International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Int. J. of Life Sciences, 2023; 11 (4): 389-395
ISSN:2320-7817(p) | 2320-964X(o)

Research Article Open Access

Screening and detection of Proteinicious Protease Inhibitor from Some Plants from MTR, Maharashtra, India

Kohli MB[1], Salve AN [2] and Jadhav AG[1]

[1]Department of Microbiology, | [2]Department of Botany, Government Institute of Science, Aurangabad, 431004, India.
Corresponding author: Jadhav A. G Email- akgjadhav@gmail.com

Manuscript details:
Received: 28.11.2023
Accepted: 27.12.2023
Published: 31.12.2023

Cite this article as:
Kohli MB, Salve AN and Jadhav AG (2024) Screening and detection of Proteinicious Protease Inhibitor from Some Plants from MTR, Maharashtra, India, *Int. J. of Life Sciences*, 12 (1): 389-395

Available online on http://www.ijlsci.in
ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

ABSTRACT

Plant protease inhibitors are widely distributed in various plants and majorly reported in seeds. Melghat Tiger Reserved (MTR), Maharashtra region is rich in plant diversity, 714 plant species are reported from the region. The plants used in this study are reported for variety of pharmacological properties and therapeutic purposes. Seeds from 30 different plants from this region were collected and analyzed for the presence of protease inhibitors. Detection of plant protease inhibitors (PI) from the defatted proteinaceous preparation from seed extracts were performed by using X-ray film method. Amongst the selected 18 were detected through X ray film method. Further found to possess the PI capable to inhibit various proteases like trypsin, pepsin and papain. These findings were further confirmed by using quantitative protease inhibition assay by caseinolytic method. The plants of this study reported first time for the presence of trypsin, pepsin and papain inhibitor.

Keywords: Protease Inhibitor, X-ray Film, Trypsin, Pepsin, Papain,

INTRODUCTION

Plant Protease inhibitors (PIs) are proteins present in plants performing regulatory functions, these are known to present in various plant materials such as seeds, leaves, flowers and tubers (Mayasa *et al.*, 2016). These play role in controlling proteolysis within the cell and thus maintain physiological regulatory cascade (Kim *et al.* 2009, Martinez *et al.* 2012). Also know to perform a role in intrinsic defense and thus have potential as drug molecules (Cid-Gallegos *et al.* 2022). These are wide spread in nature, numerous plants reported to possess protease inhibitors; however the most studied are from three family Fabaceae, Poaceae, Solanaceae (Richardson, 1991). Many studies further reported the presence of PIs in other plant families like Malvaceae, Rutaceae, Poaceae and Moringaceae (Bijina *et al.*, 2011). Majorly Plant PIs characterized are from soybeans, potatoes, squash, barley, wheat, millet, tomatoes, corn, kohlrabi and buckwheat (Fear *et al.* 2007). The plant protease inhibitors are of great interest due to their role in various stress condition like crop-protection, combating various pathogens including insects, fungi and

other phytopathogens (Carlini *et al.* 2002, Kim *et al.* 2009, Haq, *et al.* 2004, Dabhade *et al.* 2013). These are also being explored and used to cure human diseases, against viral and bacterial pathogens (Clark *et al.* 1993, Powers *et al.* 2002, Srikanth and Chen, 2016). This attracted attention as an alternative pharmacological target and thus possibility for development of future drugs. Majority of plant protease inhibitors are reported in seeds (Brady, 2003). In this preview, present study describe screening of plant seeds collected from MTR, Maharashtra (India) and assayed for the presence of protease inhibitor by rapid X-ray film method.

MATERIAL AND METHODS

Plant Material

The seeds of 30 plants were collected and investigated for detection of protease inhibitor. The plant seeds of these plants were collected from several locations of MTR, Maharashtra, India.

Protease enzyme

The protease enzyme, Trypsin (EC 3.4.21.4, Hi media; obtained from bovine pancreas), Papain (EC 3.4.22.2, Hi media, obtained from papaya latex), pepsin (EC 3.4.23.1, Hi media) were used.

Defatting of seeds

The mature dried seeds (25gm) of plants were finely powdered, depigmented and defatted with cold acetone (1:3 w/v). This was stirred for 1-2 hr on rotary shaker and kept aside for 1hr, then kept for air drying at room temperature. Further seed powder was washed with cold hexane (1:2 w/v). The solvent was removed and dried overnight at room temperature to remove all traces of solvent. (Borde *et al.*, 2012).

Extraction of Protease inhibitor (PI)

Plant materials were washed thoroughly with water and air dried. It was soaked overnight in 0.1M sodium phosphate buffer pH 7.0±0.5 (containing 1% polyvinyl pyrrolidine for removal of phenols) in a ratio of 1:4(w/v) and homogenized in an electrical blender (Tripathi *et al.,*2011). This was kept on Rotary shaker (130 rpm) for 30-60 min at room temperature and further left overnight at 4^0C for complete extraction. Homogenate obtained was filtered through muslin cloth, the filtrate further clarified by centrifugation at 10000 rpm for 15 min at 4^oC. The pellet was discarded and supernatant precipitated by adding ammonium sulphate (80%) and kept 10-12 hr at 4°C. The protein precipitate was dissolved in a minimum volume of phosphate buffer (pH 7.2) and dialyzed against same buffer. This was used for detection of protease inhibitor. The amount of protein was measured by Folin Lowry method using bovine serum albumin (BSA) as a standard (Lowry, 1951).

Detection of protease inhibitor

Preliminary detection of Protease inhibitors in the extract was performed by using X-Ray film (gelatin hydrolysis) method. Enzyme (Bovine trypsin) was preincubated for 15-20 min with protease inhibitor extract. Undeveloped gelatin coated X-ray film was immersed in the solution and incubated at 37^0C for 30min. No clearance of gelatin layer indicates protease inhibition by plant extract. This was compared with complete clearance of gelatin film (Fig.1) in control tube containing standard enzyme without inhibitor (Patil *et al.* 2014).

Protease inhibition assay

Protease inhibition was analysed by Kunitz method using casein as substrate. For caseinolytic assay, 500ul of trypsin was preincubated with 500ul of suitable dilution of crude PI extract at 37^0C for 15 minutes. To this 2.5ml of casein (0.65%) was added and incubated at 37^0C for 10min. Reaction was stopped by adding 110mM TCA and incubated at 370C for 30min and centrifuged at 4000 rpm for 5min at 40C. To the 1ml of supernatant 2.5ml of sodium carbonate (500mM) was added, to this 500ul of Folin-Ciocalteu's reagent(1:2 diluted) was added and incubated at 37^0C for 30min. Absorbance was measured at 660nm. Same was also performed for enzyme without inhibitor preparation. The assays were performed in triplicate and average is used calculate percent inhibition.

RESULTS AND DISCUSSION

MTR is having plant diversity, 714 reported from the region (Dhore, 1982, Bhogaonkar and Devkar, 1999). Many of them are known and have been characterized for their potential use in medicine. Plants investigated in this study were identified as mentioned in table 1. The plant seed contains different protease inhibitor content, if it is in a small amount then may not be detected by X-ray film. An effort is made by using three different Enzyme: Protease Inhibitor (E:PI) ratio for detection of small amount of PI in the plant extract preparation.

Fig. 1. X-ray film treated (A) with protease (B) without protease and C) Protease enzyme and protease inhibitor from the plant

Table 1: Protease inhibition activity Crude extract of dry, defatted seed powder detected through X ray film gelatin hydrolysis.

Name of plants	Family	Primary screening	E:PI		
			3:01	1:01	1:03
Crotalaria spectabilis	Fabaceae	Present	-	-	Present
Caesalpenia pulchirima	Fabaceae	Present	Present	Present	Present
Clitoria biflora	Fabaceae	-	-	-	-
Entada giga	Fabaceae	Present	Present	Present	Present
Cassia angustifolia	Fabaceae	Present	-	Present	Present
Glyricidium sepium	Fabaceae	-	-	-	-
Acacia farnesiana	Fabaceae	-	-	-	-
Cassia tora	Fabaceae	Present	-	Present	Present
Abutilon indicum	Malvaceae	Present	Present	Present	Present
Lagerstemia parviflora	Lythraceae	-	-	-	-
Astercantha longifera	Acanthaceae	-	-	-	-
Cadaba fruticosa	Capparaceae	Present	Present	Present	Present
Cleome viscosa	Cleomaceae	Present	Present	Present	Present
Merremia dissecta	Convolvulaceae	Present	Present	Present	Present
Thuja orientalis	Cupressaceae	Present	Present	Present	Present
Balanites aegyptiaca	Zygophyllaceae	-	-	-	-
Unidentified	Unidentified	-	-	-	-
Mallotus philippensis	Euphorbiaceae	-	-	-	Present
Coffea Arabica	Rubiaceae	-	-	-	-
Withania somnifera	Solanaceae	Present	-	Present	Present
Pithecellobium dulce	Fabaceae	-	-	-	-
Acacia catechu	Fabaceae	Present	Present	Present	Present
Phyllanthus emblica	Phyllanthaceae	Present	-	Present	Present
Cascabela thevetia	Apocynaceae	-	-	-	Present
Caesalpenia bonduc	Fabaceae	Present	Present	Present	Present
Abras precatorius	Fabaceae	Present	Present	Present	Present
Argyria speciosa	Convolvulaceae	Present	Present	Present	Present
Thespesia populnea	Malvaceae	-	-	-	-
Lantana camara	Verbenaceae	Present	Present	Present	Present
Bixa orellana	Bixaceae	-	-	-	-

E- Enzyme (Trypsin), PI-Plant protease Inhibitor (plant extract).

"-" Not detected on X ray film

Table 2: Protein yield and % protease inhibition activity

Name of plants	Protein (mg/ml)	%Trypsin inhibition	%Pepsin inhibition	%Papain inhibition
Crotalaria spectabilis	13.11	28.97	92.08	11.29
Caesalpenia pulchirima	12.35	53.05	90.33	0.97
Clitoria biflora	13.19	32.58	79.06	5.24
Entada giga	13.45	53.67	6.59	35.98
Cassia angustifolia	11.26	58.02	60.59	6.08
Glyricidium sepium	1.57	18	13.76	0
Acacia farnesiana	13.15	41.66	41.89	0
Cassia tora	15.42	24.52	19.48	2.20
Abutilon indicum	8.305	**98.09**	16.09	16.03
Lagerstemia parviflora	12.4	0	51.93	52.09
Astercantha longifera	5.977	32.77	96.13	13.39
Cadaba fruticosa	5.436	58.67	92.02	66.66
Cleome viscosa	11.1	83.33	84.53	25.96
Merremia dissecta	2.58	76.45	77.6	57.96
Thuja orientalis	2.083	97.05	59.65	14.34
Balanites aegyptiaca	18.36	0	74.44	41.23
Unidentified	11.33	0	79.68	5.45
Mallotus philippensis	22.83	16.25	91.45	13.70
Coffea Arabica	26.71	2.34	12.91	30.76
Withania somnifera	21.29	18.32	83.69	30.78
Pithecellobium dulce	14.83	0	25.28	13.47
Acacia catechu	24.96	66.70	75.68	**67.93**
Phyllanthus emblica	18.58	31.68	89.94	26.44
Cascabela thevetia	16.45	7.20	**96.57**	11.61
Caesalpenia bonduc	15.94	82.12	86.77	78.10
Abras precatorius	16.59	85.71	29.11	46.58
Argyria speciosa	9.53	55.55	30.57	56.26
Thespesia populnea	21.16	0	42.38	2.98
Lantana camara	6.682	94.85	35.22	15.16
Bixa Orellana	20.439	0	74.21	12.62

0- indicates no detectable activity

The quantitative inhibition was analyzed (table 2), the highest inhibition was found in *Abutilon indicum* for trypsin, *Cascabela thevetia* for pepsin and *Acacia catechu* for papain. Total 21 plant PI preparations exhibit inhibition of trypsin, pepsin and papain. The plants *Cadaba fruticosa, Merremia dissecta, Acacia catechu* and *Caesalpenia bonduc* showed more than 50%inhibition of all three proteases.

The plants used in this study are reported for variety of pharmacological properties and therapeutic purposes. Many amongst these are used by folk healers to treat several diseases and which has long been used in traditional Ayurvedic Indian medicine for various diseases. The medicinal potential of plants reported in previous studies viz; *Acacia catechu* (Modi *et al.* 2013), *Crotalaria spectabilis* (Oliveira *et al.* 2018), *Cassia angustifolia* (Boonhok *et al.* 2021, Srivastava *et al.* 2006) *Caesalpenia pulchirima* protease inhibitor *(Hase et al. 1986)* antiviral activities (Chiang *et al.* 2003), *Cassia tora* (Tripathi *et al.* 2011) *Cadaba fruticosa* (Saboo and Lahane 2020), *Withania somnifera* (Saleem *et al.* 2020) *Phyllanthus emblica (*Gaire and Subedi, 2014), Caesalpinia bonduc (Kandasamy *et al.* 2021), Abrus precatorius (Garaniya and Bapodra, 2014), *Argyreia speciosa* (Galani *et al.* 2010), *Merremia dissecta* (Austin, 2007), *Cleome viscosa* (Jane and Patil, 2012), (*Crotalaria spectabili,* de Oliveira *et al.* 2018), *Thuja* (Tsiri *et al.* 2009) and *Acacia catechu* (Adhikari *et al.* 2021) etc.

The total protein content of selected plant seed extract was assessed, every plant seed have different protein content. Total 18 plant seeds contain the protease inhibitor detected by X ray film hydrolysis (Fig 1). The protease inhibition percent calculated (Table 2), this highlighted the significant inhibition of trypsin, pepsin and papain. Among the selected plant seeds total 12 samples were from family Fabaceae, 2 each from Convolvulaceae and Malvaceae, 1 each from Apocynaceae, Acanthaceae, Bixaceae, Cupressaceae, Cleomaceae, Capparaceae, Euphorbiaceae, Lythraceae, Phyllanthaceae, Rubiaceae, Solanaceae, Verbenaceae and Zygophyllaceae.

CONCLUSION

Natural components have been extensively studied for potential applications. Medicinal plant widely explored for useful compounds eventually to develop the therapeutic product. It is indispensable to analyze various plant species or plant extracts in order to focus their potential. The research in protease inhibitors by exploration of plants is gaining importance. The MTR is rich in plant diversity, explored in present study for discovering novel protease inhibitors. The plants protease inhibitor detection revealed inhibition of trypsin, pepsin and papain proteases, indicative of its possible use for varied therapeutic applications. The competent method to detect the protease inhibitor from plant seed is endorsed in this study. By using a rapid X-ray film method, the proteinaceous protease inhibitors from *Cleome viscosa, Thuja occidentalis*, *Argyria speciosa, Entada giga* and *Merremia dissecta* were reported for the first time in this investigation.

REFERENCES

Adhikari B, Aryal B, Bhattarai BR, (2021) A Comprehensive Review on the Chemical Composition and Pharmacological Activities of Acacia catechu (L.f.) Willd., *Journal of Chemistry*, vol. 2021, Article ID 2575598, 11 pages,. https://doi.org/10.1155/2021/2575598

Austin, D.F. (2007) Merremiadissecta (Convolvulaceae): Condiment, medicine, ornamental, and weed-a review. *Econ Bot* 61, 109–120. https://doi.org/10.1663/0013-0001(2007)61[109:MDCCMO]2.0.CO;2

Bhogaonkar P Y. & Devarkar V P. (1999) ADDITIONS TO THE FLORA OF MELGHAT Directorate, Project Tiger Melghat., Paratwada., Distt. Amraveti. Maharashtra.

Bijina B, Chellappan S, Krishna JG., Basheer SM, Elyas KK, Bahkali AH, *et al.* (2011) Protease inhibitor from Moringa oleifera with potential for use as therapeutic drug and as seafood preservative. *Saudi. J. Bio. Sci.* 18, 273–281. 10.1016/j.sjbs.2011.04.002

Boonhok R, Sangkanu S, Norouzi R, *et al.* (2021) Amoebicidal activity of Cassia angustifolia extract and its effect on Acanthamoeba triangularis autophagy-related gene expression at the transcriptional level. *Parasitology* 148(9):1074-1082. doi:10.1017/S0031182021000718

Borde V, Hivrale V and Kachole M (2012) Detection and purification of Mucuna pruriens seed protease inhibitors. Elixir Int J Biotec, (49B), 10178-10181.

Brady RL. (2003) Plant protease inhibitors: significance in nutrition plant protection cancer prevention & genetic engineering. *Phytochem.* 64:1419.

Carlini CR, Grossi-de-Sá MF. (2002) Plant toxic proteins with insecticidal properties: A review on their potentialities as bioinsecticides. *Toxicon.* 40:1515–1539.

Chiang L. C., W. Chiang, M. C. Liu, C. C. Lin, (2003) *In vitro* antiviral activities of *Caesalpinia pulcherrima* and its related flavonoids, *Journal of Antimicrobial Chemotherapy*, 52(2): 194–198, https://doi.org/10.1093/jac/dkg291

Cid-Gallegos MS, Corzo-Ríos LJ, Jiménez-Martínez C, Sánchez-Chino XM. (2022) Protease Inhibitors from

Plants as Therapeutic Agents- A Review. *Plant Foods Hum Nutr*. 77(1):20-29. doi: 10.1007/s11130-022-00949-4. Epub 2022 Jan 8. PMID: 35000105.

Clark DT, Gazi MI, Cox SW, Eley BM, Tinsley GF. (1993) The effects of Acacia arabica gum on the in vitro growth & protease activities of periodontopathic bacteria*. J. Clin. Periodontol*. 20: 238–43.

Dabhade A, Patel P, Patil U. (2013) Proteinaceous protease inhibitor from Lawsonia inermis purification characterization & antibacterial activity. *Nat. Prod. Commun*. 8, 1467–1470.

de Oliveira, W.R., Rego, E.J.L., Von Buettner Ristow, P.C.L. *et al.* (2018) Isolation, characterization and analysis of the agglutinative activity of a lectin from *Crotalaria spectabilis*. *J. Plant Biochem. Biotechnol.* **27**, 373–381 https://doi.org/10.1007/s13562-018-0446-x

Dhore, MA & Joshi PA, (1988) "Flora of Melghat Reserve." Directorate Project Tiger. Melghat, Paratwada, Distt. Amravati, Maharashtra

Fear G, Komarnytsky S, Raskin I (2007) Protease inhibitors and their peptidomimetic derivatives as potential drugs. *Pharmacology & Therapeutics*. 113:354–368.

Gaire, B.P., Subedi, L. (2014) Phytochemistry, pharmacology and medicinal properties of *Phyllanthus emblica* Linn.. *Chin. J. Integr. Med.* . https://doi.org/10.1007/s11655-014-1984-2

Galani VJ, Patel BG, Patel NB (2010) Argyreia speciosa (Linn. f.) sweet: A comprehensive review. *Pharmacogn Rev.* 4(8):172-8. doi: 10.4103/0973-7847.70913. PMID: 22228958; PMCID: PMC3249918.

Garaniya N, Bapodra A. (2014) Ethno botanical and Phytophrmacological potential of Abrus precatorius L.: A review. *Asian Pac J Trop Biomed*. 4(Suppl 1):S27-34. doi: 10.12980/APJTB.4.2014C1069. PMID: 25183095; PMCID: PMC4025349.

Haq SK, Atif SM, Khan RH. (2004) Protein proteinase inhibitor genes in combat against insects pests and pathogens: natural and engineered phytoprojection. *Arch. Biochem. Biophys*. 431:145–159.

Hase S, Koyama S, Daiyasu H, Takemoto H, Hara S, Kobayashi Y, Kyogoku Y, Ikenaka T. (1986) Structure of a sugar chain of a protease inhibitor isolated from barbados pride (Caesalpinia pulcherrima Sw.) seeds. *J Biochem.* 100(1):1-10. doi: 10.1093/oxfordjournals.jbchem.a121681. PMID: 3759923.

Jane R. R., Patil S. D (2012) Cleome viscosa: An effective Medicinal herb for Otitis media. Int J of Sci and Nat vol (1) 3, 153-158.

Kandasamy V, Balasundaram U. (2021) Caesalpinia bonduc (L.) Roxb. as a promising source of pharmacological compounds to treat Poly Cystic Ovary Syndrome (PCOS): A review. *J Ethnopharmacol.* 28;279:114375. doi: 10.1016/j.jep.2021.114375. Epub 2021 Jun 27. PMID: 34192600.

Kim JY, Park SC, Hwang I, Cheong H, Nah JW, Hahm KS, Park Y. Protease inhibitors from plants with antimicrobial activity. Int. J. Mol. Sci. 2009; 10: 2860–2872.

Kunitz M. (1945) Crystallization of a trypsin inhibitor from soybean. *Science.* 101: 668–9.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. *J Biol Chem*. 1951;193(1):265–275.

Martinez M, Cambra I, Gonzalez-Melendi P, Santamaria ME, Diaz I. (2012) C1A cysteine-proteases and their inhibitors in plants. *Physiol Plant*. 145(1):85–94. doi: 10.1111/j.1399-3054.2012.01569.x.

Mayasa V, Rasal VK, Unger VS, (2016) Evaluation of phenol content, antioxidant, and proteinase inhibitory activity of plant derived protease inhibitors of eight antidiabetic plants. *Asian J. Pharm. Clin. Res.* 9 (3), 215–219.

Modi NM, Dezzutti CS, Kulshreshtha S, Rawat AK, Srivastava SK, Malhotra S, Verma A, Ranga U, Gupta SK.(2013) Extracts from *Acacia catechu* suppress HIV-1 replication by inhibiting the activities of the viral protease and Tat. *Virol J.* 18;10:309. doi: 10.1186/1743-422X-10-309. PMID: 25228267; PMCID: PMC3819669.

Ogungbenle, H. N., Oyadipe, O. T., (2015) Compositional and amino acid profile of nicker bean (Entada gigas) seeds.*British Biotechnology Journal,* 6(2) 43-50.

Patil U, Chaudhari A. (2009) Purification & characterization of solvent-tolerant thermostable alkaline metalloprotease from Alkalophilic Pseudomonas aeruginosa MTCC 7926. J. Chem. Technol. Biotechnol. 84: 1255–62.

Richardson M. J. (1991). Seed storage proteins: the enzyme inhibitors, in Methods in Plant Biochemistry, ed Richardson M. J. (New York, NY: Academic Press;), 259–305.

Saboo S and Lahane H (2020) Cadaba fruticosa Druce: Medicinal plant Journal of Pharmacognosy and Phytochemistry 2020; 9(1): 2331-2334

Saleem S, Muhammad G, Hussain MA, Altaf M, Bukhari SNA. (2020) Withania somnifera L.: Insights into the phytochemical profile, therapeutic potential, clinical trials, and future prospective. *Iran J Basic Med Sci.* 23(12):1501-1526. doi: 10.22038/IJBMS.2020.44254.10378. PMID: 33489024; PMCID: PMC7811807.

Shamsi, TN, Parveen R, Fatima S, (2016) Characterization, biomedical and agricultural applications of protease inhibitors: a review. *Int. J. Biol. Macromol*. 19, 1120–1133.

Srikanth S, Chen Z. (2016) Plant Protease Inhibitors in Therapeutics-Focus on Cancer Therapy. *Front Pharmacol.* 8;7:470. doi: 10.3389/fphar.2016.00470. PMID: 28008315; PMCID: PMC5143346.

Srivastava M, Srivastava S, Khatoon S A.K.S. Rawat, Mehrotra S and Pushpangadan P (2006) Pharmacognostical Evaluation of Cassia angustifolia. Seeds, *Pharmaceutical Biology,* 44:3, 202-207, DOI: 10.1080/13880200600686442

Tripathi VR., Shailendra Kumar and Garf SK (2011) A study on trypsin, Aspergillus flavus and Bacillus sp. Protease inhibitory activity in Cassia tora(L) syn Senna tora(L) Roxb. Seed extract. J BMC comp &Alte Med. 12;11:56. doi: 10.1186/1472-6882-11-56. PMID: 21749682; PMCID: PMC3144011.

Tsiri D, Graikou K, Pobłocka-Olech L, Krauze-Baranowska M,

Spyropoulos C, Chinou I. Chemosystematic value of the essential oil composition of Thuja species cultivated in Poland-antimicrobial activity. Molecules. 2009 Nov 19;14(11):4707-15. doi: 10.3390/molecules14114707. PMID: 19935470; PMCID: PMC6254788.

Acknowledgement : AGJ gratefully acknowledges financial support from DST SERB (NO SB/EMEQ-305/2013)

Conflict of interest: The authors declare that they have no conflict of interest.

Publisher's Note
IJLSCI remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Correspondence and requests for materials should be addressed to AGJ.

Peer review information
IJLSCI thanks the anonymous reviewers for their contribution to the peer review of this work. A peer review file is available.

Reprints and permissions information is available at https://www.ijlsci.in/reprints