Influence of solvents and techniques for extraction of phenolic phytochemicals linked with antioxidant and enzyme inhibition potential of *Ocimum tenuiflorum* Linn.

Amrita Chatterjee, Prashanta Kumar Deb, Biswatrish Sarkar*
Department of Pharmaceutical Sciences & Technology
Birla Institute of Technology, Mesra, Ranchi-835215
Email ID: *chatterjee.amrita95@gmail.com / biswatrishsarkar@bitmesra.ac.in*

ABSTRACT

Ocimum tenuiflorum Linn. (Synonym: *O. sanctum*; Common name: Krishna Tulsi; Purple Basil) is a household herb of India which is traditionally revered for its diverse medicinal properties. The therapeutic properties of this plant have been linked to its array of polyphenolic repository. Thus, the current study was designed to optimize the suitable solvent and extraction technique for maximum recovery of the polyphenols from this herb, to achieve maximum therapeutic benefits linked with its antioxidant and enzyme inhibition properties. Dried aerial parts of the plant were powdered and extracted repeatedly 3 times with different solvents (methanol, 95% methanol, ethanol, 95% ethanol, ethyl-acetate and acetone) at ambient temperature. Different extraction techniques like cold maceration, soxhlet extraction, heat reflux, microwave assisted extraction, ultrasonic assisted extraction and ultrasonic assisted ionic liquid-based extraction were also performed. Variation in the extractive yield, recovery of the total polyphenol, total flavonoid, total tannin and total anthocyanin content in these extracts were estimated along with antioxidant (DPPH, ABTS, FRAP, PMD) and enzyme inhibition (α-glucosidase and pancreatic lipase) properties. Maximum yield of crude extract (23.5%) was obtained with 95% methanol with highest content of phenolic phytochemicals which also exhibited superior antioxidant and enzyme inhibition capacity. Whereas, cold maceration was found to be the best technique to recover maximum polyphenolic compounds with highest *in-vitro* bioactivities. Thus, it can be concluded that hydro-methanolic solvent and cold maceration technique can be used for the extraction of *O. tenuiflorum* L. as it yielded maximum polyphenolic phytochemicals and best therapeutics properties.

Key words: Antioxidant; Enzyme inhibitions; Extraction; *Ocimum tenuiflorum*; Polyphenol; Solvent Optimization

INTRODUCTION

- ***Ocimum tenuiflorum*** **Linn. (OT) is commonly known as Krishna Tulsi is one of the most important traditional and medicinal herb of India**
- **The therapeutic properties of OT has been linked with its repository of diverse range of phytochemicals, mostly polyphenols**
- **Polyphenols are the major class of phytochemical responsible for antioxidant and other wide ranges of biological activities**
- **Choice of solvent and extraction techniques are most important factor for extraction and recovery of bioactive phytochemicals**
- **This comparative study will give the idea to use suitable solvent and most effective technique to extract and recover bioactive molecules**

METHODOLOGY

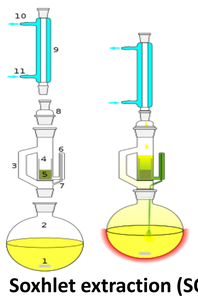
Ocimum tenuiflorum Linn.

OT aerial part coarse powder

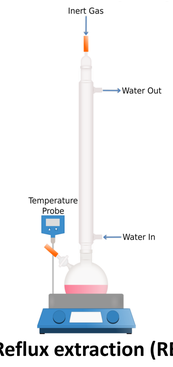
Optimization of Extraction technique

Microwave assisted extraction (MAE)

Cold maceration (CME)



Soxhlet extraction (SOX)



Reflux extraction (REF)

Ultrasonic assisted extraction (UAE) and UAE with ionic liquid (ILE)

Optimization of Extraction Solvents

Cold maceration technique was employed for extraction with different solvents at room temperature (25 ± 2 ºC) for 24 h

Solvents

- **Methanol (ME)**
- **95% Methanol (MH)**
- **Ethanol (ET)**
- **95% Ethanol (EH)**
- **Acetone (Ac)**
- **Ethyl acetate (EA)**

Estimation of extractive yield

Estimation of Phytochemical Contents

- Total polyphenol (TPC)
- Total flavonoid (TFC)
- Total proanthocyanidin (TPAC)
- Total anthocyanin (TAC)

Antioxidant Assay:

- FRAP
- DPPH
- ABTS
- PMD

Enzyme inhibition Assay:

- Pancreatic lipase
- α-glucosidase

RESULTS

Comparison of extraction recovery

Solvents used	% Yield
ME	19.7
ET	9.65
Ac	9.05
EA	4.95
MH	23.5
EH	18.74

Techniques	% Yield
CME	23.5
MAE	18.96
UAE	14.01
SOX	17.8
REF	14.07
ILE	15.4

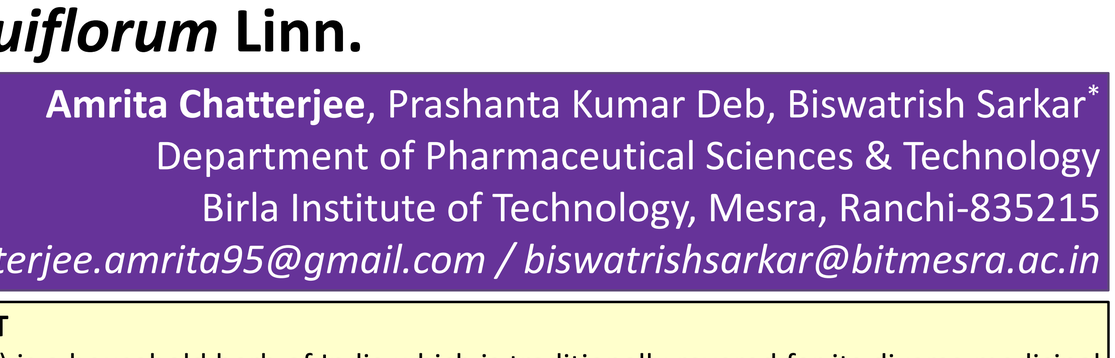


Figure 1: Comparison of different extraction solvents. A-Total phenolic content (TPC) and flavonoid content (TFC) of the different extracts; B-Total proanthocyanidin content (TPAC) and anthocyanin content (TAC) of the different extracts; The data are expressed as mean ±SD (n=3). Different alphabets above the bars indicates significant difference (P<0.05) when compared between each other.

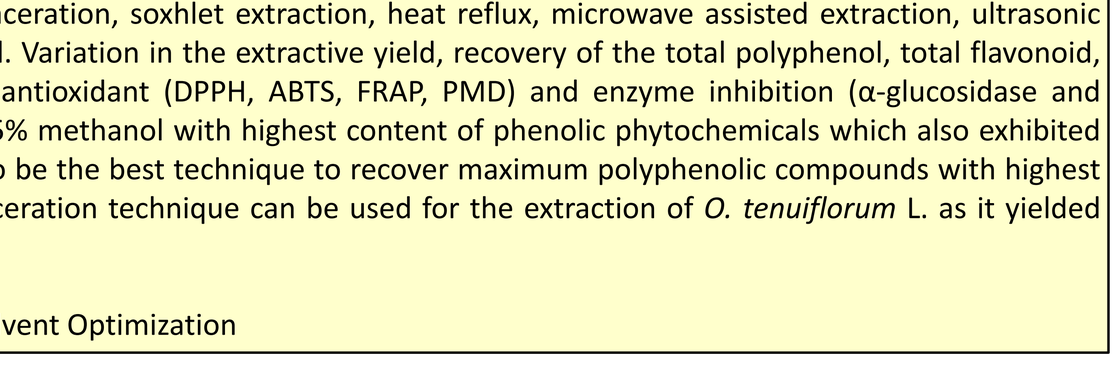


Figure 2: Comparison of different extraction solvents. A-DPPH and ABTS radical scavenging potential; B- Ferric reducing assay power (FRAP) and phosphomolybdenum assay (PMD); C- Pancreatic lipase enzyme inhibition assay; D- α-glucosidase enzyme inhibition potential. The data are expressed as mean ± SD (n=3). Different alphabets above the bars indicates significant difference (P<0.05) when compared between each other.

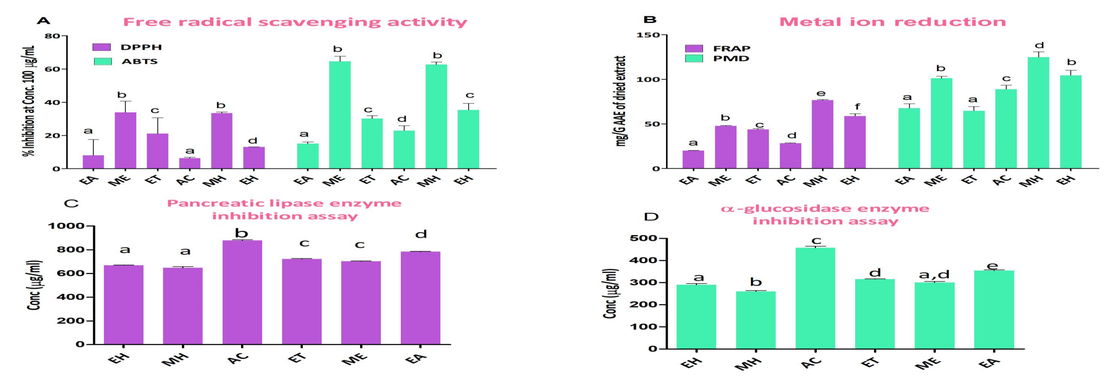


Figure 3: Comparison of different extraction techniques. A-Total phenolic content (TPC) and flavonoid content (TFC) of the different extracts; B-Total proanthocyanidin content (TPAC) and anthocyanin content (TAC) of the different extracts; The data are expressed as mean ±SD (n=3). Different alphabets above the bars indicates significant difference (P<0.05) when compared between each other.

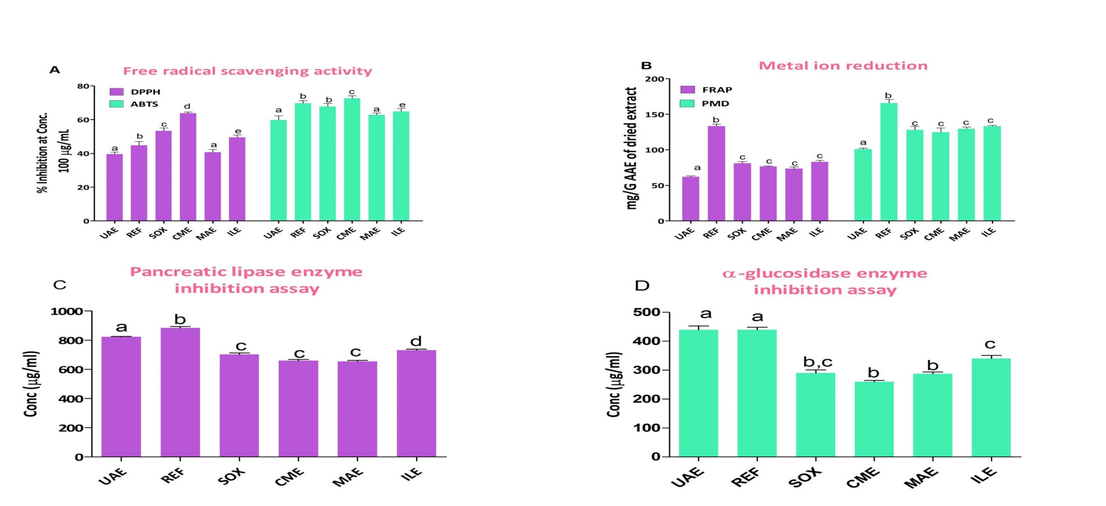


Figure 4: Comparison of different extraction techniques. A-DPPH and ABTS radical scavenging potential; B- Ferric reducing assay power (FRAP) and phosphomolybdenum assay (PMD); C- Pancreatic lipase enzyme inhibition assay; D- α-glucosidase enzyme inhibition potential. The data are expressed as mean ± SD (n=3). Different alphabets above the bars indicates significant difference (P<0.05) when compared between each other.

CONCLUSION

From the findings it can be concluded that, cold maceration extraction technique using 95% methanol as a solvent recovers significantly high content of phenolic phytochemical and exhibited strong antioxidant potential. Hence it could be used for bulk extraction of polyphenols from Krishna Tulsi.

Science & Engineering Research Board
Department of Science & Technology
Government of India

Acknowledgment: **DST, SERB Project (File No. EMR/2016/ 005695)**