0.50 | 16.6 | 0.52 | 30.4 | 9.90 | 0.55 | 0.4 | 718.1 | 11.97 |
| 7 | 2 | 0.36 | 16.3 | 0.38 | 109.2 | 39.16 | 0.43 | 1.0 | 3012.8 | 48.57 |
| 7 | 4 | 0.49 | 19.3 | 0.52 | 47.7 | 17.11 | 0.56 | 0.5 | 1267.2 | 20.43 |

DISCUSSION

High performance thin layer chromatography (HPTLC)

Methanol, petroleum ether and ethyl acetate fruit extracts of *W. tinctoria* R.Br and methanol and petroleum ether corm extracts of *A. campanulatus* (Roxb.) were screened by using high performance thin

layer chromatography (HPTLC) techniques for detection of bioactive compound at 200 nm, 254 nm and 366 nm (before derivatization) and at 580 nm (after derivatization) the results were found to be different separation pattern. However, compounds separated at RF value 0.37 (± 0.02) and 0.51 (± 0.02) were observed characteristic color reactions with anisaldehyde-sulphuric acid reagent. These compounds were

identified as beta-sitosterol and lupeol, respectively. The results were in accordance with⁷ who reported the bio-compounds like Lupeol and beta-sitosterol as major compounds are statistically extreme significance. Reference standards for beta-sitosterol and lupeol were also applied to high performance thin layer chromatography (HPTLC) for comparison. Methanol and petroleum ether fruit extracts of *W.tinctoria R.Br* and corm *A. campanulatus (Roxb.)* were studied for detection of lupeol, as crude drug compound was separated at Rf value of 0.51 (\pm 0.02) and exhibited a characteristic purple-violet colour with anisaldehyde-sulphuric acid reagent. Similarly, beta-sitosterol was observed in presence of methanol and petroleum ether fruit extracts of *W. tinctoria R.Br* and in corm of *A. campanulatus (Roxb.)*^{12 and 13} found the beta-sitosterol from methanol and petroleum ether fruit extract of *W. tinctoria* and corm of *A. camapnulatus*. Ramchandra¹⁰ isolated beta-sitosterol from the pod (fruit) of *W. tinctoria* were found the presence of beta-sitosterol at Rf value of 0.37 (\pm 0.02) and exhibited a characteristic violet-blue colour with anisaldehyde-sulphuric acid reagent. Beta-

Sitosterol was found as the major compound in corm of *A. campanulatus (Roxb.)*. Similar finding was supported by report of Bigonia⁸ and Dey³. While, ethyl acetate fruit extract of *W. tinctoria R.Br* was devoid of beta-sitosterol and lupeol.

CONCLUSION

Beta- sitosterol was recorded a major bioactive compounds from corm extracts of *A. campanulatus (Roxb.)*. Whereas, beta-sitosterol and lupeol were not observed in the ethyl acetate fruit extract of *W. tinctoria R.Br*. Methanol and petroleum ether extracts belongs to fruit of *W. tinctoria* and corm of *A. campanulatus* were supported to determine the beta-sitosterol as bioactive compounds.

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Alam Khan, Moizur Rahman, Islam MS. Antibacterial, antifungal and cytotoxic activities of amblyone isolated from *Amorphophallus campanulatus*. Indian J Pharmacol, 2008. 40(1): 41.
2. Bharat NS. Ph.D. thesis submitted to Swami Ramanand Teerth Marathwada University, Nanded. 2015. p. 85-87.
3. De S, Dey YN, Ghosh AK. Phytochemical investigation and chromatographic evaluation of the different extracts of tuber of *Amorphophallus paeoniifolius (Araceae)*. International Journal on Pharmaceutical and Biomedical Research (IJPBR), 2010; 1(5): 150-157.
4. Ghani A, Medicinal plants of Bangladesh. Bangladesh, Asiatic society of Bangladesh, 1998: 77-78.
5. Hallivel H, Lancet. Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? 1994. 344(8924):721-4.
6. Larson R. The antioxidants of higher plants. Phytochemistry. 1988;27: 969-78.
7. Srivastava M, Chakravarty R, Kumar A. Analytical aspects of the seed polysaccharide of *Wrightia tinctoria R.Br (roxb.)*; The Pharma Research. 2010, 3:99-111.
8. Bigonia P, Shukla A, Agrawal P and Rana AC. Pharmacological screening of *Wrightia tinctoria* bark hydroalcoholic extract. 2008. Asian J. Exp. Vol. 22(3): 235-244.
9. Ansil PN, Wills PJ, Varun R, Latha MS. Cytotoxic and apoptotic activities of *Amorphophallus campanulatus (Roxb.)* Bl. tuber extracts against human colon carcinoma cell line HCT-15. Saudi j. of Biol. Sci. 2014 Dec; 21(6): 524-531.
10. Srivastava R. A review on phytochemical, pharmacological, and pharmacognostical profile of *Wrightia tinctoria*: Adulterant of kurchi. Pharmacognosy Review. 2014 Jan-Jun; 8(15): 36-44.
11. Ramchandra P, Basheermiya M, Krupadanam GLD, Srimannarayana G. Wrightial, a new terpene from *Wrightia tinctoria*. J Nat Prod; 1993. 56:1811-2.
12. Ramalingam R, Hima Bindu K, Bindu Madhavi B, Ravinder Nath A, David Banji D. Phyto chemical and anthelmintic evaluation of corm of *Amorphophallus campanulatus*. Int J Pharma Bio Sci 2010;1:1-9
13. Rice-Evans, Packer, LC. In Flavonoids in health and disease. Eds.; Marcel Dekker: New York. 1998: 66-110.
14. Tripathi S, Chitra V, Sheikh NW, Mohale DS, Dewani AP. Immunomodulatory activity of methanolic extract of the tuber of *Amorphophallus companulatus (Araceae)* tuber Tropical journal of Pharmaceutical research. 2010 October; 9 (5): 451-454.
15. Jain S, Dixit VK, Malviya N and Ambawatia V. Antioxidant and hepatoprotective activity of ethanolic and aqueous extracts of *Amorphophallus campanulatus (Roxb.)*. Acta Poloniae Pharmaceutical and drug research. 2009;66(4):423-428.
16. Shilpi JA, Ray PK, Sarder MM, Uddin SJ. Analgesic activity of *Amorphophallus companulatus* tuber. *Fitoterapia*. 2005 Jun;76(3-4):367-9.
17. Shinde SR. Ph.D. Thesis submitted to Swami Ramanand Teerth Marathwada University, Nanded. 2008. P.71-72.
18. Gajare SM, *Amorphophallus Campanulatus* : Review of medicinal properties. An international journal of Pharmaceutical Sciences., Pharm. Sci. Monitor. 2014 Jul-Sep 5(3):122-130.
19. Dey YN and Ghosh AK. Evaluation of anthelmintic activity of the methanolic extracts of *Amorphophallus paeoniifolius* tuber. IJPSR 2010; 1(11):111-117.

Reviewers of this article

DR.Bhagwat.N.Poul

Principal and Professor, Department of
Pharmacy,
Maharashtra College of Pharmacy,
adhav Nagar, Nilanga, Maharashtra
413521, India



Asst.Prof.Dr. Sujata Bhattacharya

Assistant Professor, School of Biological
and Environmental Sciences, Shoolini
University, Solan (HP)-173212, India



Prof.Dr.K.Suriaprabha

Asst. Editor , International Journal
of Pharma and Bio sciences.



Prof.P.Muthuprasanna

Managing Editor , International
Journal of Pharma and Bio sciences.

We sincerely thank the above reviewers for peer reviewing the manuscript