The 7th International Electronic Conference on Medicinal Chemistry (ECMC 2021)
01–30 NOVEMBER 2021 | ONLINE

HPLC based fractionation and microtiter plate based bioactivity profiling of *Ocimum sanctum* Linn. leaves for potential pancreatic lipase inhibitor(s)

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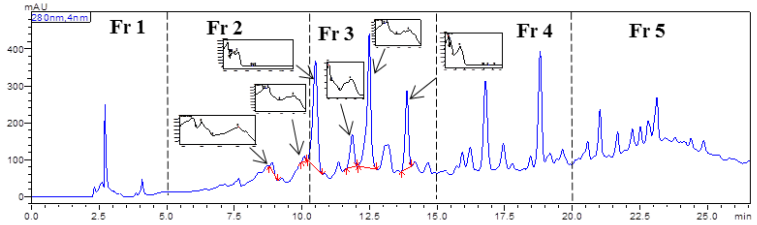
Graphical Abstract

Ocimum sanctum

Ethanolic extract

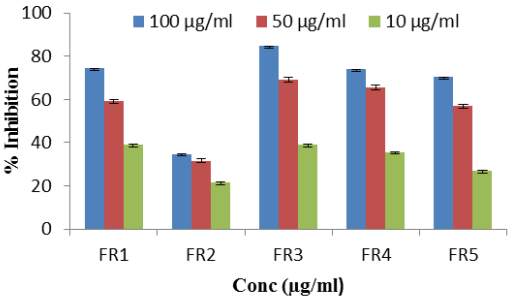
Screening of *O.sanctum* for lipase inhibition



HPLC based bioactivity fractionation

Enzymatic (spectrophotometric assay)

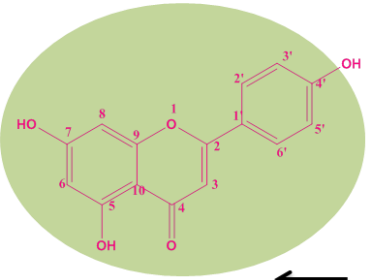
Bioactivity fractionation through Semi-prep / Analytical method



Isolated compound

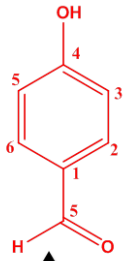
Structure elucidation through U.V, NMR, Mass, HR-MS

in -vitro PL inhibiton of isolated compound

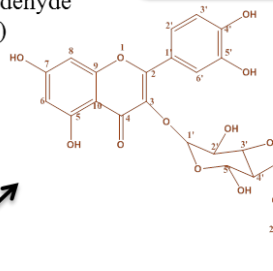


Apigenin (**API**)

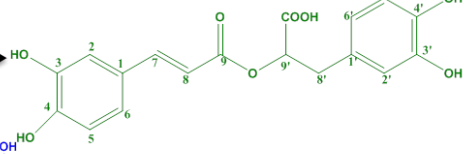
Most potent PL Inhibitor (IC_{50} =7.80 µg/mL)



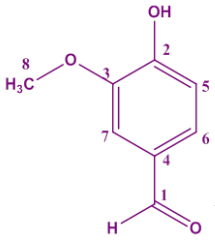
4-Hydroxy benzaldehyde (**4-HB**)



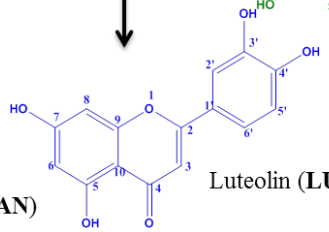
Rutin (**RUT**)



Rosmarnic acid (**RA**)



Vanillin (**VAN**)



Luteolin (**LUT**)

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ABSTRACT

Background: *Ocimum sanctum* (family- Lamiaceae) is used in the traditional medicinal systems of India, China, Malaysia, and Thailand. The aerial parts are used to treat various health ailments viz. stomachache, common colds, headaches, obesity, and heart disease.

Aim: The objective of the present study is to explore pancreatic lipase inhibitory activity of *Ocimum sanctum* leaves fractions and to identify the bioactive phytochemical(s).

Method: Air-dried (*O.sanctum*) plant material (200 g) was collected and extracted in ethanol (3×500 ml) at room temperature (25±5°C) by cold percolation. The solvent was filtered and evaporated under a vacuum. The prepared extract was investigated for '*in-vitro*' lipase activity using the spectrophotometric assay. *OS* leaves part shows potent PL inhibition. Isolation of compounds through up scaling in semi-preparative HPLC with isocratic elution of solvent-A (0.1% v/v, AcOH) and Solvent-B (MeOH) at flow rate 3.0 mL/min was performed at 280 nm.

Results: HPLC-based profiling was developed and activity-guided fractionation using *in-vitro* pancreatic lipase inhibition. In the present investigation, three flavone and two phenolic aldehyde compounds were isolated from *OS* and tested PL inhibitory activity. These compounds were characterized by spectroscopic methods HPLC, ESI-MS, HR-MS, and NMR. Among isolated compounds, apigenin was most potent PL inhibitor (IC_{50}= µg/ml)

Conclusion: The PL inhibitory activity of *OS* extract (IC_{50} < 100 µg/ml) and its phytomolecules (IC_{50} = 7.80 - 95.31 µg/ml). The study confirms that *OS* leaves for potential PL inhibitor(s). *Ayurvedic* plants may be useful for the management of obesity which correlates with ethnomedicinal data on the use of these plants in Indian traditional medicines.

Keyword: PL; pancreatic lipase, OS; *Ocimum sanctum*, HPLC; High performance liquid chromatography

Introduction

- *Ocimum sanctum* (family- Lamiaceae) also known as Holy basil or Tulsi has been used for medicinal purposes in traditional system of India, China, Malaysia and Thailand.
- It is an erect annual herb extremely pungent and bitter in taste, worldwide approx.150 varieties of OS were found.
- Two spicies of Ocimum, *O. sanctum* and *O. basilicum*, are most widely distributed and studied variety.
- It is found to contain important phytoconstituents such as oleanolic acid, ursolic acid, rosmarinic acid, eugenol, carvacrol, linalool.
- It has been regulate metabolic stress through normalization of blood glucose, blood pressure and lipid levels, and psychological stress through its anxiolytic and anti-depressant properties.
- Tulsi acts as a safe and effective alternative to the prescription of antibacterial, antimicrobial activity, wound healing, antibacterial, antiviral, antifungal, mosquito repellent, anti-diarrheal, anti-oxidant, immunomodulatory and heptoprotection.
- The inhibition of pancreatic lipase activity of *O. sanctum* extracts and orlistat (positive control) tested following standard *in-vitro* protocol.
- We have evaluated the lipase inhibitory activity of *O. sanctum* extracts & its isolated compound for potent lipase inhibitory activity.

Methodology

- The *Ocimum* aerial parts were collected from research farm of CIMAP, Lucknow, India.

- Air-dried (*O. sanctum*) plant material (200.0 g) was collected and extracted in ethanol (3×500 ml) at room temperature (25±5°C) by cold percolation. The solvent was filtered and evaporated under a vacuum.

- The prepared extract was investigated for '*in-vitro*' lipase activity using the spectrophotometric assay

- *OS* leaves part shows potent PL inhibition. Isolation of compounds through up scaling in semi-preparative HPLC with fraction was performed using RP-HPLC column C_{18} (10 cm×10 mm,10µm) isocratic elution of solvent-A (0.1% v/v, AcOH) and Solvent-B (MeOH) at flow rate 3.0 mL/min was performed at 280 nm.

Reference- Podsedek, A., Majewska, I., *Journal of agricultural and food chemistry*,2014,*62*(20), 4610-4617.

O. sanctum (200 g)

Alcoholic Extract (16.30 g)

In-vitro pancreatic lipase inhibition

Isolation of compounds (semi-preparative HPLC/Analytical HPLC)

Characterization of compoundsby spectroscopic methods HPLC, ESI-MS, HR-MS, and NMR

Bioactivity based fractionation of *O.sanctum*

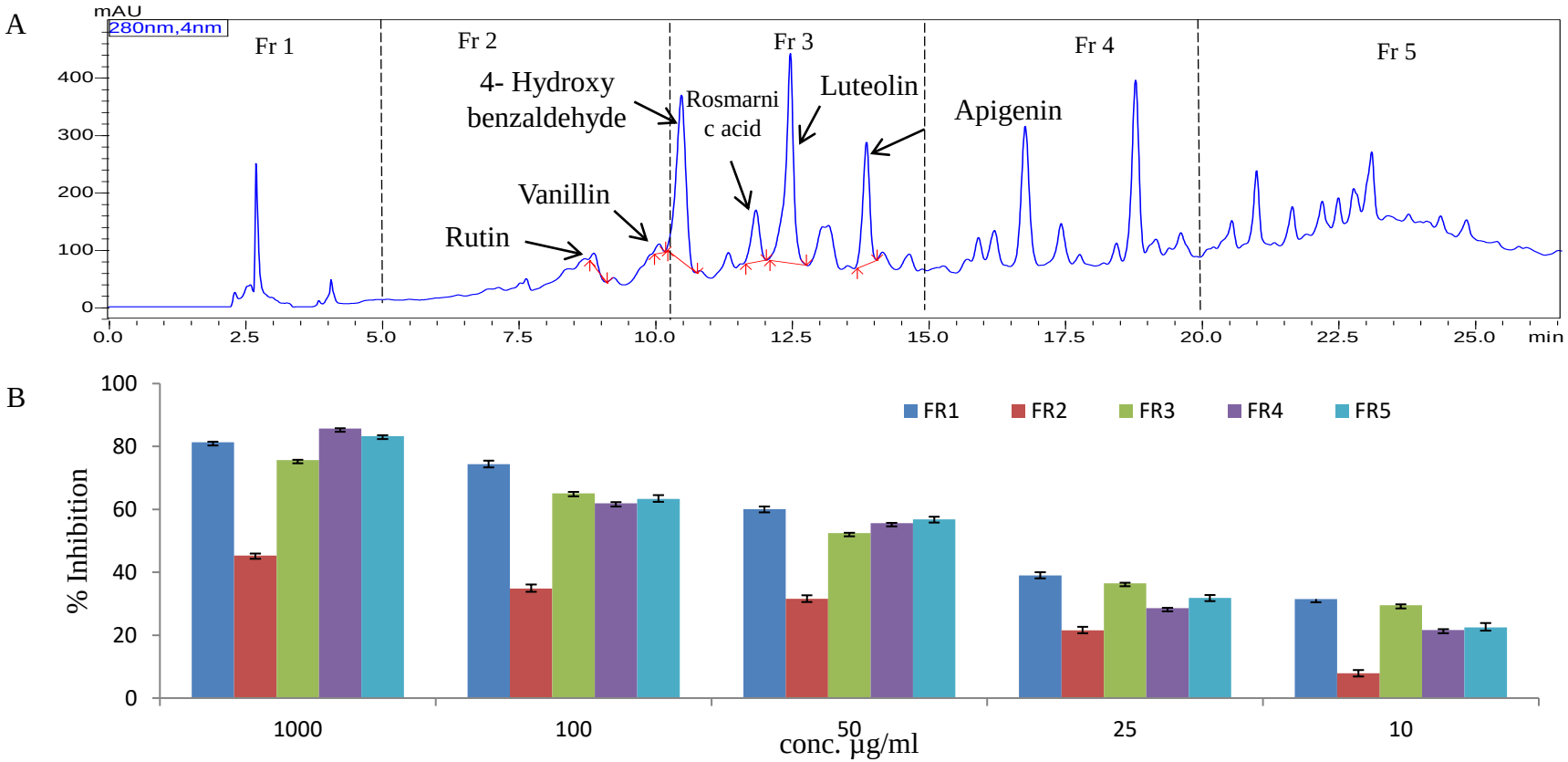
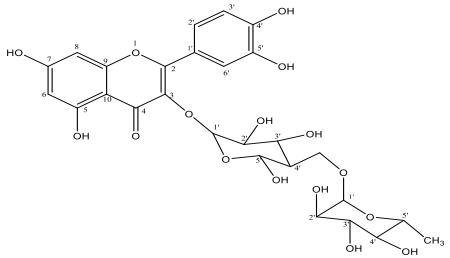


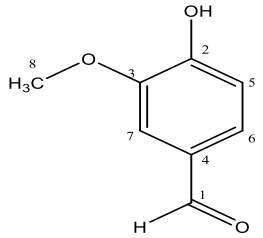
Fig. **A** HPLC based bioactivity fractionation of OS ethanol extract recorded at 280 nm with time segments (5min) of fractionation (I-V) shown in figure. **B** Pancreatic lipase activity was measured using 4-methylumbelliferyl oleate as a substrate. Fractions relative lipase activity (%) was calculated as (activity of compound with substrate – negative control of compound without substrate)/(activity without compound and with substrate – negative control without compound and substrate)×100.

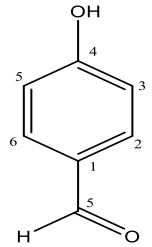
Isolation of secondary metabolites of *O.sanctum* through Prep-HPLC and Preperative-TLC

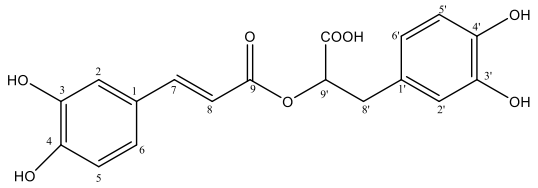
- In Prep-HPLC fractionation method was developed.
- *Ocimum sanctum* (1.0 g) extract fraction are collected at 30 min in 5 min interval.
- Third fraction (10-15 min) are the most efficient fraction showd PL inhibition activity proceed for isolation of compounds through Prep-HPLC and purify through Prep-TLC .
- In Prep-HPLC, isolation of six compound were isolated on the basis of Rt and U.V pattern.
- Confirmation and structure elucidation of four compound are done through HR-MS,1H NMR spectroscopy.

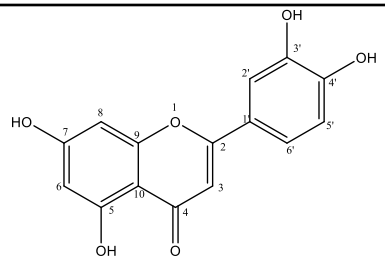
Structure elucidation of *O.sanctum*

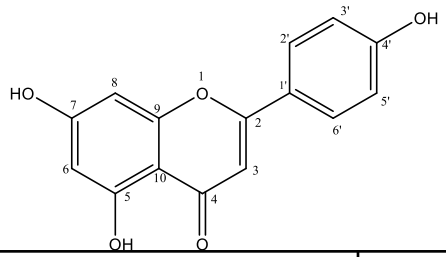
Compound B	**Rutin**		
Appearance	Yellow powder		
Molecular formula	$C_{27}H_{30}O_{16}$		
HR-MS (m/z)	610.52		
Melting point	243º C		
^{1}HNMR (500 MHz $CDCl_3$)	δH 6.21(1H, d, J=2, C6-H), 6.38 (1H, d, J=2, C8-H),7.62 (1H, d, J=2.1,C2'-H),6.89(1H, d J=9,C5'-H),	7.64 (1H, dd, J=9,2.1, C6'-H),8.07 (1H, s, C4'-OH), 8.05 (1H, s, C3'-OH), 12.62 (1H, s, C5-OH),	7.88 (1H, s, C7-OH), 5.12 (1H, d, J=7.4, H1-G),4.55 (1H, d, J=1.9, H1-R), 1.15 (3H, d, J=6.1,CH3- R)
^{13} C NMR (125 MHz $CDCl_3$)	158.50 (C-2),135.75 (C-3), 179.41 (C-4), 159.40 (C-5), 100.06 (C-6), 166.01(C-7), 95.03 (C-8), 162.92 (C-9), 105.68(C-10),	123.71 (C-1'), 116.17(C-2'), 145.6 (C-3'), 149.3 (C-4'), 117.85 (C-5'), 123.19 (C-6'),104.88 (C1-G), 75.82(C2-G),	78.23(C3-G), 74.03(C4-G), 77.21(C5-G), 69.80 (C6-G), 102.49 (C1-R), 71.45 (C2-R), 72.16 (C3-R), 72.33 (C4-R), 68.66(C5-R), 18.00 (C6-R) 128.45 (C-6')

Compound E	**Vanillin**		
Appearance	White crystalline powder		
Molecular formula	**$C_8H_8O_3$**		
HR-MS (M/z)	152.15		
Melting point	83º C		
^{1}HNMR (500 MHz $CDCl_3$)	δ 9.81(1H,s,H-1),9.78(1H,s,H-2), 7.02 (1H,d,J-8.2Hz, H-5),	7.40 (1H,d,J-2.0Hz,H-6),	6.64 (1H,s,H-7), 3.91(1H,s,8)
^{13} C NMR (125 MHz $CDCl_3$)	δ 191.11 (C-1), 151.89 (C-2), 147.28 (C-3), 129.76 (C-4), 127.58 (C-5),	114.52 (C-6), 108.93 (C-7),	56.09 (C-8), 30.0 (OCH3) 128.45 (C-6')

Compound F	**4-Hydroxybenzaldehyde**		
Appearance	Yellow powder		
Molecular formula	**$C_7H_6O_2$**		
HR-MS (M/z)	122.02		
Melting point	310º C		
^{1}HNMR (500 MHz $CDCl_3$)	δ 9.75 (C-5,s,1H),	6.92 (C-3,d, 2H);	7.77 (C-2,d,2H),
^{13} C NMR (125 MHz $CDCl_3$)	δ 129.14 (C-1), 133.57 (C-2), 116.99 (C-3),	165.26 (C-4), 115.96 (C-5),	130.55 (C-6), 193.03(C-5');

Compound A	**Rosmarinic acid**		
Appearance	White powder		
Molecular formula	$C_{18}H_{16}O_8$		
HR-MS (m/z)	360.32		
Melting point	172º C		
^{1}HNMR (500 MHz $CDCl_3$)	δH 3.34 (d,13.8 Hz,OH) 7.04 (d,1.9Hz,C-2), 6.78 (d,8Hz, C-5), 6.96 (dd,8 Hz,C-6), 6.71(dd,8.0Hz, C-6’	7.56 (d,16 Hz,C-7), 6.28 (d,16 Hz,C-8), 6.88 (br,s,C-2’), 6.75 (d,8.0Hz,C-5’),	3.11(dd,13.8Hz, C-7’), 5.19 (d,8.8Hz,C-8’)
^{13} C NMR (125 MHz $CDCl_3$)	^{13}C NMR (125 MHz, MeOD): δ 123.28 (C-1), 115.39 (C-2), 145.41 (C-3),;	146.30 (C-4), 116.46 (C-5), 124.1 (C-6), 149.86 (C-7), 114.60 (C-8), 168.63 (C-9),	129.46 (C-1’), 117.73 (C-2’), 146.95 (C-3’), 148.85 (C-4’), 116.66 (C-5’), 127.83 (C-6’), 38.08 (C-7’), 74.82 (C-8’), 173.73 (C-9’) 128.45 (C-6’)

Compound D	**Luteolin**		
Appearance	Yellow crystal		
Molecular formula	$C_{15}H_{10}O_6$		
HR-MS (m/z)	286.04		
Melting point	330º C		
^{1}HNMR (500 MHz $CDCl_3$)	δH 6.91(d,1H,J= 7.5Hz,C-6), 6.54 (s,1H,C-2’),	6.44 (d,1H,J=2Hz,C-5’),6.21 (d,1H,J=2.5Hz,C-6’)	
^{13} C NMR (125 MHz $CDCl_3$)	163.9 (C-2), 102.5 (C-3), 182.5 (C-4), 161.8 (C-5), 98.7 (C-6), 164.8 (C-7),	93.6 (C-8), 158.0 (C-9), 103.9 (C-10), 122.3 (C-1’), 112.8 (C-2’), 145.6 (C-3’),	149.6(C-4’), 115.4 (C-5’), 118.9 (C-6’). 128.45 (C-6’)

Compound C	**Apigenin**		
Appearance	Yellow crystalline solid		
Molecular formula	**$C_{15}H_{10}O_5$**		
ESI-MS (M/z)	271.21		
Melting point	345º C		
^{1}HNMR (500 MHz $CDCl_3$)	δH 6.78 (1H, s, C2-H), 6.19(1H, d, J=2.1, C6-H),6.48 (1H, d, J=2.1,C8-H),	7.93 (2H, d, J=8.8,C2', 6'-H), 6.93 (2H, d, J=8.8, C3',5'-H),10.39 (1H, s, C4'-OH),	12.96 (1H, s, C5-OH), 10.80 (1H, s, C7-OH);
^{13}C NMR (125 MHz $CDCl_3$)	164.13 (C-2),102.83 (C-3), 181.72 (C-4), 157.30 (C5), 98.83 (C-6), 163.74 (C-7),	93.95 (C-8), 161.44 (C-9),103.68 (C-10), 121.17 (C-1'), 128.45 (C-2'),	115.95 (C-3'), 161.14 (C-4'), 116.8 (C5'), 128.45 (C-6')

Pancreatic lipase inhibitory potential (IC_{50}) of *O. sanctum* isolated compounds

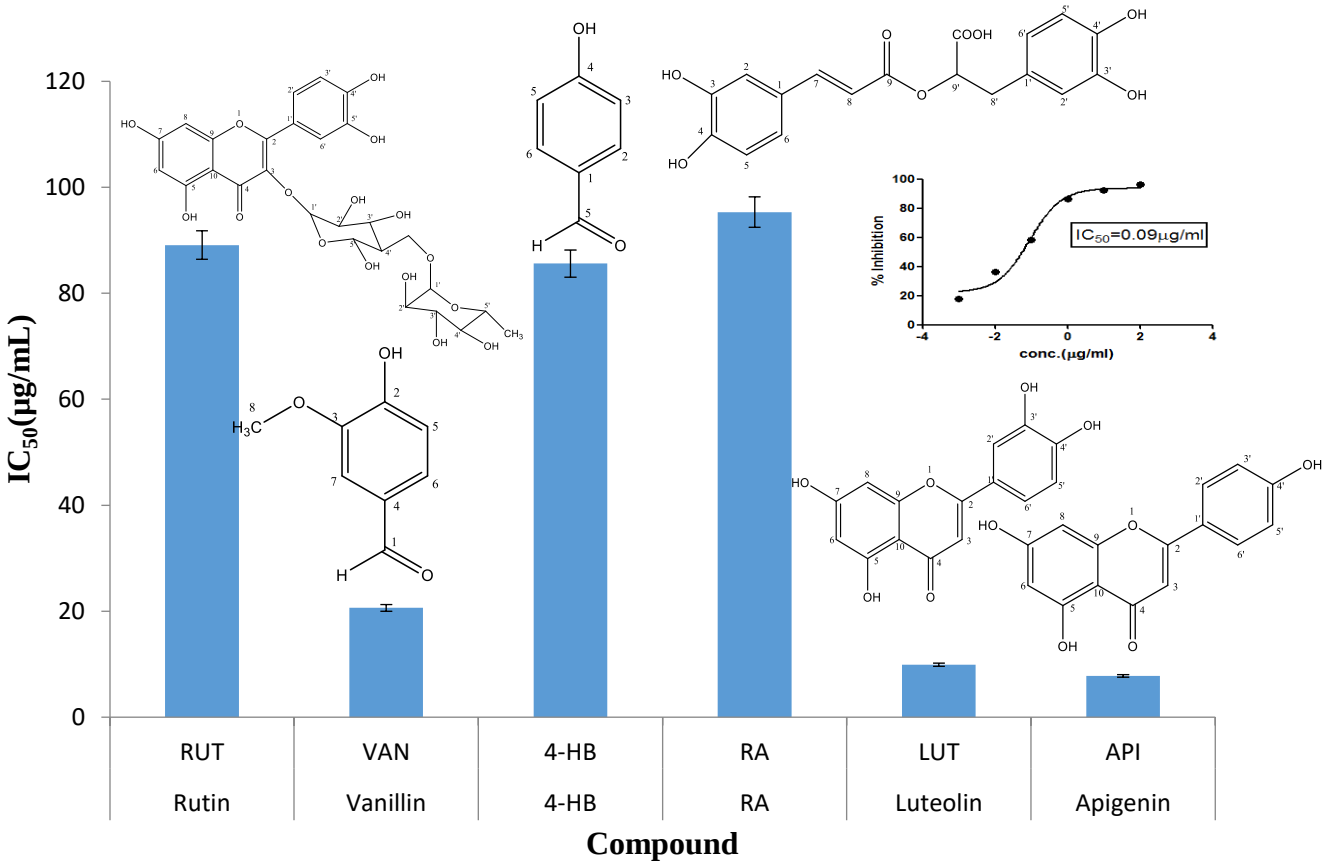


Fig. Pancreatic lipase inhibitory potential of *O. sanctum* isolated compunds Rutin (**RUT**), Vanillin (**VAN**), 4-Hydroxybenzaldehyde (**4-HB**), Rosmarnic acid (**RA**), Luteolin (**LUT**),, Apigenin (**API**). Results are expressed as the mean ± S.D. of three independent experiments, shown in set

Results and discussion

- Alcoholic extract of *O.sanctum* aerial part (IC_{50} = 41.35±1.45 µg/ml) will be selected for processing and isolation of compounds, according to their lipase inhibitory activity.

- Ethanolic extract of *O.sanctum* aerial part fractionation was done, develop HPLC based bio-assay fractionation and *in-vitro* PL inhibition activity.

- Isolation of six compound from ethanol fraction of OS, three flavone, two phenolic aldehyde and one phenolic acid class of compounds.

- PL inhibition of Apigenin (IC_{50}=7.80±0.25 µg/ml) showed potent lipase inhibition.

- The chemical characterization of isolated compounds done through HR-MS, NMR , IR, and U.V.

CONCLUSION

- The aerial part of *O.sanctum* showed the lipase inhibitory potential less than (IC_{50} < 100 µg/ml).
- Overall study proves that Apigenin (**API**) is the most potent PL inhibitor and can be considered as a potential natural supplement for the management of obesity.
- The study indicates lipase inhibition potential of *Ayurvedic* plants, may be useful for the management of obesity which correlate with Ethanobotanical data on the use of these plants in Indian folklore.

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ACKNOWLEDGEMENT

The author is very thankful to Director, CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow for providing research facilities and CSIR–SRF for supporting this work through fellowship [CSIR-SRF(31/34/(0202)/2018-EMR-1)].