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

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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.***

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INTRODUCTION

W. tinctoria (R.Br) and A. campanulatus (Roxb.) are medicinal potentials which has phytochemicals[9] and are ethno-medico-botanical significance[14]. The phytochemicals like flavonoids, alkaloids, steroids, phenol and tannins and are multi potentially and used for the treatment of various diseases[15]. The extracts of fruit and corm extracts of *W. tinctoria (R.Br.)* and *A. campanulatus (Roxb.)* respectively, play important role in antimicrobial activity[1] heapato-protective activity[15,19], antitumor activity[9]. The study emphasizes on isolation of phytocompounds which are biologically active and multi dimensional their significances. *A. campanulatus (Roxb.)* belongs to family *Araceae* as corm or tuber producing plant largely cultivated through the Indian plain parts and commonly known as Suran[4]. It is tuberous, stout, indigeneous herb having 1.5-1.5 m in length. The corm of *A. campanulatus (Roxb.)* contain an active digestive enzyme amylase, betulinic acid, b-sitpsterol, stigmasterol, b- sitosterol palmite, lupeol, triacontane, amino acid, carbohydrates, saponin, thiamine, riboflavin, niacin and carotene and beneficial for treatment of common ailments[12]. *W. tinctoria (R.Br)* belongs to family *Apocynaceae.* It is well known by common name as Indrajav. It is very important traditionally useful for healing on different ailments. *W. tinctoria (R.Br)* is considered as therapeutically very effective Jaundice plant in indigenous system of medicine. Fruit extracts of *W. tinctoria (R.Br.)* have qualitative and quantitative presence of various bioactive compounds have been reported in literature. The present work on the line of the estimation of bioactive compounds from 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 in 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 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 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)

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 precoated 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) finger printing. Lupeol and beta-sitosterol were used as standard triterpenoids.

RESULTS

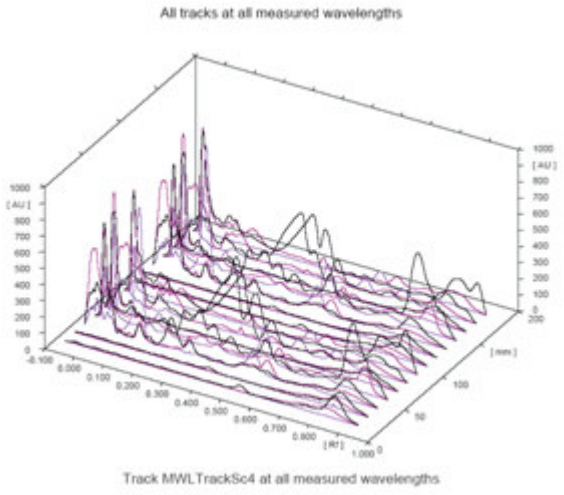


Figure 1
3-D view of HPTLC Plate scanned at multi-wavelength (200 nm – 400 nm) for Standard drugs (Lupeol and Beta-Sitosterol) and test drugs (Wrightia tinctoria R.Br. and Armophophallus campanulatus Roxb. extracts).

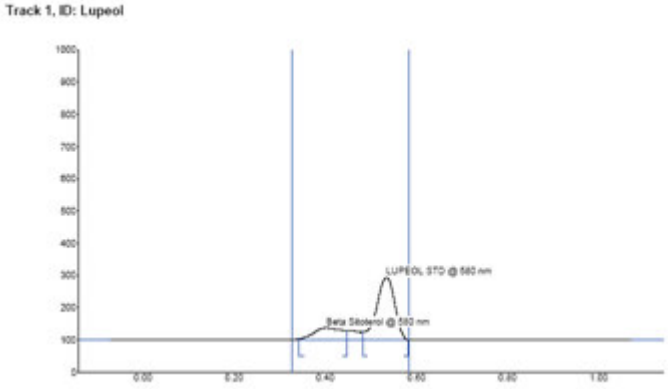


Figure 2
HPTLC spectrum of Lupeol scanned 580 nm

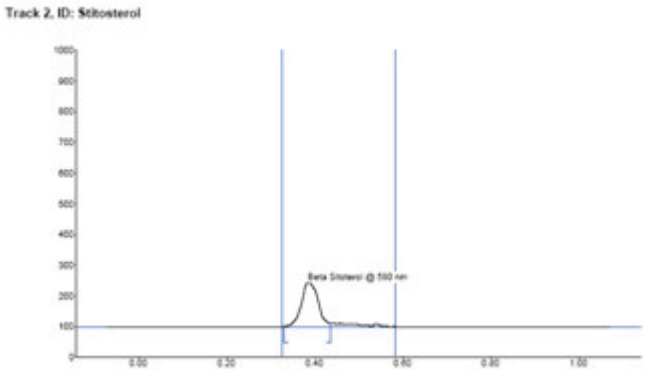


Figure 3
HPTLC spectrum of Beta-Sitosterol scanned 580 nm

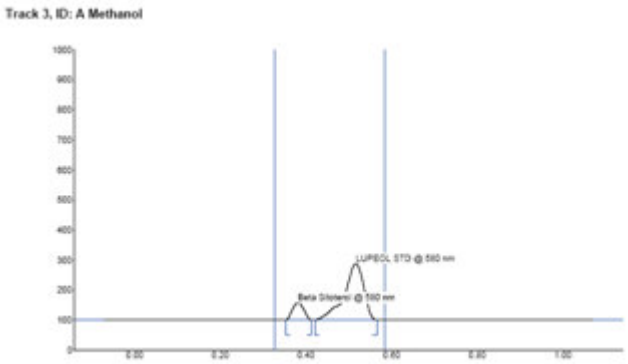


Figure 4
HPTLC spectrum of Methanol extract of Wrightia tinctoria R.Br. scanned 580 nm

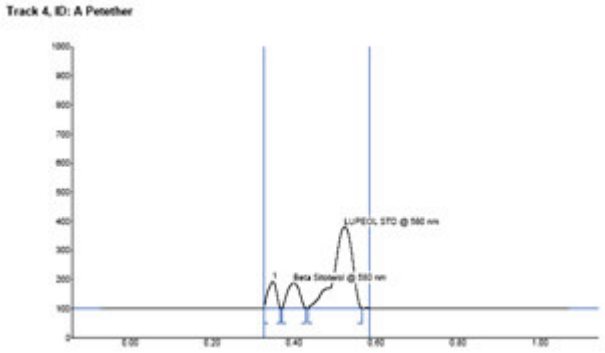


Figure 5
HPTLC spectrum of Pet ether extract of Wrightia tinctoria R.Br. scanned 580 nm

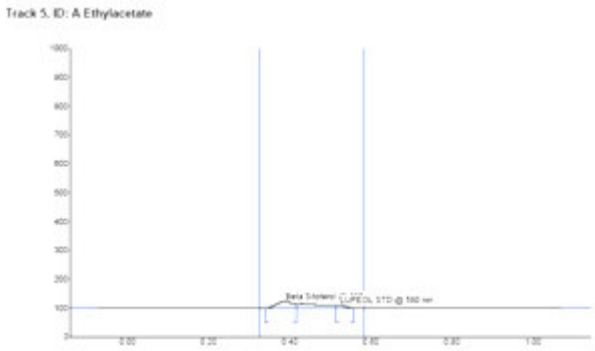


Figure 6
HPTLC spectrum of Ethyl acetate extract of Wrightia tinctoria R.Br. scanned 580 nm

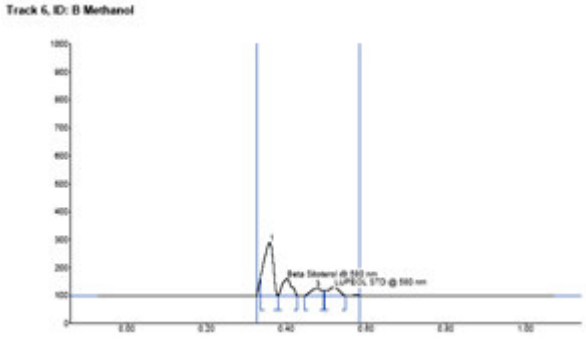


Figure 7
HPTLC spectrum of Methanol extract of *Amorphophallus campanulatus Roxb.* scanned 580 nm

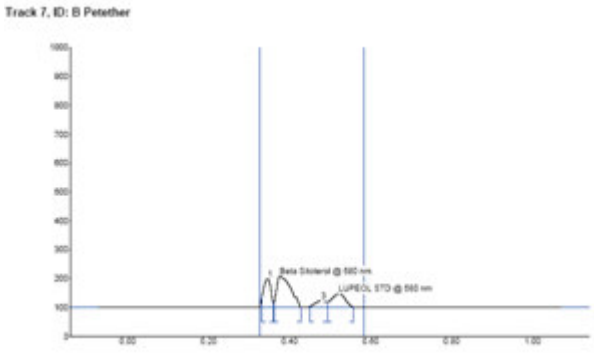


Figure 8

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

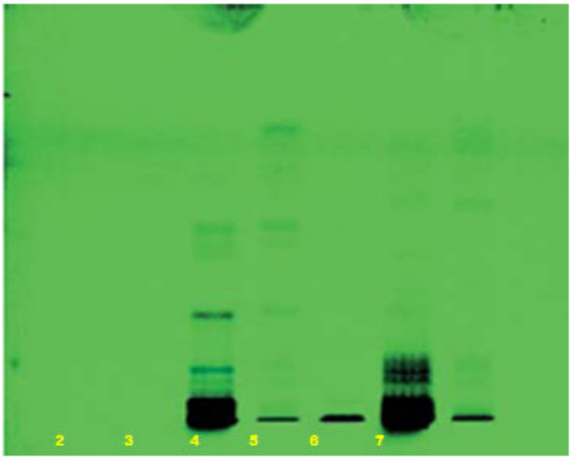


Figure 9

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 W*rightia tinctoria R.Br.* 4 = Pet. Ether extract of W*rightia tinctoria R.Br.,* 5 = Ethyl Acetate extract of W*rightia tinctoria R.Br.* 6 = Methanol extract of *Amorphophallus campanulatus Roxb.,* 7 = Pet Ether extract of *Amorphophallus campanulatus Roxb.*

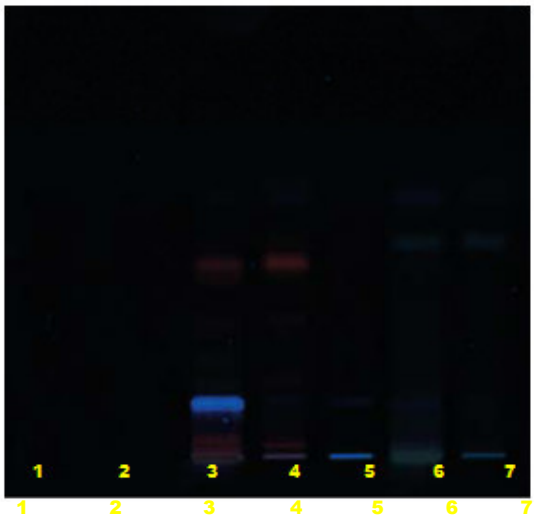


Figure 10

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 W*rightia tinctoria R.Br.,* 4 = Pet. Ether extract of W*rightia tinctoria R.Br.,* 5 = Ethyl Acetate extract of W*rightia tinctoria R.Br.,* 6 = Methanol extract of *Amorphophallus campanulatus Roxb.* 7 = Pet Ether extract of *Amorphophallus campanulatus Roxb.*

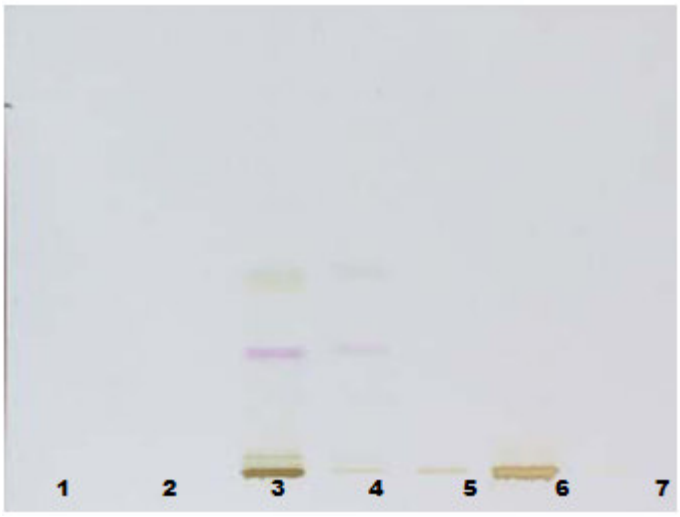


Figure 11
HPTLC plate for Wrightia tinctoria R.Br. and Amorphophallus campanulatus Roxb. extracts scanned at 400 nm (in daylight) before derivatization.

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

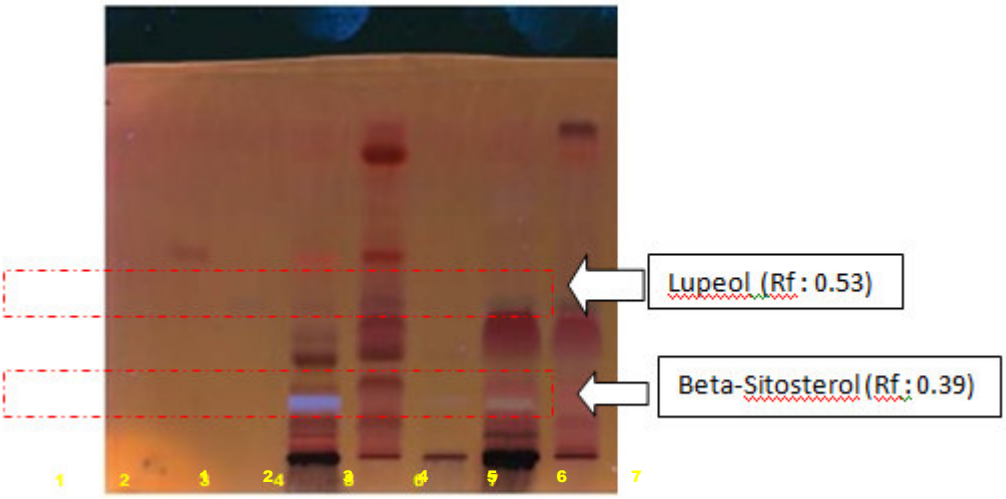
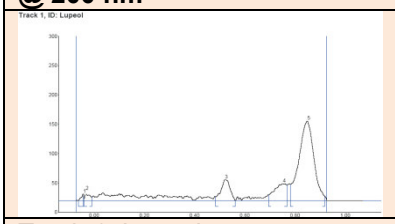
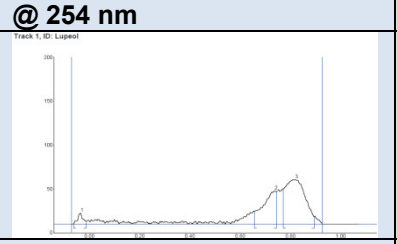
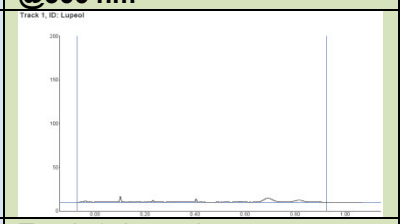


Figure 12
Photo of HPTLC plate for W. tinctoria and Amorphophallus campanulatus Roxb. extracts scanned at 580 nm after derivatization with Anisaldehyde-sulphuric acid reagent.
[Note: Compounds separated at Rf value 0.39 (±0.02) and 0.53 (±0.02) were identified as beta-Sitosterol and Lupeol, respectively, by comparison with standard drugs used (Track 1 and 2, respectively).]

1 = Standard Drug, 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.

@ 200 nm	@ 254 nm	@366 nm
		
Track 1: Lupeol	Track 1: Lupeol	Track 1: Lupeol

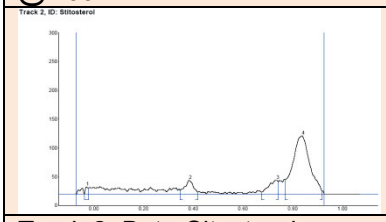
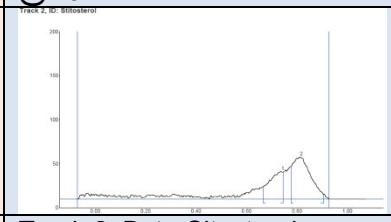
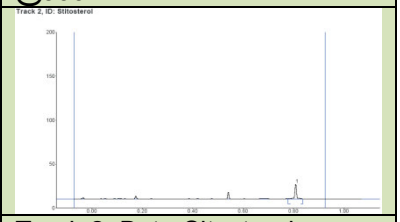
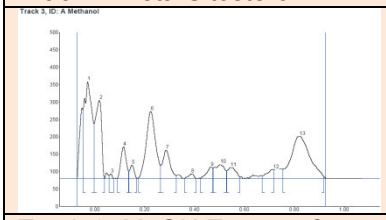
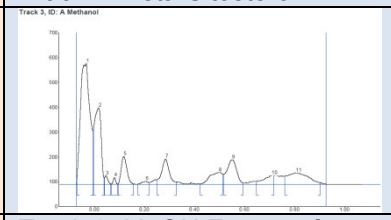
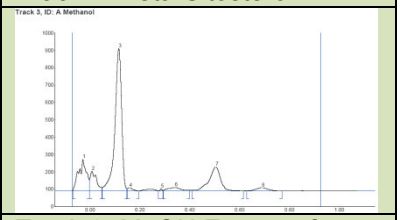
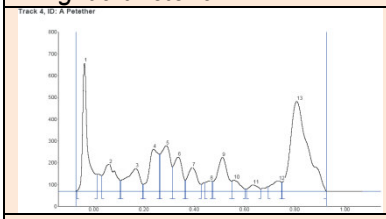
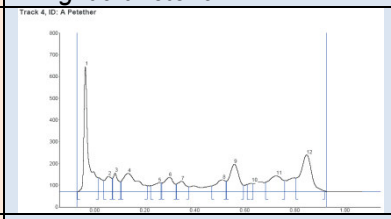
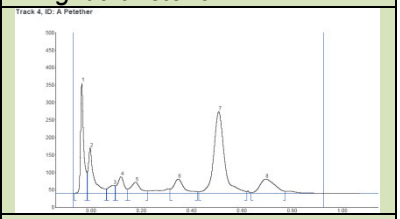
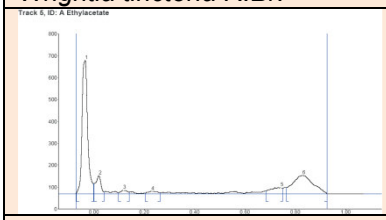
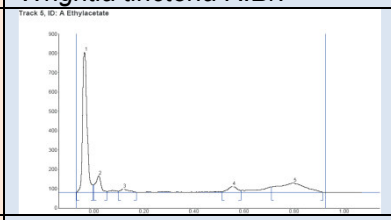
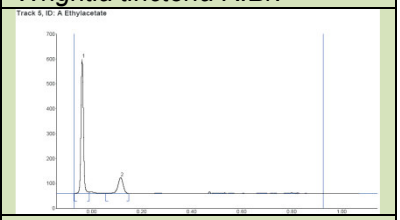
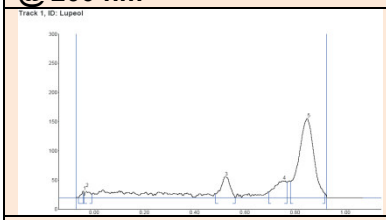
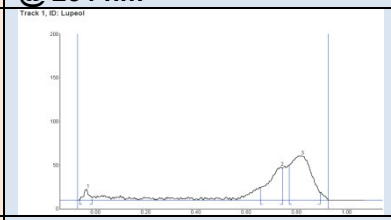
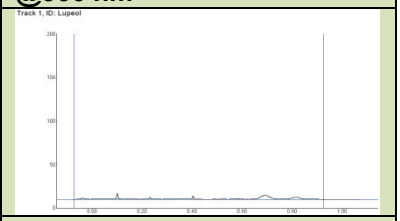
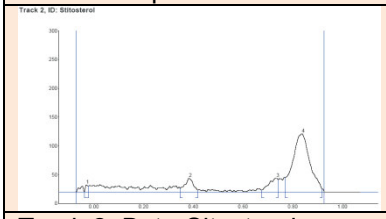
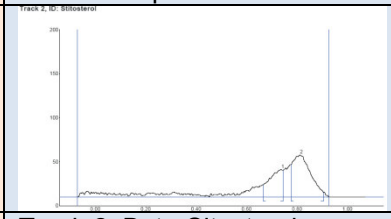
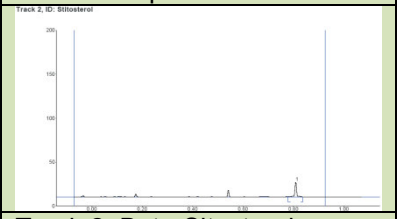
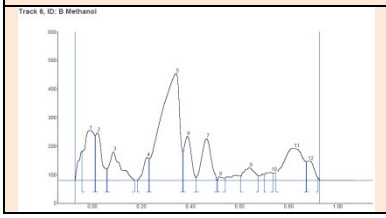
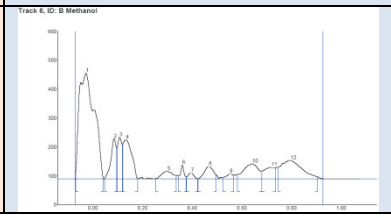
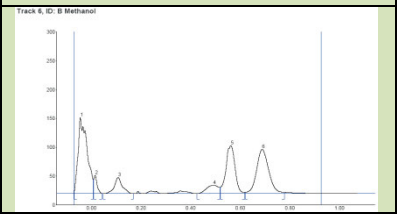
@ 200 nm	@ 254 nm	@366 nm
		
Track 2: Beta-Sitosterol	Track 2: Beta-Sitosterol	Track 2: Beta-Sitosterol
		
Track 3: MeOH Extract of *Wrightia tinctoria R.Br.*	Track 3: MeOH Extract of *Wrightia tinctoria R.Br.*	Track 3: MeOH Extract of *Wrightia tinctoria R.Br.*
		
Track 4: Pet Ether Extract of *Wrightia tinctoria R.Br.*	Track 4: Pet Ether Extract of *Wrightia tinctoria R.Br.*	Track 4: Pet Ether Extract of *Wrightia tinctoria R.Br.*
		
Track 5: EtOAc Extract of *Wrightia tinctoria R.Br.*	Track 5: EtOAc Extract of *Wrightia tinctoria R.Br.*	Track 5: EtOAc Extract of *Wrightia tinctoria R.Br.*

Figure 13

Comparative HPTLC chromatograms scanned at 200 nm, 254 nm and 366 nm for Standard drugs (Lupeol and Beta-Sitosterol) and test drugs (*Wrightia tinctoria R.Br.)*

Abbreviations: MeOH = Methanol; EtOAc = Ethyl acetate

@ 200 nm	@ 254 nm	@366 nm
		
Track 1: Lupeol	Track 1: Lupeol	Track 1: Lupeol
		
Track 2: Beta-Sitosterol	Track 2: Beta-Sitosterol	Track 2: Beta-Sitosterol
		

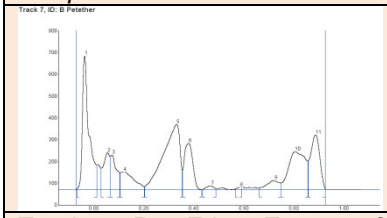
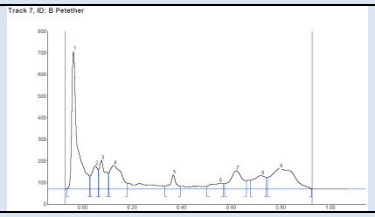
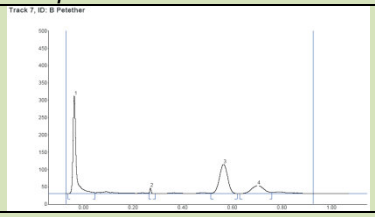
@ 200 nm	@ 254 nm	@366 nm
Track 6: MeOH Extract of *Amorphophallus campanulatus Roxb.*	Track 6: MeOH Extract of *Amorphophallus campanulatus Roxb.*	Track 6: MeOH Extract of *Amorphophallus campanulatus Roxb.*
		
Track 7: Pet Ether Extract of *Amorphophallus campanulatus Roxb.*	Track 7: Pet Ether Extract of *Amorphophallus campanulatus Roxb.*	Track 7: Pet Ether Extract of *Amorphophallus campanulatus Roxb.*

Figure 14

Comparative HPTLC chromatograms scanned at 200 nm, 254 nm and 366 nm for Standard drugs (Lupeol and Beta-Sitosterol) and test drugs (*Amorphophallus campanulatus Roxb.)*
Abbreviations: MeOH = Methanol; EtOAc = Ethyl acetate

Table2
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 with[7] 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 ((± 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*. Ramchandra[10] isolated beta-sitosterol from the pod (fruit) of *W. tinctoria* were found the presence of beta-sitosterol at Rf value of 0.37 (± 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 Bigonia[8] and Dey[3]. 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.

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