Presentation on

STANDARDIZATION OF EXTRACTS OF *OPERCULINA TURPETHUM* (LINN.) SILVA MANSO ROOTS AND EVALUATION OF ITS EFFECT ON INFLAMMATION

Presented by

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CONTENTS

- *Column Chromatography*
- *HPTLC*
- *Pharmacological Screening*
  - *Acute Toxicity Studies*
  - *Cell Viability Assay on HepG2 Cells*
  - *In Vitro inhibition of albumin denaturation*
  - *Egg albumin-induced inflammation model in rats*
  - *Cotton pellet-induced granuloma model in rats*

5. CONCLUSION

6. FUTURE PROSPECTS.

1. INTRODUCTION

- Medicinal plants constitute an effective source of both traditional and modern medicine.
- Natural products are regarded as the origin of new chemical variety, and today it is inclined towards world's choice. The reservoirs of these products are plants, animals, and microorganisms.
- Many natural products identified from medicinal plants, or its secondary metabolites such as phenolic acids, terpenoids, coumarins, lignans, tannins, steroids, flavonoids, quinones, alkaloids, which show the important therapeutic activities, have performed a significant role in the treatment of diseases. [*Kaur et al., 2011*]

OPERCULINA TURPETHUM (LINN.) SILVA MANSO

- *Operculina turpethum* (Linn.) Silva Manso, belonging to the family Convolvulaceae, also named as *Tihudi*/*Trivrit* because of the triangular shape stem.

- It has been recognized as "Virechan" or a laxative in Ayurveda. [*Kohli et al., 2010*]

- It is known by other names such as-

English : Indian jalap/Turpeth root
Hindi : Nisoth/Tarbut/Panila/Pithori
Sanskrit : Trivrit
Telugu : Tegada
Kan. : Sigade
Tamil : Kumbham/Sivatai
Malayalam : Trikolpakkonna/Triputa/Sivata.

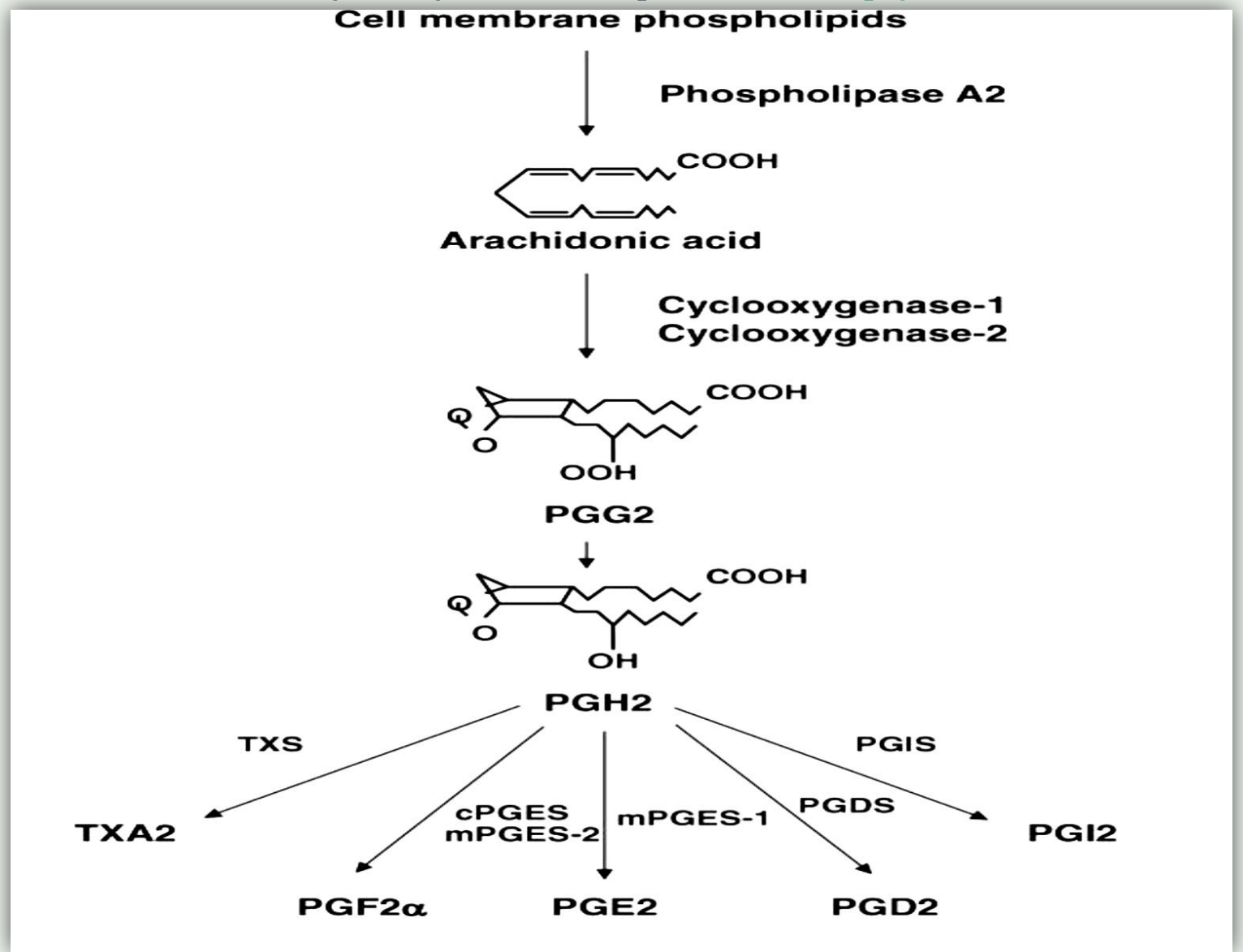
Pictorial representation of *Operculina turpethum* (Linn.) Silva Manso

INFLAMMATION

- Inflammation is non-specific, localized immune reaction of the organism, which tries to localize the pathogenic agents.
- It consists in vascular, metabolic, cellular changes, triggered by the entering pathogenic agents in healthy tissues of the body.
- Inflammation is caused by a variety of stimuli such as physical injury, ultraviolet (UV)-irradiation, microbial attack and immune reactions and it is the response of living tissues to damage or burn. [*Habibur Rahman et al., 2012*]
- Inflammation begins when phospholipase A_2 (PA_2) enzyme is activated to hydrolyse membrane phospholipids to arachidonic acid, which further forms various mediators that are vasoconstrictive and increase vascular permeability.

MECHANISM OF ACTION

- A schematic representation of the arachidonic acid metabolism pathway. [mPGEs-1/2, cPGEs: Microsomal or cytosolic prostaglandin E synthase, TXS: Thromboxane synthase, PGIS: Prostacyclin synthase; Adapted from *Sampey et al., 2005*]



2. OBJECTIVE OF WORK

- To standardize the extracts of *Operculina turpethum* roots by carrying out physicochemical and phytochemical studies; further to isolate, identify and quantify the phytoconstituent by column chromatography, TLC, and HPTLC.
- To determine the *in-vitro* cytotoxic activity of the methanol extract of *O. turpethum* roots on HepG2 cells by using WST-1 Cell Proliferation Kit.
- To evaluate the effect of the methanol extract/fraction of *O. turpethum* roots on inflammation.

3. MATERIALS AND METHODS

Root powder/ Extracts of OT → Percentage yield → Organoleptic evaluation *(Color, Odor, Taste and Surface characteristics)*

↓

Physicochemical investigations
- *pH*
- *Loss on Drying/Moisture Content*
- *Total Ash, Water-soluble Ash, and Acid-insoluble Ash*
- *Hot Water and Ethanol-Extractable Matter*
- *Volatile Oil Content*

← Preliminary phytochemical screening

↓

Chromatographic separation
- *TLC*
- *Column Chromatography*
- *HPTLC*

→

Pharmacological screening
- *Acute toxicity studies*
- *Cytotoxic activity on HepG2 cells*
- *In vitro inhibition of albumin denaturation*
- *Egg albumin-induced inflammation model in rats*
- *Cotton pellet-induced granuloma model in rats*

PROCUREMENT & AUTHENTICATION OF PLANT MATERIAL

- *The root part of plant Operculina turpethum (Linn.) Silva Manso was purchased from Surya Bahadur Shrestha, Nepali Kothi, Phoolwali Gali, near Chowk, Lucknow and it was botanically authenticated by Dr. Priyanka Agnihotri, CSIR - National Botanical Research Institute, Lucknow, India, and where a voucher specimen (authentication number: LWG-75) has been deposited after ethnobotanical identification of species.*

EXPERIMENTAL ANIMALS

- *The Wistar albino rats of 150-200 g of either sex were obtained from Mahatma Gandhi Institute of Pharmacy, Lucknow, India and used for the acute toxicity and anti-inflammatory activity.*
- *The Wistar albino rats were isolated in a group of five per cage in polypropylene cages randomly with paddy husk as bedding. The animals were housed under standard environmental provisions of temperature (25 ± 2°C) and relative humidity (50 ± 5%) with 12 h light and dark cycle. The animals were fed with standard diet and water ad libitum.*

PREPARATION OF PLANT MATERIAL

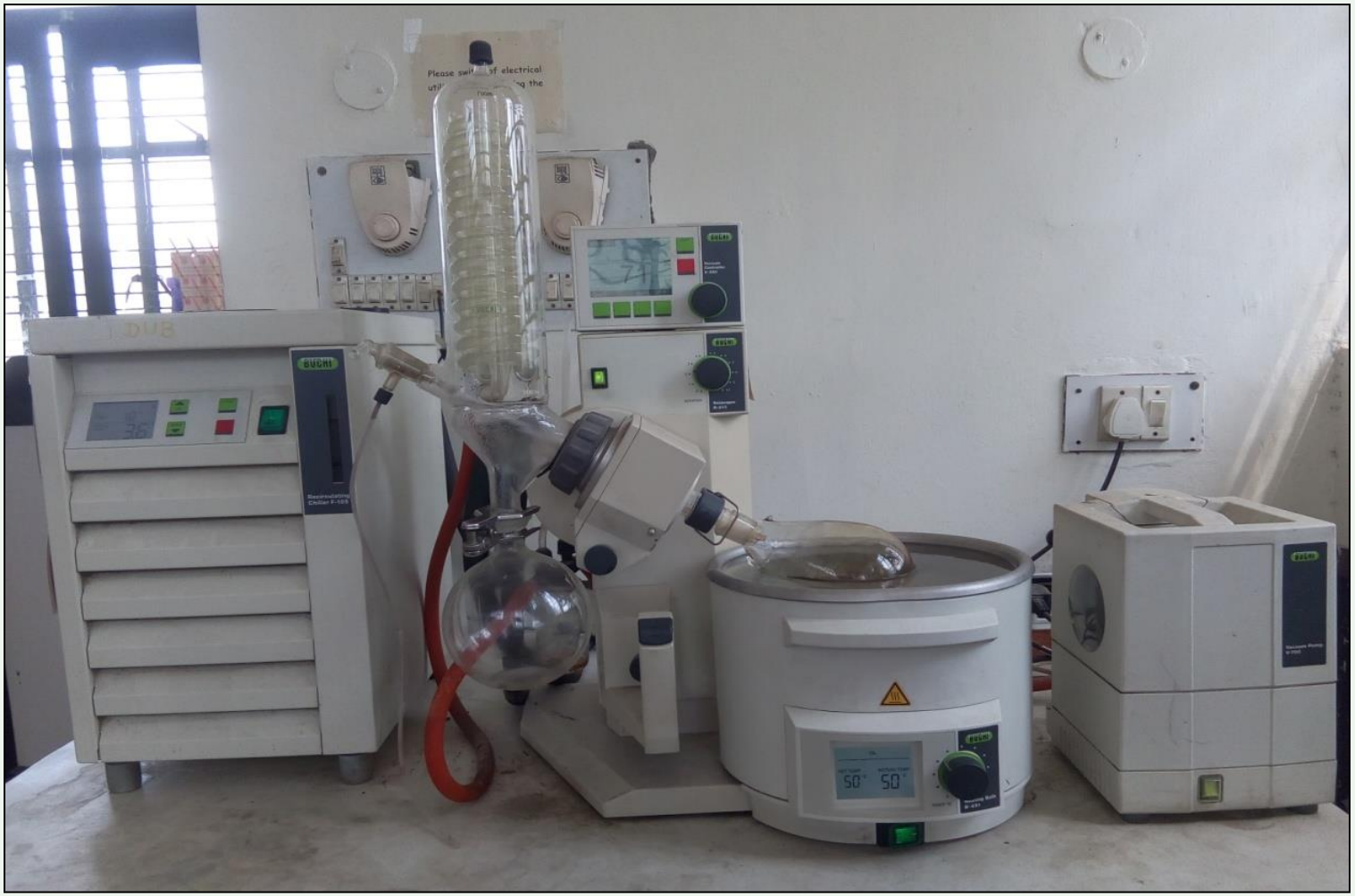
- *The collected root of O. turpethum was washed with tap water and cut into small portions and shade dried for two months to avoid direct loss of phytoconstituents from sunlight. The shade dried materials were powdered using the electric grinder and passed through 80 mesh sieve. It was then homogenized to the fine powder and put in an airtight container for further studies.*

PREPARATION OF EXTRACTS

- *The powdered root material of O. turpethum was extracted successively with different solvents ranging from non-polar to polar using Soxhlet apparatus by continuous hot percolation method.*
- *The extracts were collected and further concentrated under reduced pressure with a rotary evaporator. Dried extracts were collected at 5°C in airtight containers for further studies.*

Extraction in Soxhlet apparatus

$$\% \text{ Extraction Yield} = \frac{\text{Weight of the plant extract}}{\text{Weight of the initial plant material}} \times 100$$



Concentrating the Extract using Rotary Evaporator

CHROMATOGRAPHIC SEPARATION

- Chromatography is an analytical method that is broadly used for the separation, isolation, identification, and quantification of components in a mixture.
- Components of the mixture are separated through the stationary phase by the flow of a mobile phase.
- Based on differences in migration rates among the sample components, components of the mixture are separated. [*Harwood and Moody, 1989*]
- There are following methods used for chromatography separation:
  - Thin Layer Chromatography (TLC)
  - Column Chromatography
  - High-Performance Thin Layer Chromatography (HPTLC)

PHARMACOLOGICAL SCREENING

Acute Toxicity Studies

- **Experimental design**
  - **Group I** Normal control: 1% v/v Tween 80, 5 mL/kg body weight per oral.
  - **Group II** MEOT, 50 mg/kg body weight per oral.
  - **Group III** MEOT, 300 mg/kg body weight per oral.
  - **Group IV** MEOT, 2000 mg/kg body weight per oral.

- **Body weight**

The body weight of the animals was noted before the start of the experiment and was also noted at the end of the experiment and expressed in grams.

$$\% \text{ Change in the body weight} = \frac{\text{Change in the body weight}}{\text{Initial body weight}} \times 100$$

In Vitro Anti-inflammatory Activity

Inhibition of albumin denaturation

- **Control solution (50 mL):** 2 mL of egg albumin (protein), 28 mL of phosphate buffer of pH 6.4 and make the volume to 50 mL with 20 mL distilled water.
- **Standard drug (50 mL):** 2 mL of egg albumin, 28 mL of phosphate buffer and various concentrations of standard drug (Aspirin) concentration of 100, 200, 300 and 400 µg/mL.
- **Test solution (50 mL):** 2 mL of egg albumin, 28 mL of phosphate buffer and various concentration of plant extract (MEOT) concentration of 100, 200, 300 and 400 µg/mL.

- All of the above solutions were set to pH using a little amount of 1 N HCl, incubated at 37°C for 15 minutes and heated at 70°C for 5 minutes. After cooling the samples, the turbidity (absorbance) of the solutions was taken spectrophotometrically at a wavelength of 660 nm.
- The percentage of inhibition of protein denaturation was calculated by using the following formula [*Chandra et al., 2012; Sangeetha et al., 2011*]:

$$\text{Percentage inhibition} = \frac{(\text{Abs of control} - \text{Abs of sample})}{\text{Abs of control}} \times 100$$

In Vivo Anti-inflammatory Activity

Egg albumin-induced inflammation model

- The Wistar albino rats were randomized into five groups of 5 rats each.
- **Experimental design**
  - **Group I** Normal control: 1% Tween 80, 5 mL/kg body weight per oral.
  - **Group II** Reference control: Indomethacin, 10 mg/kg body weight per oral.
  - **Group III** MEOT, 100 mg/kg body weight per oral.
  - **Group IV** MEOT, 200 mg/kg body weight per oral.
  - **Group V** MEOT, 400 mg/kg body weight per oral.
- One hour after the drug treatment, all the animals of all groups were injected 0.1 mL of 1% w/v egg albumin (suspended in normal saline) subcutaneously into the plantar region of the left hind paw.
- Paw edema volumes were measured at every 1 h after the administration of egg albumin by using plethysmometer. [*Kulkarni, 1987*]
- The reading was taken for the total of 4 h. Edema volumes were calculated, and % reduction in the edema volume due to drug treatment was determined by comparing with the control group.

Cotton pellet-induced granuloma model

- Cotton pellet-induced granuloma model was carried out for estimating the anti-inflammatory activity. [*Winter and Porter, 1957*]
- The Wistar albino rats were randomized into five groups of 5 rats each. The furs of the axilla area of animals were shaved, wiped with 70% v/v ethanol and anesthetized by giving proper dose of Ketamin, intraperitoneal. After 10 minutes of anesthesia established, sterile pre-weighed cotton pellets (50 ± 1 mg) were implanted in the axilla area of each rat through a small needle incision by an aseptic method. The incisions were sutured by sterile catgut/biodegradable surgical stings.
- **Experimental design**
  - **Group I** Normal control: 1% Tween 80, 5 mL/kg body weight per oral for 7 days.
  - **Group II** Reference control: Indomethacin, 10 mg/kg body weight per oral for 7 days.
  - **Group III** MEOT, 100 mg/kg body weight per oral for 7 days.
  - **Group IV** MEOT, 200 mg/kg body weight per oral for 7 days.
  - **Group V** MEOT, 400 mg/kg body weight per oral for 7 days.

- On the 8th day, the animals were anesthetized again, and the cotton pellets covered with the granulomatous tissue were removed surgically and after that pellets made free from extraneous tissues.
- The pellets were incubated at 37°C for 24 h and dried at 60°C to constant weight. The increment in the dry weight of the pellets was regarded as a measure of granuloma formation.
- Then the net weight was calculated by the following formula:

$$\text{Percentage inhibition} = \frac{(Wc - Wd)}{Wc} \times 100$$

Where, W_c = Difference in the weight of control group

W_d = Difference in the weight of extract group

4. RESULTS AND DISCUSSION

PERCENTAGE YIELD OF DIFFERENT EXTRACTS OF *O. TURPETHUM* ROOTS

- Percentage yield of extraction of different extracts of *O. turpethum* roots is tabulated in the following table:

Solvents	Yield of extract / 50 g roots (% w/w)	Color
Petroleum Ether	2.6	Light Brown
Benzene	4.9	Brown
Chloroform	2.9	Brown
Methanol	9.4	Dark Brown

- The extractive yield value was found to be high in methanolic extract of roots of *O. turpethum*, i.e., 9.4%.

ORGANOLEPTIC EVALUATION OF ROOT POWDER AND EXTRACTS OF *O. TURPETHUM*

- Organoleptic properties of coarse powder; and various extracts of *O. turpethum* roots are tabulated in the following table:

Parameters	Raw (coarse powder)	Petroleum ether extract	Benzene extract	Chloroform extract	Methanolic extract
Color	Yellowish Brown	Light Brown	Brown	Brown	Dark Brown
Odour	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic
Taste	Slight pungent	Bitter	Bitter	Bitter	Bitter
Appearance (Texture)	Powder	Liquid	Liquid	Liquid	Liquid
Touch	Coarse	Smooth	Sticky	Sticky	Sticky

- All the extracts of *O. turpethum* roots had almost similar organoleptic properties except for the color of all the extracts.

PHYSICOCHEMICAL INVESTIGATIONS OF ROOT POWDER OF *O. TURPETHUM*

Determination of pH

Sample	pH	
Coarse Powder	1% w/v formulation solution	10% w/v formulation solution
	6.83 ± 0.046	5.58 ± 0.035

Determination of Loss on Drying/Moisture Content

Sample	Loss on drying / Moisture content
Coarse Powder	13 ± 0.20% w/w

Value is expressed as the Mean ± S.D.; n = 3

- The less value of moisture content of drugs could prevent bacterial, fungal or yeast growth through storage. [*Pandey et al., 2012*]

Determination of Total Ash, Water-soluble Ash, and Acid-insoluble Ash

- Total ash, acid-insoluble ash, and water-soluble ash value of coarse powder are tabulated in the following table:

Sample	Total ash	Water-soluble ash	Acid-insoluble ash
Coarse Powder	8.25 ± 0.05% w/w	4.12 ± 0.08% w/w	2.23 ± 0.03% w/w

Value is expressed as the Mean ± S.D.; n = 3

- Ash values used to find out quality, authenticity, and purity of unsophisticated drug and also these values are essential quantitative standards. [*Paramjyothi and Syed, 2010*]

Determination of Hot Water and Ethanol-Extractable Matter

Sample	Water soluble (hot) extractive value	Ethanol soluble (hot) extractive value
Coarse Powder	7.05 ± 0.03% w/w	12.07 ± 0.31% w/w

Value is expressed as the Mean ± S.D.; n = 3

- The extractive values are valuable to determine the chemical constituents present in the crude drug and furthermore assist in the evaluation of specific constituents soluble in a particular solvent. [*Jain et al., 2011*]

Determination of Volatile Oil Content

Sample	Volatile oil content
Coarse Powder	2.35 ± 0.13% v/w

Value is expressed as the Mean ± S.D.; n = 3

- The volatile oil content in the root of *Operculina turpethum* coarse powder was found to be 2.35 ± 0.13% v/w.

PRELIMINARY PHYTOCHEMICAL SCREENING OF DIFFERENT EXTRACTS OF *O. TURPETHUM* ROOTS

Phytochemicals		Petroleum ether Extract (PEEOT)	Benzene extract (BEOT)	Chloroform extract (CEOT)	Methanolic extract (MEOT)
Flavonoids	Shinoda test	-	-	-	++
	Alkaline reagent test	+	+	-	++
	Lead acetate test	-	-	+	+
Alkaloids	Mayer's test	-	-	-	-
	Dragendorff's test	+	-	+	++
	Wagner's test	-	-	+	+
	Hager's test	-	-	+	+
	Tannic acid test	-	+	+	-
Tannins	Ferric chloride test	+	+	++	+
	Gelatin test	-	-	-	-
	Vanillin HCl test	+++	+	+++	+

Cont.

Phenolic compounds		-	-	-	+
Cardiac glycosides	Keller killiani test	++	+	+++	+++
	Salkowski test	+	+	+	++
Steroids and Triterpenoids	Liebermann-Burchard test	+	-	++	++
	Salkowski test	+	++	+	++
Proteins and Amino acids	Xanthoproteic test	-	-	-	-
	Ninhydrin test	-	-	-	-
Saponins	Foam (Froth) test	+	+	+	+++
Reducing sugar	Fehling's test	++	++	+	++
Carbohydrates	Molisch's test	-	-	-	++
Quinones		-	-	-	-

(+++) appreciable amount;
(++) moderate/average amount;
(+) trace amount and
(-) completely absent.

PEEOT- Petroleum ether extract of *O. turpthum*;
BEOT- Benzene extract of *O. turpthum*;
CEOT- Chloroform extract of *O. turpthum*;
MEOT- Methanolic extract of *O. turpthum*.

CHROMATOGRAPHIC SEPARATION

TLC Fingerprinting Profiling of Different Extracts of O. turpethum Roots

- R_f value of various extracts of *O. turpethum* roots in different mobile phase is tabulated in the following table:

Figure No.	Extracts	Mobile phase	R_f value (Iodine)
(A)	Petroleum ether	Benzene: Chloroform (9: 1)	0.48
(B)	Benzene	Toluene: ethyl acetate (9: 1)	0.54
(C)	Chloroform	Benzene: Chloroform (9: 1)	0.58
(D)	Chloroform	Toluene: Chloroform: Methanol (5: 4.5: 0.5)	0.90, 0.31
(E)	Methanol	Toluene: Ethyl acetate: Methanol (9: 1: 0.1)	0.32, 0.41, 0.44 & 0.84
(F)	Methanol	Chloroform: Acetone (9: 1)	0.34

Cont.

(G)	Methanol	Toluene: Ethyl acetate: Methanol (9: 1: 0.1)	0.32, 0.78
(H)	Methanol	Ethyl acetate: Formic acid: Glacial acetic acid: Water (100: 11: 11: 26)	0.39
(I)	Methanol	Toluene: Ethyl acetate: Methanol (9: 1: 0.1)	0.32, 0.47, 0.53 & 0.70
(J)	Methanol	Benzene: Chloroform (9: 1)	0.38 & 0.89
(K)	Methanol	Ethyl acetate: Benzene (4.5: 5.5)	0.77
(L)	Methanol	Toluene: Ethyl acetate (9: 1)	0.44

(A) TLC for Terpenoids
(Petroleum ether extract)

(B) TLC for Glycosides
(Benzene extract)

(C) TLC for Terpenoids
(Chloroform extract)

(D) TLC for Alkaloids
(Chloroform extract)

(E) TLC for Steroids
(Methanol extract)

(F) TLC for Steroids
(Methanol extract)

(G) TLC for Steroids (Methanol extract)

(H) TLC for Flavonoids (Methanol extract)

(I) TLC for Steroids (Methanol extract)

(J) TLC for Steroids (Methanol extract)

(K) TLC for Tannins/Phenols (Methanol extract)

(L) TLC for Oils (Methanol extract)

Figure: TLC plates of various extracts *O. turpethum* roots

Column Chromatography of Methanol Extract (ME) of O. turpethum Roots

- The column was run with different mobile phases, i.e., hexane, hexane: ethyl acetate, ethyl acetate, ethyl acetate: methanol and methanol in different concentration ratios based on gradually increasing polarity which gave 21 different fractions.
- These were further analyzed by TLC using mobile phase toluene: ethyl acetate: methanol (9: 1: 0.1 v/v/v) and the single spot were observed in the fraction number 14 to 21 with R_f value 0.32.

- The fractions obtained with same R_f value were tabulated in the following table:

S. No.	Fractions	Rf value
1.	Fraction – 14	0.32
2.	Fraction – 15	0.32
3.	Fraction – 16	0.32
4.	Fraction – 17	0.32
5.	Fraction – 18	0.32
6.	Fraction – 19	0.32
7.	Fraction – 20	0.32
8.	Fraction – 21	0.32

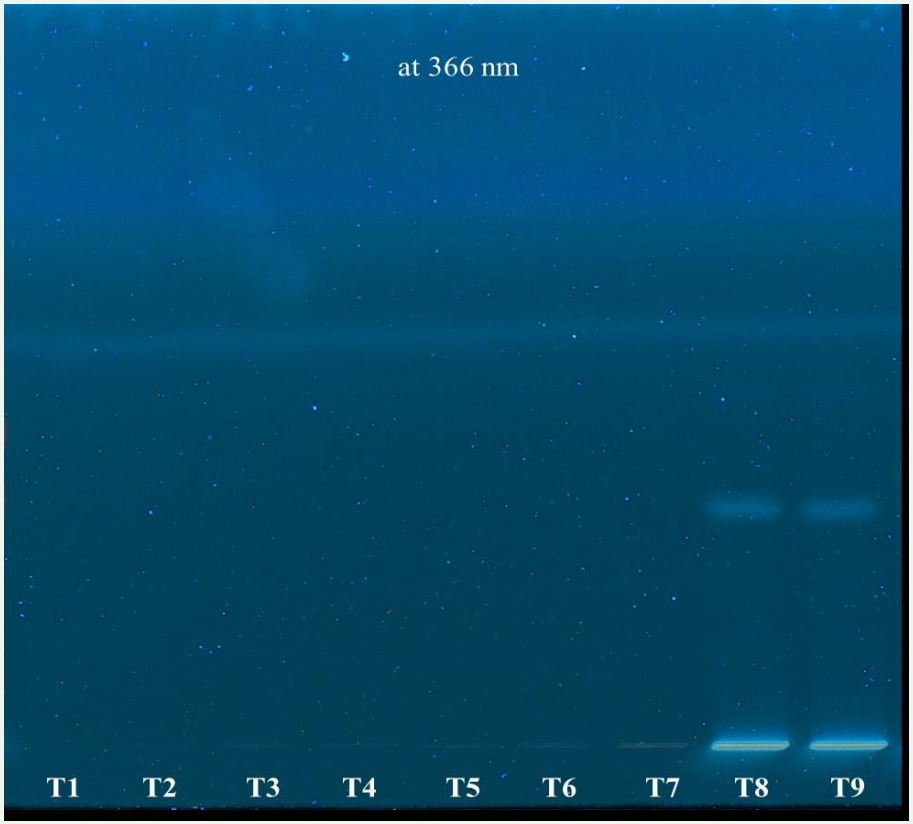
- The fractions 14-21 which showed the R_f value of 0.32 were pooled. The solvent present in it was evaporated, and the residue was then subjected to HPTLC for the detection and quantification of the isolated compound.

High-Performance Thin Layer Chromatography (HPTLC) of Methanol Extract (ME) of O. turpethum Roots

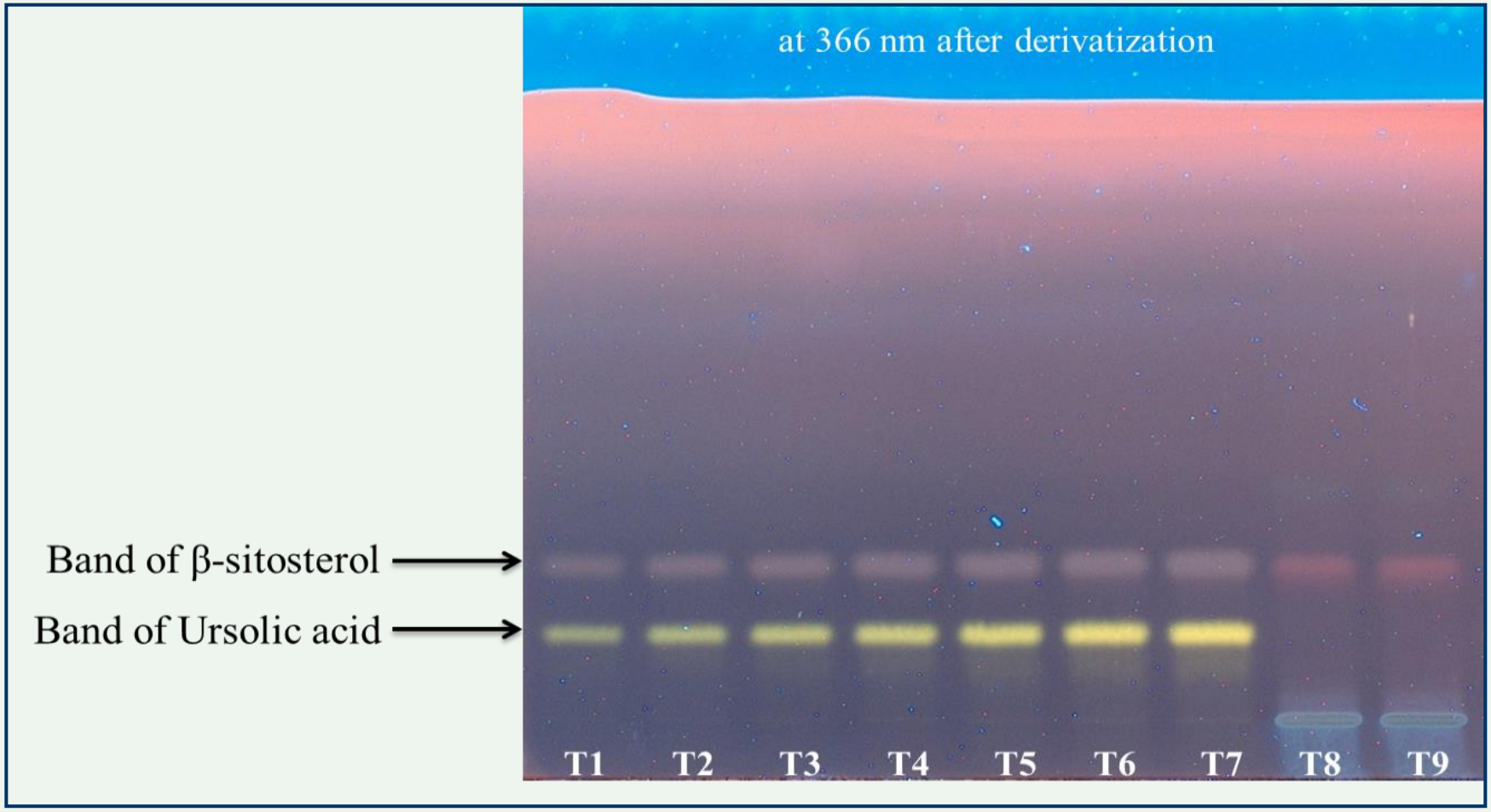
- The isolated compound was confirmed to be beta-sitosterol by comparing with reference standard by HPTLC studies.
- 2.0 µL, 3.0 µL, 4.0 µL, 5.0 µL, 6.0 µL, 7.0 µL, and 8.0 µL of the mixed standard working solution containing 100 µg/mL of ursolic acid and β-sitosterol as well as 15.0 µL of sample solution were spotted on the HPTLC plate using Linomat 5 applicator and developed in a mobile phase.
- HPTLC fingerprinting profile of standard ursolic acid and β-sitosterol as well as fraction of the methanolic extract of *O. turpethum* roots in different wavelength is shown in following Figure (A-C).

(A)

(B)



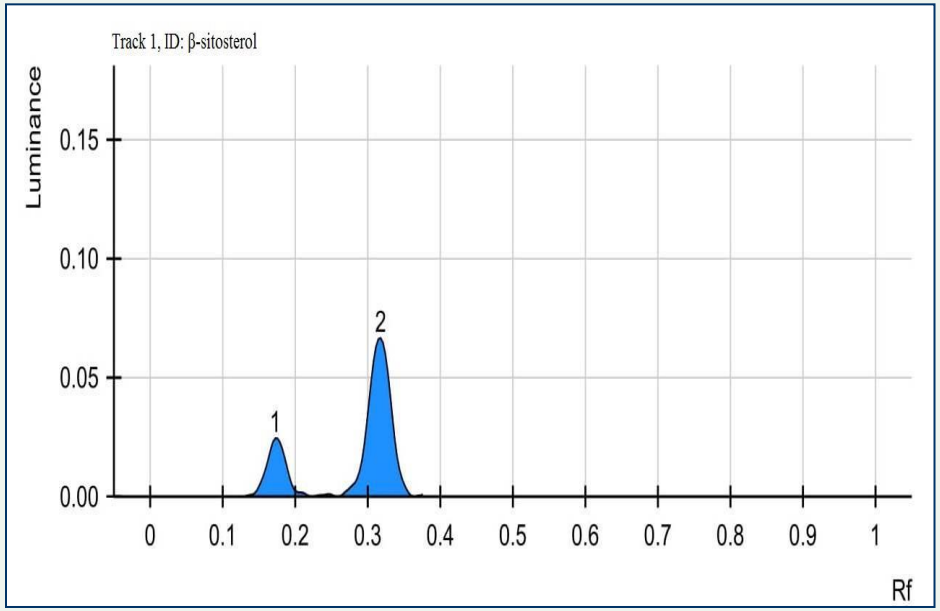
HPTLC fingerprinting profile of standard ursolic acid and β-sitosterol as well as the fraction of the methanolic extract of *O. turpethum* roots in visible light **(A)**, at 366 nm **(B)**.



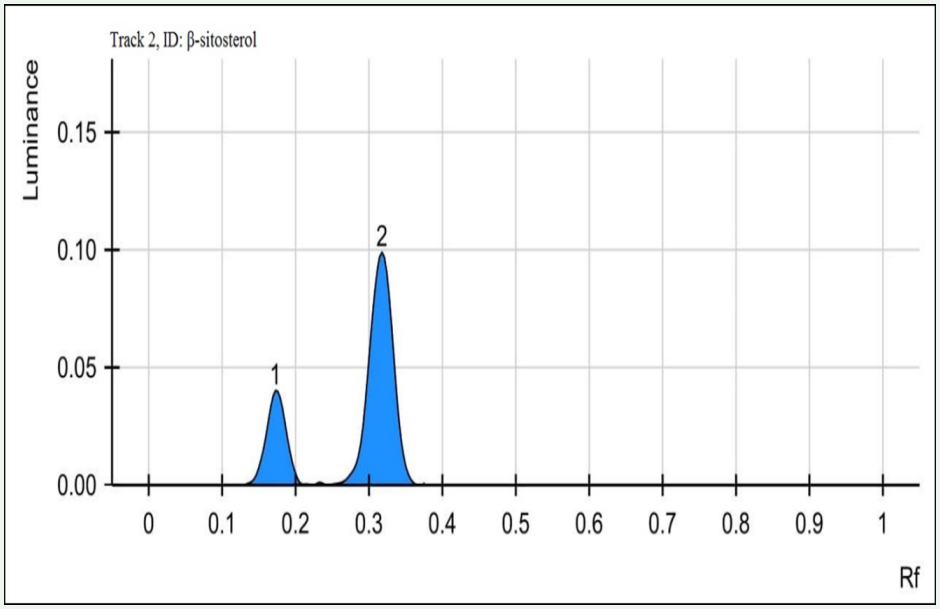
(C)

HPTLC fingerprinting profile of standard ursolic acid and β-sitosterol as well as the fraction of the methanolic extract of *O. turpethum* roots at 366 nm after derivatization **(C)**.

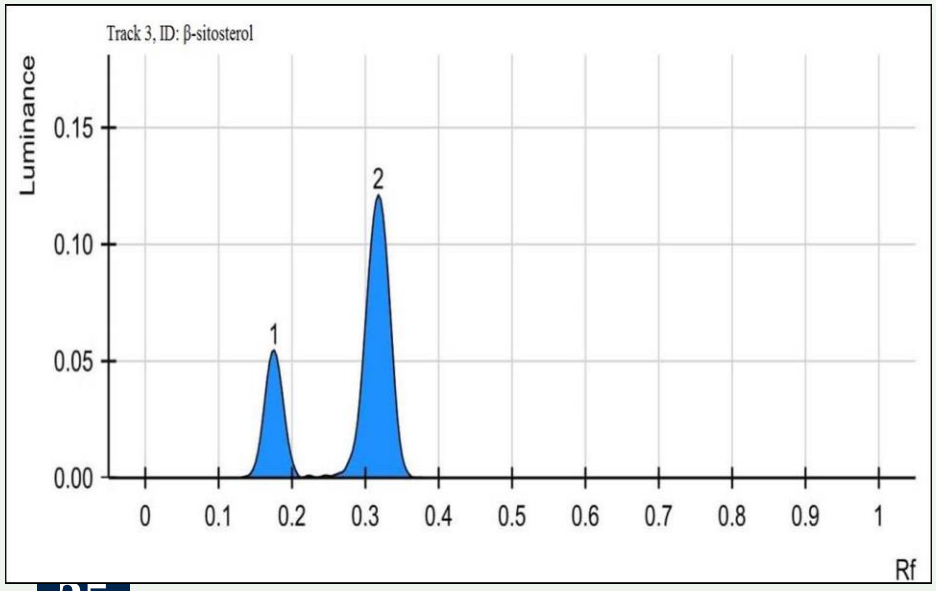
[T1, T2, T3, T4, T5, T6, and T7: Mixture of standard ursolic acid and β-sitosterol; T8 and T9: fraction of the methanolic extract of O. turpethum roots (MEOT)].


Track 1, ID: β-sitosterol
Luminance
0.15
0.10
0.05
0.00
1
2
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1
Rf

(D)


Track 2, ID: β-sitosterol
Luminance
0.15
0.10
0.05
0.00
1
2
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1
Rf

(E)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0.00
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2
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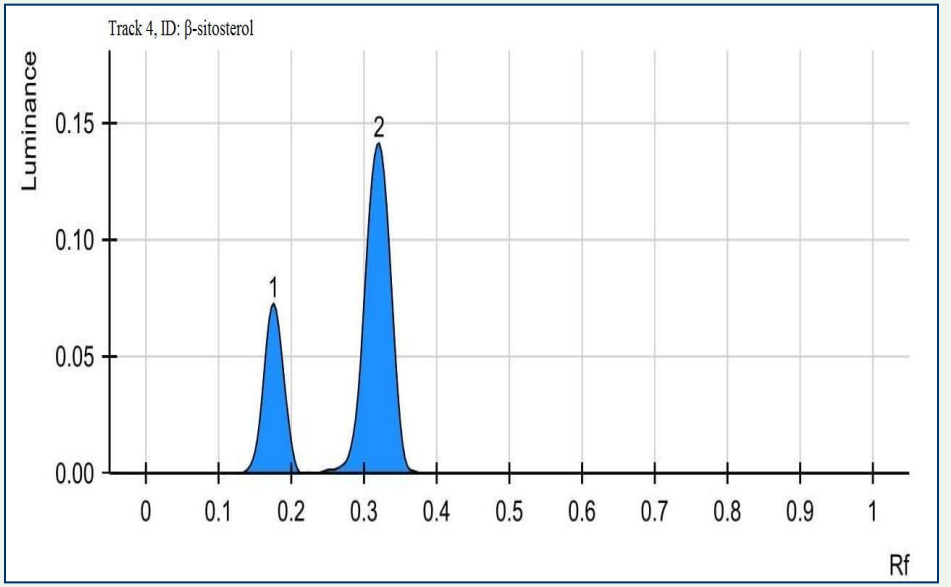
Rf

(F)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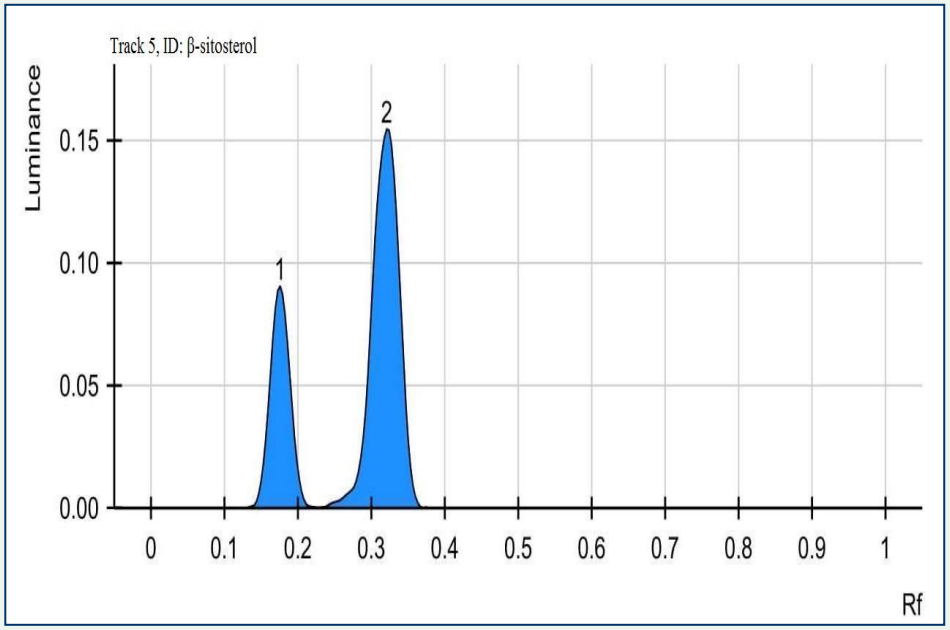
Rf

(G)



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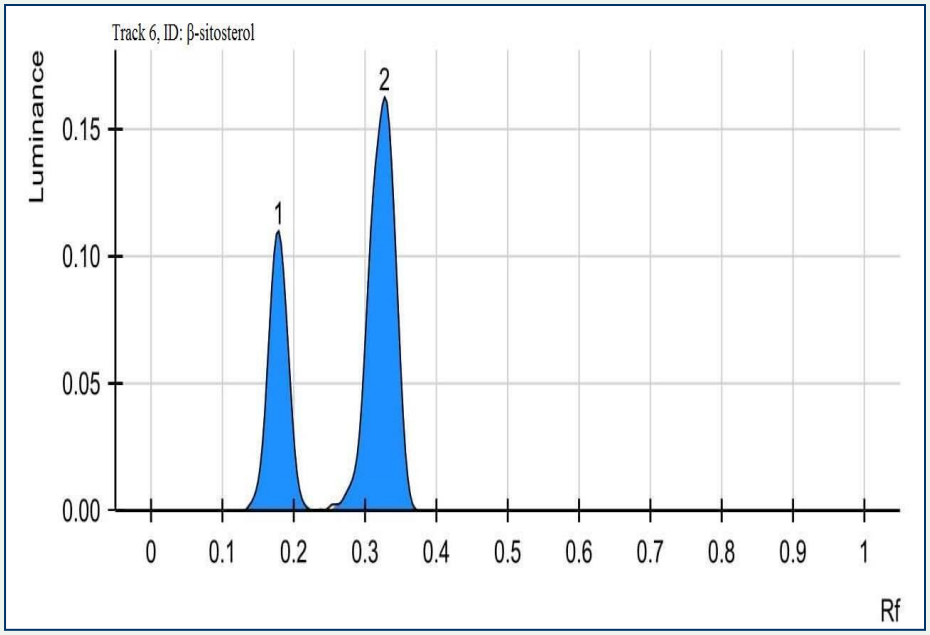
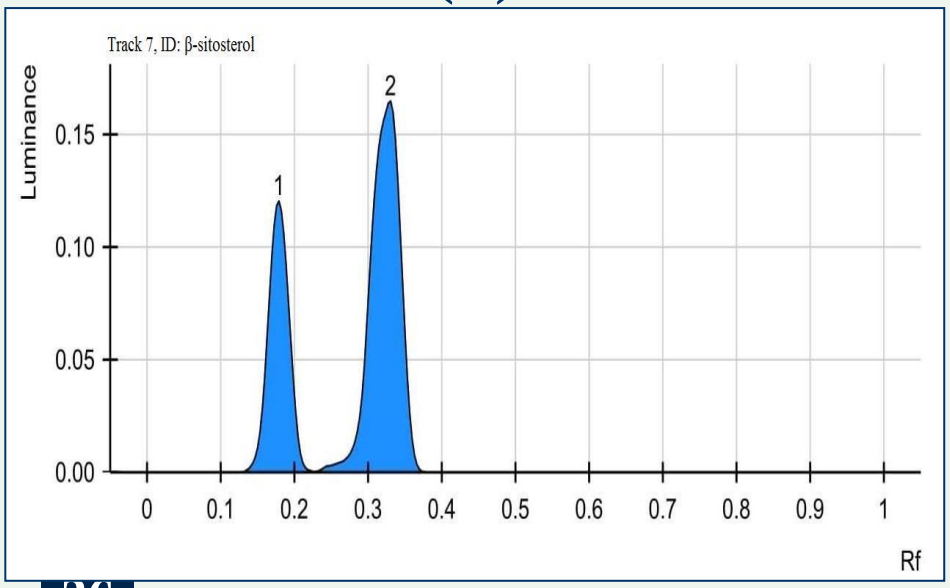


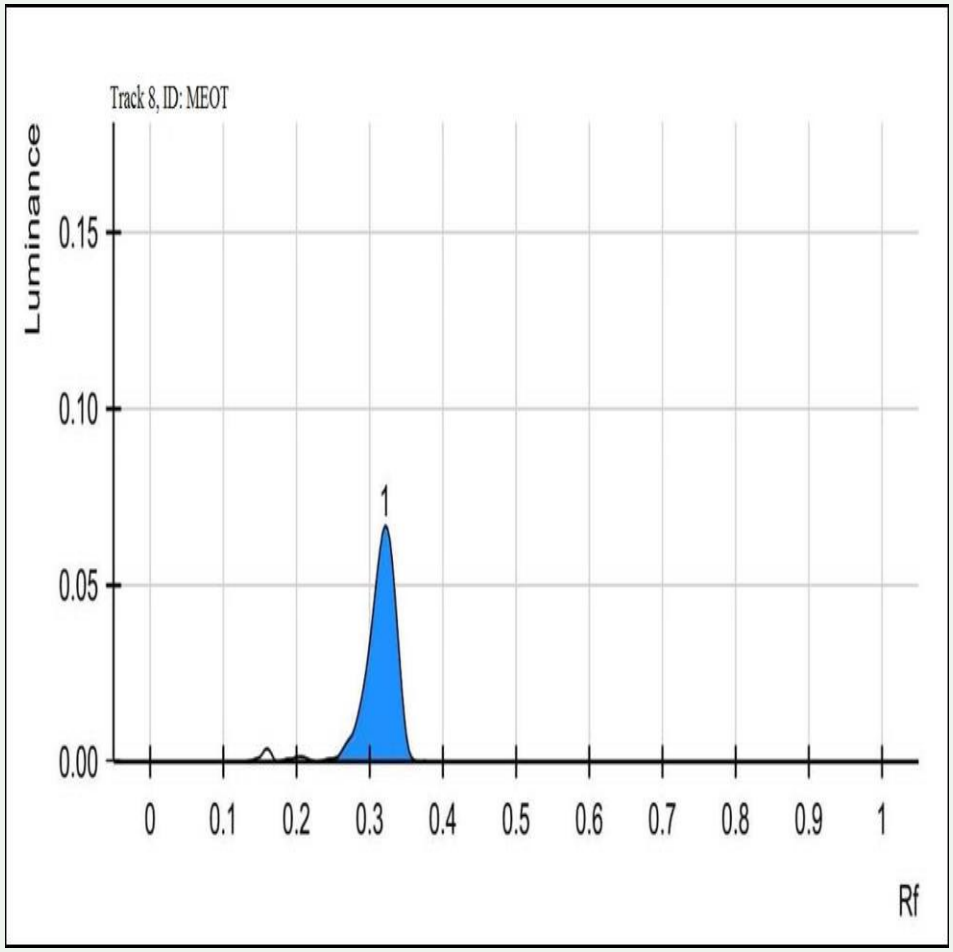
(I)



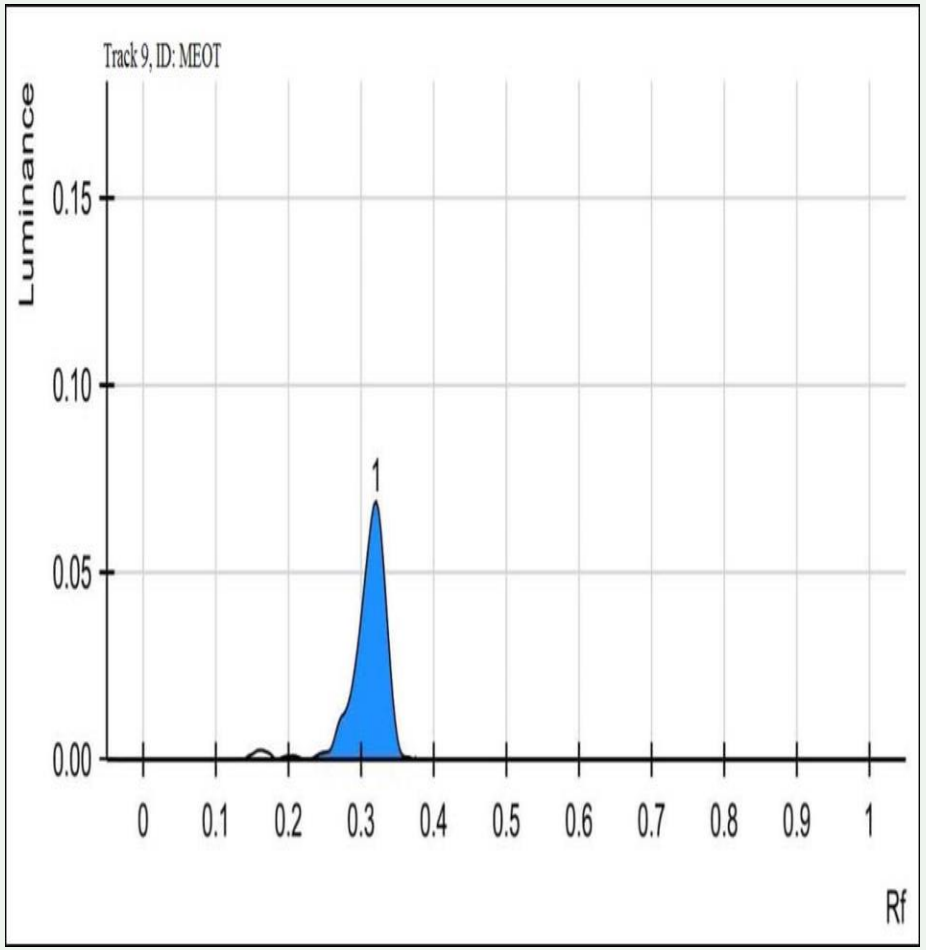
(J)

- HPTLC chromatogram of standard ursolic acid [R_f: 0.18] and β-sitosterol [R_f: 0.32] in different track by spotting different volume of mixture of standard solutions- Figure (D-J);

[1 Peak of ursolic acid and 2 Peak of β-sitosterol]



(K)



(L)

Figure (K) and (L): HPTLC chromatogram of fraction of methanolic extract of *O. turpethum* roots (MEOT) showing peak of β-sitosterol [R_f: 0.32] in different track by spotting 15.0 µL volume of sample solution.

- Results obtained from the HPTLC studies of the fraction of the methanolic extract of *O. turpethum* showed that MEOT showed only one spot at R_f 0.32, which was coinciding with the standard compound β-sitosterol (R_f value 0.32) indicating that the phytoconstituent present in it is a phytosterol (β-sitosterol). Thus by CAMAG HPTLC system with Win CATS programming software, one compound β-sitosterol identified and quantified.

Quantification of β-sitosterol in the fraction of MEOT (using 2 replicates of sample application)

- The amount of β-sitosterol was found to be 14.09 µg in 10.00 mg fraction of MEOT and which is reported in the following table:

Sample	Track	Volume applicate (µL/mL sample solution)	Coefficient of Variation (CV)	Amount of β-sitosterol
Fraction of MEOT	Track 8	15.0 µL	203.4 ng	13.56 µg/mL
	Track 9	15.0 µL	219.2 ng	14.61 µg/mL
			5.282%	14.09 µg/mL

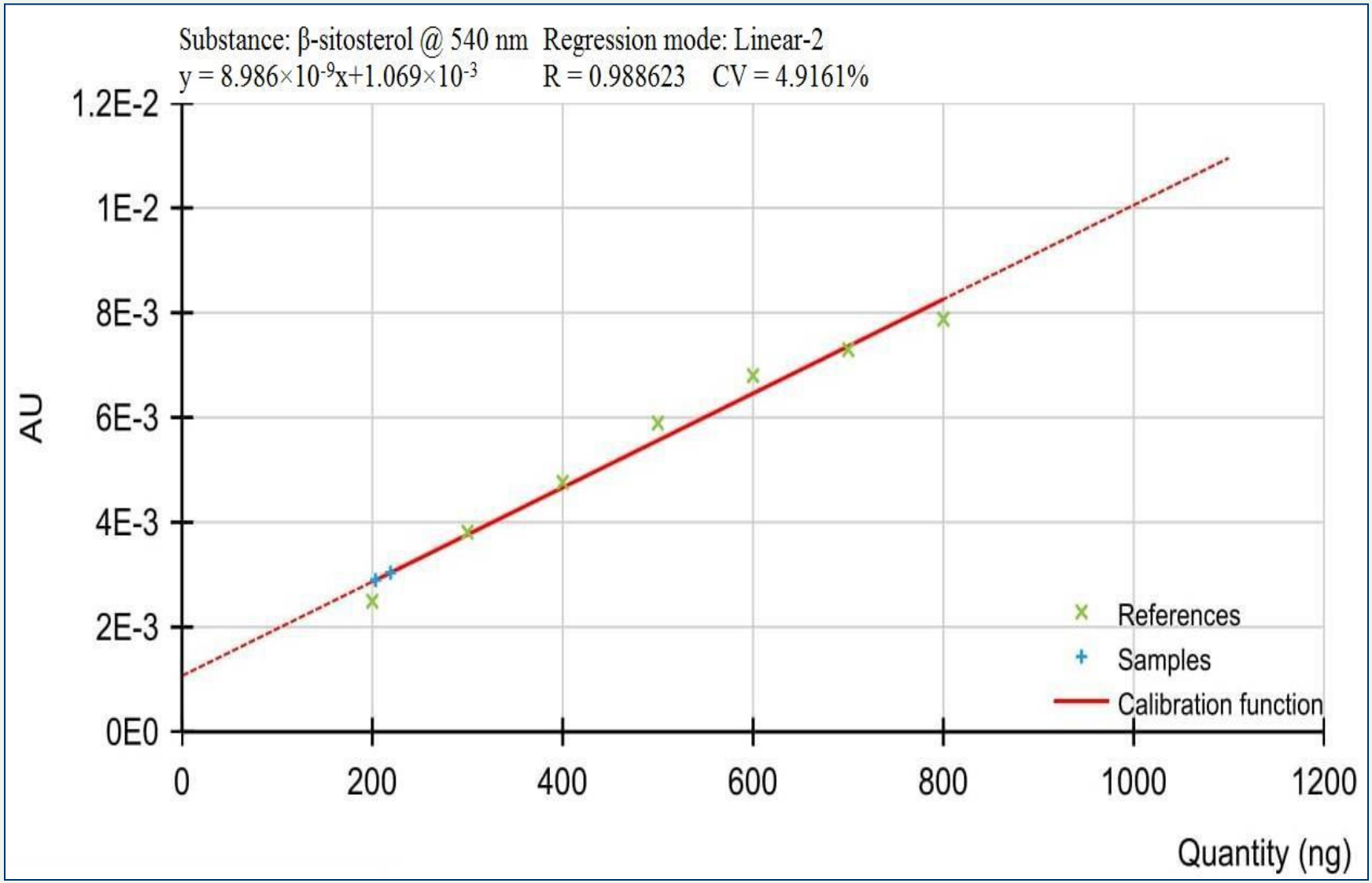


Figure : Calibration curve for beta-sitosterol

PHARMACOLOGICAL SCREENING

Acute Toxicity Studies

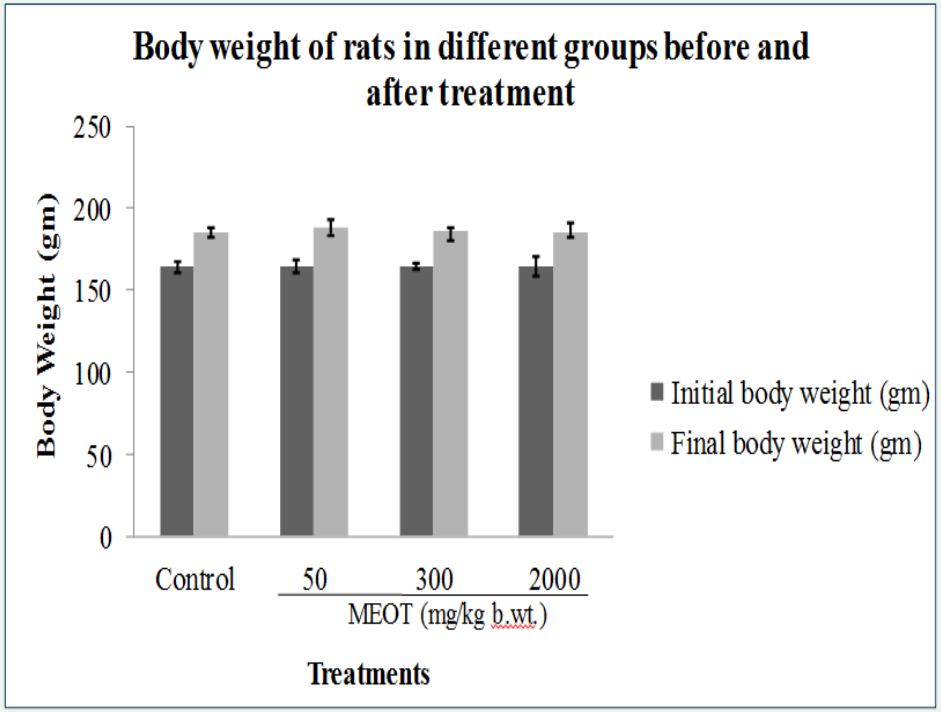
- Over the study duration of 14 days, there was no mortality observed after oral administration of MEOT up to a dose level of 2000 mg/kg body weight. Animals did not produce any toxic symptoms or any significant changes in the behavioral (general appearance) or autonomic responses.

Effect on the body weight

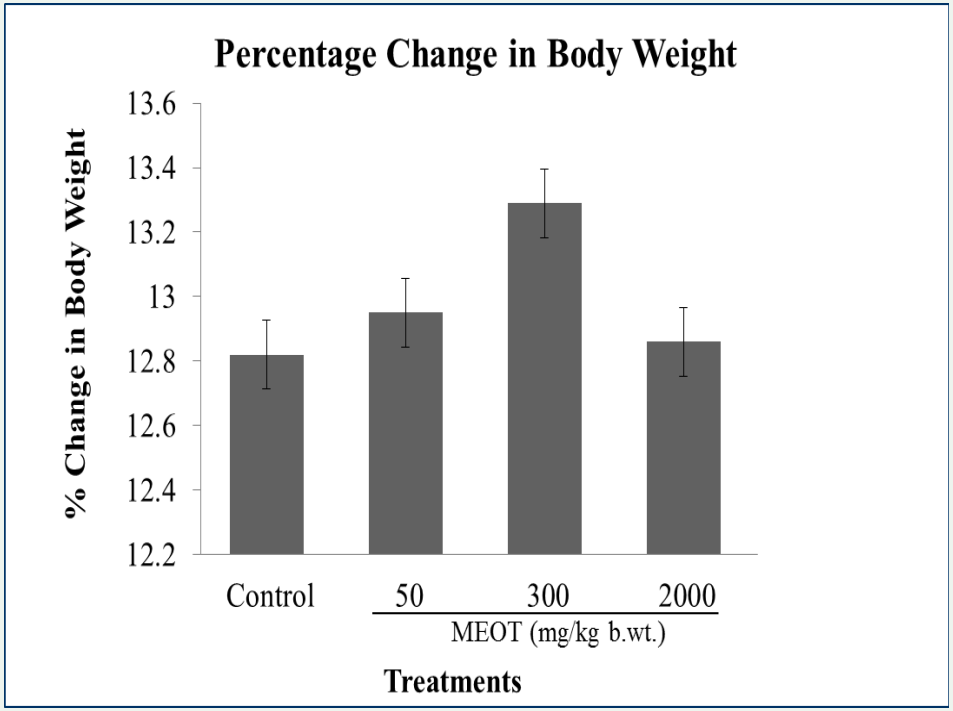
Groups	Treatment	Initial body weight (gm)	Final body weight (gm)	% Change in body weight
I	Control (vehicle)	164.23 ± 3.21	185.28 ± 3.20	12.82
II	MEOT (50 mg/kg b.wt.)	164.29 ± 3.97^{ns}	185.56 ± 5.02^{ns}	12.95
III	MEOT (300 mg/kg b.wt.)	164.22 ± 2.12^{ns}	186.04 ± 5.83^{ns}	13.29
IV	MEOT (2000 mg/kg b.wt.)	164.45 ± 5.45^{ns}	185.59 ± 2.95^{ns}	12.86

All the values are expressed as the Mean ± SEM, n = 3 animals in each group.
ns - Statistically not significant (p-value > 0.05) as compared to control.
Data were analyzed by One-way ANOVA followed by Dunnett Multiple Comparisons Test.

- MEOT treated groups were not significantly different (P > 0.05) when compared with vehicle control group, indicating that it did not have any adverse effects on the body weight.



Graphical representation of the acute toxicity of O. turpethum showing body weight of rats.



Graphical representation of the acute toxicity of O. turpethum showing % change in body weight of rats.

In Vitro Anti-inflammatory Activity

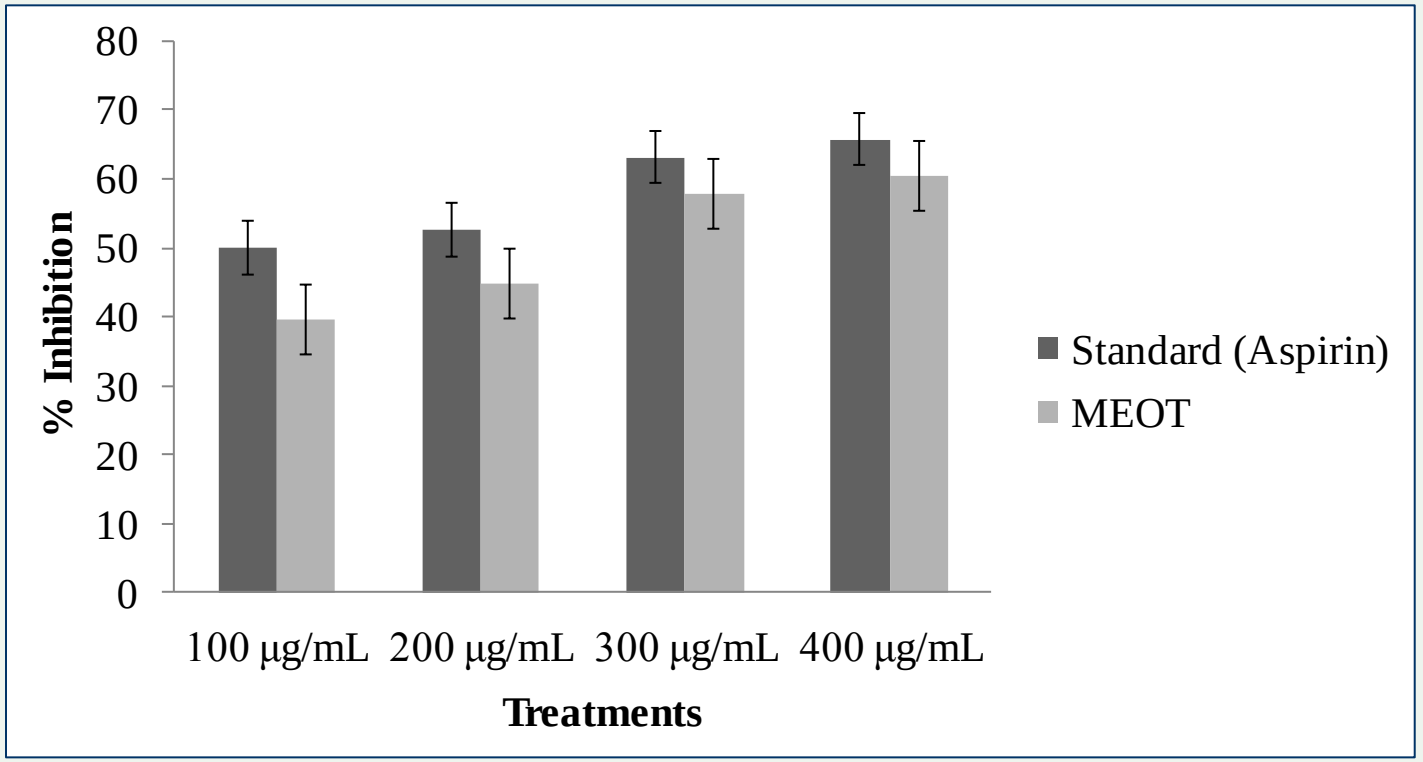
Inhibition of albumin denaturation

- *In vitro* anti-inflammatory activity of ME of *O. turpethum* by egg albumin denaturation inhibition method is tabulated in the following table:

Treatment	Concentration (µg/mL)	Absorbance at 660 nm	% Inhibition of albumin denaturation
Control	-	0.38 ± 0.026	-
Standard (Aspirin)	100	0.19 ± 0.019**	50.00
	200	0.18 ± 0.021**	52.63
	300	0.14 ± 0.016**	63.15
	400	0.13 ± 0.018**	65.78
MEOT	100	0.23 ± 0.021**	39.47
	200	0.21 ± 0.018**	44.73
	300	0.16 ± 0.022**	57.89
	400	0.15 ± 0.016**	60.52

All the values are expressed as the Mean ± standard error of the mean (SEM), n = 5.
** - Statistically significant (p-value < 0.01) as compared to control.
Data were analyzed by One-way Analysis of Variance (ANOVA) followed by Dunnett Multiple Comparisons Test.

- MEOT at a concentration of 400 µg/mL showed maximum activity, while aspirin, a standard anti-inflammatory drug at a concentration of 400 µg/mL showed the maximum inhibition of 65.78% as compared with control.



Graphical representation of the in-vitro anti-inflammatory activity of O. turpethum showing percentage inhibition of albumin denaturation.

In Vivo Anti-inflammatory Activity

Egg albumin-induced inflammation model

- *In vivo* anti-inflammatory activity of ME of *O. turpethum* (Paw volume change in different group) by egg albumin-induced paw edema testing in rats is tabulated in the following table:

Groups	Treatment	Dose (mg/kg)	Time intervals (h) and Paw Volume (mL)				
			0	1	2	3	4
I	Control	-	4.42±0.05	5.59±0.03	5.86±0.03	5.99±0.03	6.15±0.02
II	Standard	10	4.02±0.03**	4.65±0.02**	4.95±0.02**	4.30±0.02**	3.00±0.07**
III	MEOT	100	4.21±0.03**	5.05±0.04**	5.64±0.03**	4.75±0.03**	4.02±0.03**
IV	MEOT	200	4.19±0.03**	5.00±0.04**	5.51±0.05**	4.50±0.04**	3.68±0.05**
V	MEOT	400	4.09±0.04**	4.95±0.04**	5.35±0.04**	4.34±0.05**	3.03±0.02**

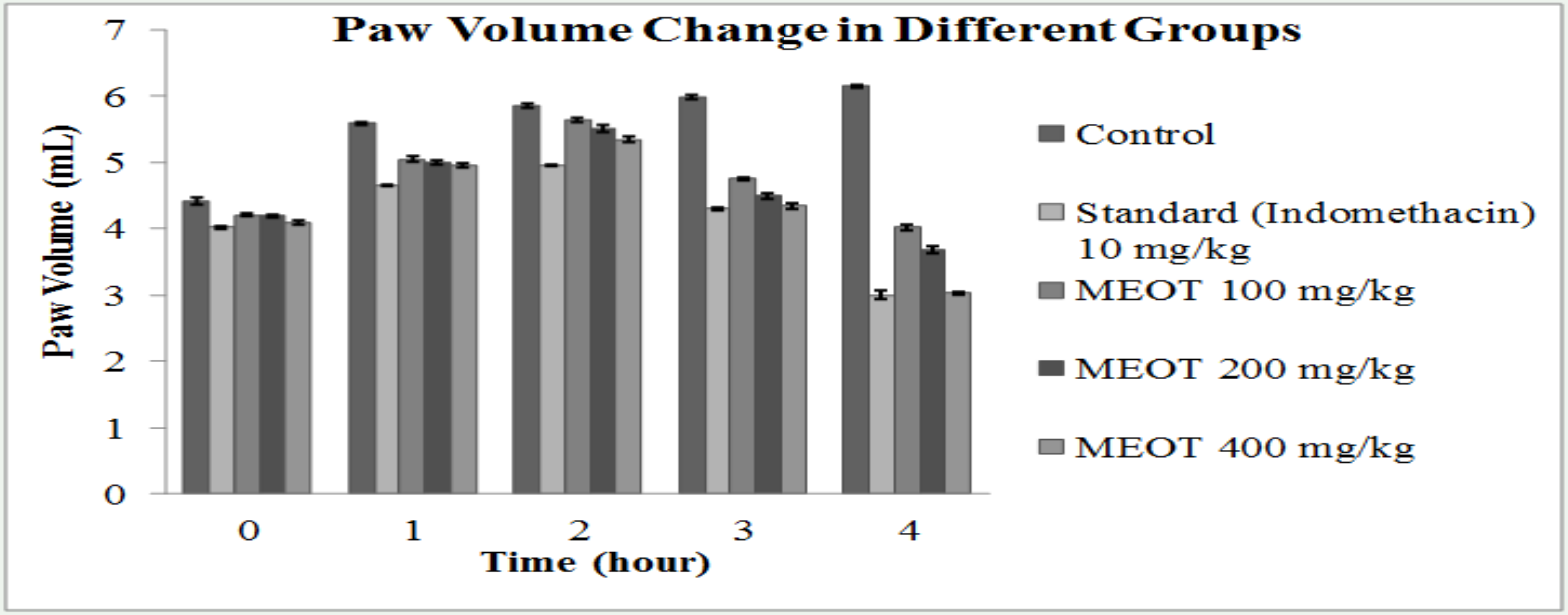
All the values are expressed as the Mean ± SEM, n = 5.
** - Statistically significant (p-value < 0.01) as compared to control group.
Data were analyzed by one-way ANOVA followed by Dunnett's Test.

- In egg albumin-induced edema in rats, ME caused a significant ($p < 0.01$) dose-dependent anti-inflammatory effect against edema indicated by the reduced paw volume.

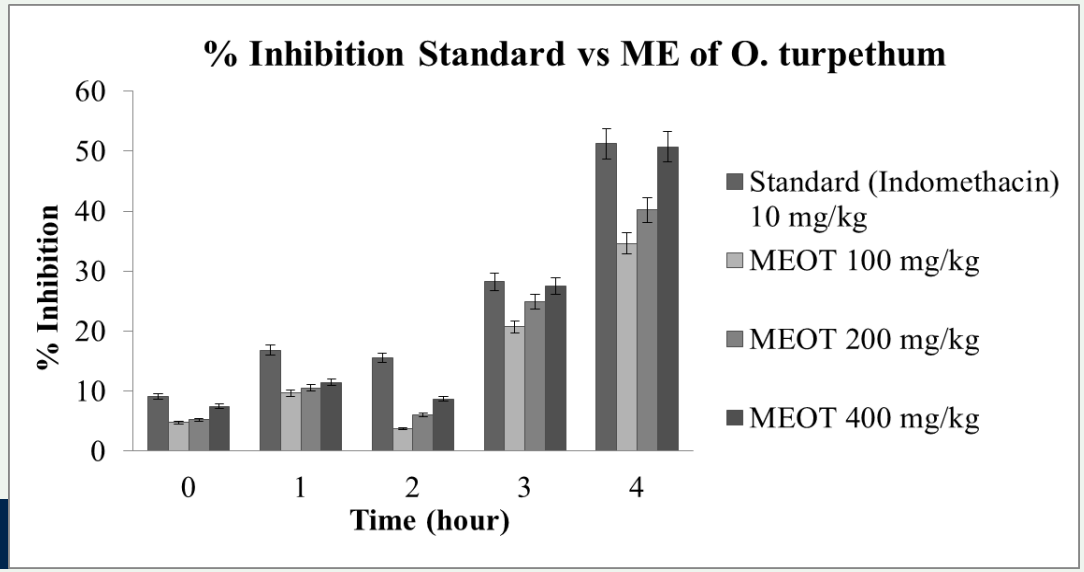
- Percentage edema inhibition of ME of *O. turpethum* in comparison with standard in egg albumin-induced paw edema testing in rats is tabulated in the following table:

Groups	Treatment	Dose (mg/kg)	Time intervals (h) and % Edema Inhibition				
			0	1	2	3	4
I	Control	-	-	-	-	-	-
II	Standard (Indomethacin)	10	9.04	16.81	15.52	28.21	51.21
III	MEOT	100	4.75	9.66	3.75	20.70	34.63
IV	MEOT	200	5.20	10.55	5.97	24.87	40.16
V	MEOT	400	7.46	11.44	8.70	27.54	50.73

All the values are expressed as the Mean%, n = 5.



Graphical representation of the in-vivo anti-inflammatory activity of O. turpethum showing paw volume change in different groups at the different time.



Graphical representation of the % Inhibition of ME of O. turpethum in comparison with the standard in egg albumin-induced paw edema.

Cotton pellet-induced granuloma model

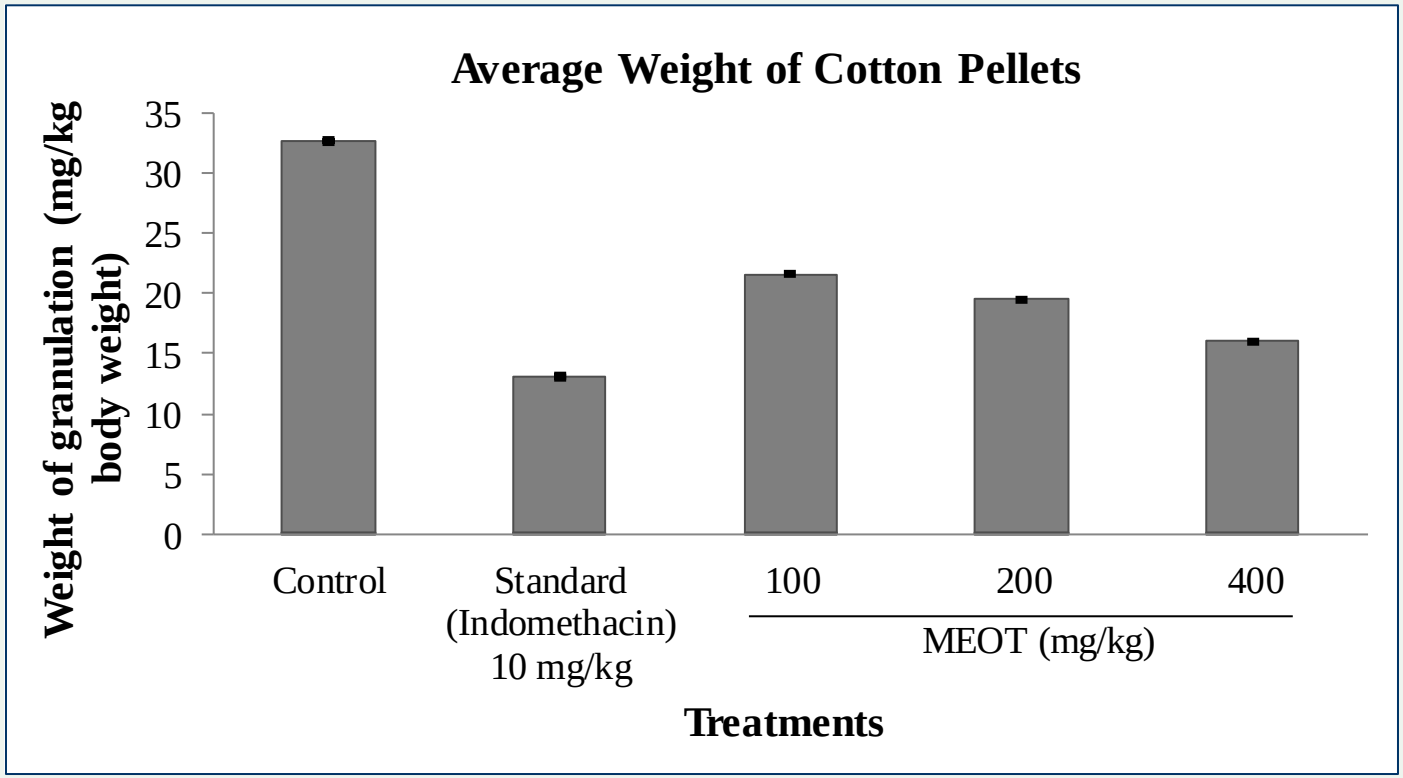
- *In vivo* anti-inflammatory activity of ME of *O. turpethum* by cotton pellet-induced granuloma in rats is tabulated in the following table:

Groups	Treatment	Dose (mg/kg)	Weight of granulation (in mg)	% Inhibition
I	Control	-	32.74 ± 0.055	-
II	Standard (Indomethacin)	10	13.06 ± 0.021**	60.10
III	MEOT	100	21.59 ± 0.010**	34.15
IV	MEOT	200	19.50 ± 0.013**	40.43
V	MEOT	400	16.02 ± 0.011**	51.06

All the values are expressed as the Mean ± SEM, n = 5.
** - Statistically significant (p-value < 0.01) as compared to control.
Data were analyzed by one-way ANOVA followed by Dunnett's Test.

Cont.

- In cotton pellet-induced granuloma, MEOT significantly ($p < 0.01$) diminished the formation of granuloma (i.e., 16.02 ± 0.011) in a dose-dependent manner. MEOT at the dose of 400 mg/kg displayed maximum granuloma inhibition (51.06%) and which is comparable to that of standard drug, indomethacin.



Graphical representation of the effect of the average weight of cotton pellets for different groups.

CONCLUSION

- *The present study has reported that the several extracts of Operculina turpethum roots were qualitatively examined for the presence of the preliminary phytochemicals and it was confirmed that these plant extracts contain metabolites named as alkaloids, flavonoids, steroids, terpenes, tannins, phenols, cardiac glycosides, saponins, carbohydrates, reducing sugar; and did not indicate the presence of proteins, amino acids, and quinones.*

- *MEOT is destitute of toxicity up to 2 g/kg in Wistar albino rats. The extract showed dose-dependent anti-inflammatory activity, which was found to be statistically significant.*

- *From the present study, the obtained findings confirm that Operculina turpethum contains β-sitosterol which is responsible for potent anti-inflammatory without any cytotoxicity of the plant.*

FUTURE PROSPECTS

- *From this study, it is concluded that the roots of the plant Operculina turpethum contains β-sitosterol which possess anti-inflammatory activity.*
- *This study presents an idea that the compound of plant O. turpethum can be employed as a lead compound for designing an effective anti-inflammatory drug which can be employed to cure edema.*

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