Vol 16 | Issue 1| 2025 | 1-7.

e - ISSN 2249-7544
Print ISSN 2229-7464

INTERNATIONAL JOURNAL
OF
PHYTOPHARMACY RESEARCH
www.phytopharmacyresearch.com

IN-VITRO EVALUATION OF ANTI-UROLITHIATIC ACTIVITY OF EXTRACTS OF PARMOTREMA PERLATUM BY USING TITRIMETRIC METHOD & MOLECULAR DOCKING STUDIES

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ABSTRACT
The present study explored the anti-urolithic activity of *parmotrema perlatum* extract. It was carried out with the help of non polar to polar solvent by using soxhlet extraction method. This extraction was performed until the solvent become colorless and solvents were evaporated to get active compound. By using this extract preliminary phytochemical screening was performed to identify the phytoconstituent present in the extract are alkaloids, flavonoids, essential oil, glycosides, phenol, saponin, terpenoids, tannins, usnic acid etc. There after extract was evaluated for anti-urolithic activity using titrimetric method (semi-permeable egg membrane). In which cystone used as standard and calcium oxalate crystal was prepared in laboratory. Blank, standard, samples are packed into semi-permeable membrane of egg along with calcium oxalate crystal and then placed incubator 37°Cfor 2 hours, for about 7-8 hours. Removed the contents of semi-permeable membrane from each group into a test tube. Added 2 ml of 1 N sulphuric acid and titrated with 0.9494 N KMnO4 till a light pink color end point obtained.1ml of 0.9494 N KMnO4 equivalent to 0.1898mg of Calcium. The amount of undissolved calcium oxalate is subtracted from the total quantity used in the experiment in the beginning, to know how much quantity of calcium oxalate actually test substance(s) could dissolve. Finally, percentage dissolution was calculated which helps to determine the antiurolithic activity of *parmotrema perlatum*. In addition, molecular docking studies were performed to confirm the antiurolithiasis activity.

Keywords: *Parmotrema perlatum*, Calcium oxalate crystals, Cystone, Molecular docking, Anti-urolithiatic activity.

INTRODUCTION

Urine has many dissolved minerals and salts. Stones may form when urine has high levels of some of these minerals and salts. [1-4] Kidney stones may start small and not cause any issues at first. However, kidney stones can grow larger in size, even filling the inner hollow structures of the kidney. Some stones stay in the kidney and will never cause any problems. Kidney stones can travel down the ureter sometimes. (The ureter is the tube between the kidney and the bladder.) If the stone reaches your bladder, it can be passed out of the body through your urine. If the stone becomes lodged in the ureter, it blocks urine flow from that kidney. [5-8] This may be painful. There are many types of kidney stones i.e, calcium, uric acid, struvite, and cystine. They can vary in size, shape, and color. Calcium stones is the most common type of kidney stone that Can be calcium oxalate or calcium phosphate stones which is caused by high levels of calcium in the urine, or low levels of citrate in the urine. [9-11]

AIM AND PLAN OF WORK

AIM: To determine the antiurolithiatic activity of *Parmotrema perlatum* (black stone flower) by titrimetric method. In this wvaluation the anti urolithiatic activity of standard drug (Cystone) is compared with sample. Phytochemical screening also performed to find out the chemical constituent present in the extract of *Parmotrema perlatum*. [12-15] Molecular docking is carried out to find out the docking capacity of the *Parmotrema perlatum*.

PLAN OF WORK

- Collection and authentication of the plant.
- Extraction by using various solvents of increasing order of polarity.
- Perform the preliminary phytochemical studies for the identification of active constituents.
- Carried out the in vitro evaluation to determine the antiurolithic activity using *Parmotrema perlatum* extract by titrimetry.
- Results are determined by subtracting the initially used quantity of calcium oxalate crystal to that of un dissolved calcium oxalate. [16-19]
- This evaluation is carried out to determine the antiurolithic activity of Parmotrema perlatum.
- Carried out the molecular docking by auto dock

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MATERIALANDMETHODS

Chemicals required:

Calcium chloride dehydrate, Sodium oxalate, Potassium permanganate, 2N Sulphuric acid, 1N Sulphuric acid , Ammonia solution , 2N HCL, CYSTONE TABLET , Trisbuffer solution/Phosphate buffer, Sodium hydroxide, Egg (semipermeable membrane), Distilled water.[20-22]

Apparatus required:
Beaker, Conical flask, Incubator, Refrigerator, Burette, aluminium foil, thread.

Cystone Tablet:

Cystone tablet contain ingredient that possess diuretic, antimicrobial properties. [23-26] The formulation helps in the treatment and prevention of crystals in the urine. Moreover, it helps in the removal of kidney stones and prevents the recurrence of stones. These tablets contain pashanabheda and shilapushpa which are narural diuretics. They well dissolve small kidney stones naturally. The tablets also help in the treatment of ailments like painful urination, blood in urine and burning urination. [27-30]

Step 1: Preparation of calcium oxalate:

1.47 gm of calcium chloride dihydrate was dissolved in 100ml distilled water and 1.34 gm of sodium oxalate was dissolved in 100ml of 2N sulphuric acid both were mixed equally in a beaker to precipitate out calcium oxalate with stirring. The result of sulphuric acid by ammonia solution; washed with distilled water and dried at a temperature 60-degree celcius for 2 hour.

Figure: 1 Decalcified egg

Step 2: Preparation of semi permeable membrane:

The semi permeable membrane of egg lies in between the outer calcified shell and the inner content likes albumin &yolk. [31-34] Shell was removed chemically by placing the egg in 2M HCL for an overnight, which caused complete decalcification. Further, washed with distilled water, and hole is made on the top and the content squeezed out completely from the decalcified egg. [35- 38] Then egg membrane washed thoroughly with distilled water, and placed it in ammonia solution, in the moistened condition for a while & rinsed it with distilled water. Stored in refrigerator at a Ph of 7-7.4.

Figure 2: Crystal and Extraction Soaked in the Tris Buffer Solution

Step 3: Investigation of in vitro anti-urolithic activity by titrimetric method

Totally 3 semi permeable egg membrane were collected. Exactly 5mg of calcium oxalate and 2ml of distilled water were packed together in a semi-permeable membrane and the suture was made. The sample was served as negative control. [39-41]

The second group was contained 5mg of Calcium oxalate and along with 2ml of 10mg/ml Cystone solution of served as a positive control. The third group was contained 5mg of Calcium oxalate and along with 2ml of 10mg/ml of *Parmotrema perlatum* extract. These were allowed to suspend in a conical flask containing 100ml of Trisbuffer. All the conical flasks were kept in an incubator to 37 °C for 7 hours. Remove the contents of semi-permeable membranes from each group into separate test tubes, add 2 ml of 1N sulfuric acid to each test tube and titrated with 0.9494N KMnO4 till a light pink colour end point obtained. Consequently, 1ml of 0.9494 N KMnO4 was equivalent to 0.1898 mg of calcium the experiments

were performed in replicate. Finally, the undissolved Calcium oxalate was subtracted from the total quantity used in the experiment, in the beginning, to know the total quantity of dissolved Calcium oxalate by various dosage. The following formulas were used to calculate dissolved Calcium oxalate and Percentage dissolution respectively.

Percentage dissolution= dissolved Calciumoxalatex100
=8.6x 100 =86%

Figure: 3 Crystal Dissolved in the Extraction

MOLECULAR DOCKING

Docking is an attempt to find the best matching between two molecules. Docking is a method which predicts the preferred orientation of one ligand when bound in an active site to form a stable complex. [42-45] Molecular docking studies are a vital part of the in-silico drug discovery process. They help to understand the interaction between proteins and ligands, and can be used to predict the binding energetics between the two molecules. A docking program is employed to position the ligand molecule within the target structure across a range of positions, conformations, and orientations. Each docking configuration is referred to as a pose. The scoring of each pose is determined by its complementarity to the target regarding shape and properties, including electrostatics, aiming to pinpoint the most energetically favorable pose.

MOLINSPIRATION

The designed and docked molecules are screened in silico using MOLINSPIRATION Chemo informatic software to assess drug likeness. [46] This tool is user-friendly and efficient, accessible online for computing significant molecular properties such as log P, polar surface area, number of hydrogen bond donors and acceptors, among others. Additionally, it predicts the bioavailability score for crucial drug targets, including.

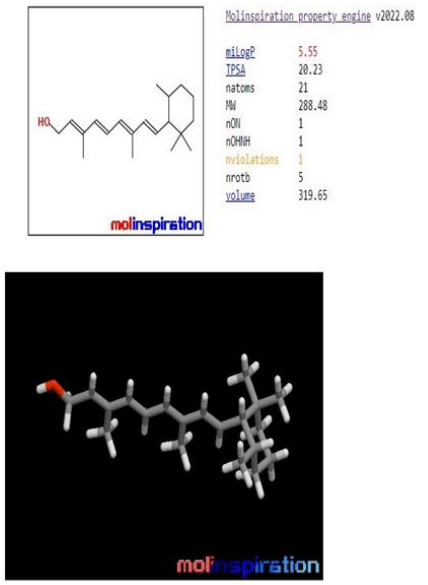

Figure: 4 Interaction with Hydrogen Bond

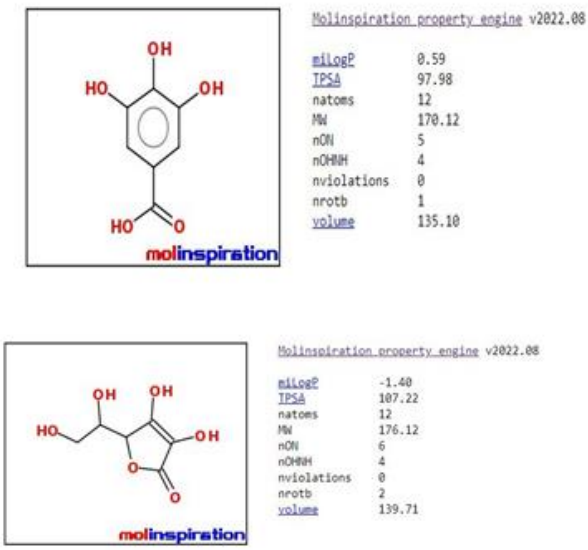

Figure: 5 Docking View For 3pl1

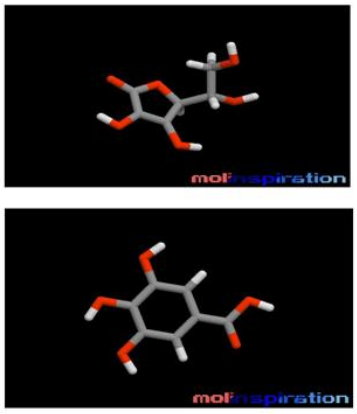

Figure: 6 Interaction With Amino acids

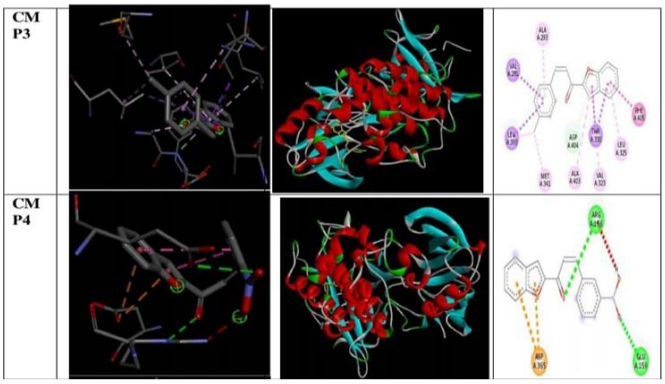

Figure: 7 Docking Structure

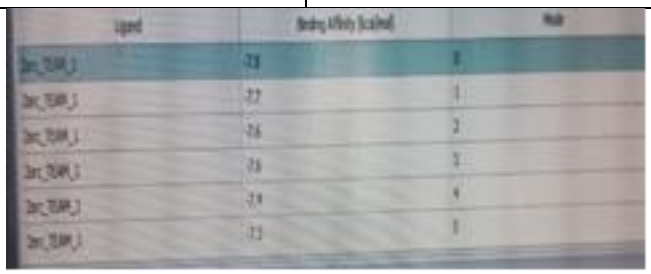

Figure: 8 Docking Score of atranorin

RESULT AND DISCUSSION

After the collection and identification of the plant, were cleaned and dried. Later the dried plant parts were ground to powder and extracted with petrolieum ether, chloroform, acetone, ethanol, and aqueous solvent using Extraction technique. [47] Next the acquired extract underwent an initial screening to identify different constituents using phytochemical methods.

Alkaloids, Glycosides, Carbohydrates, Proteins, Tannins, Flavanoids, Steroids, Terpenoids, Phenol, Saponin, and reducing sugars were found in the aqueous extract according to the preliminary phytochemical screening as shown in phytochemical screening table.

After performing the phytochemical analysis, the plant extract was subjected to assess the anti-urolithiasis activity of the *Parmotrema perlatum*. [48] The assessment was carried out by using the titrimetry method, in which artificial kidney stones from calcium oxalate were prepared and the percentage dissolution of stones was calculated.

The percentage dissolution was calculated for the positive control (cystone), and the aqueous,ethanol extract, and the results were summarised. The test sample (Aqueous extract) dissolves the 86% calcium oxalate whereas the positive contol shows 83% dissolution of calcium oxalate. This shows that *Parmotrma perlatum* extract can be used successfully for the treatment of urolithiasis. [49-50]

In a docking study project, the "results and discussion" section would typically present the top-ranked ligand poses identified through docking simulations, analyzing key interactions with the protein target, comparing different ligand molecules based on their binding affinities, and discussing the implications of these findings for further drug development, highlighting potential lead compounds based on the docking results and their predicted binding modes.

Docking Parameters and Methodology:

Briefly describe the protein structure used, the ligand library, the docking software and scoring function employed, and the parameters used during the docking process.

Top-Ranked Ligands:

Present the top few ranked ligands based on their docking scores (e.g., binding affinity).Visualize the docked poses of these ligands within the protein binding site using 2D and 3D representations.

Ligand Structural Features:

Analyze the structural features of the top-ranked ligands that contribute to their high binding affinity. Discuss the potential for further optimization based on these feature.

Comparison of Different Ligands:

Compare the docking scores and binding modes of different ligands within the same chemical series or with different functional groups. Explain how these differences in binding modes might affect their biological activity.

Validation and limitation

Discuss the limitations of the docking study, such as the reliance on scoring functions and potential

inaccuracies in protein structure. If applicable, mention any experimental validation data that supports the docking predictions. Standard drug docking score for anti-wrolithic activity depending on specific docking software of autodock and protein targetted.

Comparison to known standard drugs:

When evaluating new compounds, comparing Their docking scores to a known standard drug of (cystone) Provides useful reference comparison studies of molecular docking score Between Test and standard drug: Higher negative values indicating better Binding affinity and potential mas anti-urolithic activity of standard drug:

Higher negative values indicating better Binding affinity and potential anti-urolithic activity of standard [cystone] drug than the test drug

Score Interpretation:-

A more negative docking Score signifies a stronger interaction between the molecules and the protein target, suggesting greater potential for anti-urolithic activity

Table1: comparison of STD and Test Binding Score

S.No	Ligands	Target Protien of Anti-urolithic Activity	Binding score
1(Test)	Atranorin	5FBH	-7.8
2(STD)	Cystone	5FBH	-8.6

CONCLUSION

We anticipated that this study would stimulate more research into novel medications for the management and prophylaxis of urolithiasis. The current studies offer important details regarding *Parmotrema perlatum* anti-urolithiatic activity. Some constituents like Alkaloids, glycosides, carbohydrates, proteins, tannins, flavanoids, steroids, terpenoids, phenol, saponin, and reducing sugar etc. were found after the phytochemical evaluation of the plant.

The aqueous and ethanol extract demonstrated the calcium oxalate stones disintegration by using titrimetric method analyzing the % dissolution of calcium oxalate crystals.

Additional research in pharmacology and clinical settings is necessary to fully comprehend the workings and true effectiveness of the *Parmotrema perlatum* plant in the treatment of urolithiasis.

(For Docking Studies):

Molecules designed to inhibit these target enzymes exhibited [invitro] anti - urolithic activity as demonstrated by pyrx - virtual screening tool.

Notably there exists a significant correlation Between the insilico molecular docking scores and the (invitro) anti-urolithic activity.

The synthesized compounds of *Parmotrema perlatum* ligand activities were assessed through (invitro) evaluation using several analytical assay methods word with the Non-pathogenic -3pl1, 2 src, etc... Strain

Further structural Features and refinement of the synthesized compounds is expected to yield a promising drug candidate against *Parmotrema perlatum*.

REFERENCES

1. Jarald EE, Kushwah P, Edwin S, Asghar S, Patni SA. Effect of unex on ethylene glycol-induced urolithiasis in rats. *Indian J Pharmacol*, 43(4), 2011, 466-468.
2. Mayilsamy M, Geetharamanan K. Cytotoxic activity of Parmelia perlata extracts against Artemia salina. *J Exp Sci*, 7, 2016, 36-39.
3. Leela K, Iyer P. Extraction and application of secondary metabolites from Parmelia perlata. *Asian J Biol Life Sci*, 5(2), 2016, 211-216.
4. Gehlot V, Mahant S, Vijayraghwan P, *et al*. Therapeutic potential of lichen Parmelia perlata against dual drug-resistant Helicobacter pylori isolates. *Int J Pharm Pharm Sci*, 8, 2016, 205-208.
5. Shailajan S, Joshi M, Tiwari B. Hepatoprotective activity of Parmelia perlata (Huds.) Ach. against CCl_4-induced liver toxicity in Albino Wistar rats. *J Appl Pharm Sci*, 4, 2014, 70-74.
6. Diwakar Y, Chitra V, Evelyn S. Study of Parmelia perlata for its potential as an anti-inflammatory and antiarthritic agent using an in vitro model. *Asian J Pharm Clin Res*, 12(1), 2019, 95-97.
7. Goyal PK, Verma SK, Sharma AK. Evaluation of antiurolithiatic effects of Parmelia perlata against calcium oxalate calculi in hyperoxaluric rats. *J Appl Pharm Sci*, 8(01), 2018, 129-135.
8. Jothi G, Brindha P. Antidiabetic and antihyperlipidemic effect of Parmelia perlata Ach. in alloxan-induced diabetic rats. *Int J Pharm Pharm Sci*, 6, 2014, 43-46.
9. Chawla AS, Sharma AK, Handa SS, Dhar KL. Chemical investigation and anti-inflammatory activity of Vitex negundo seeds. *J Nat Prod*, 55(2), 1992, 163-167.
10. Leelaprakash G, Dass SM. In vitro anti-inflammatory activity of methanol extract of Enicostemma axillare. *Int J Drug Dev Res*, 3(3), 2011, 189-196.

11. Kardile MV, Mahajan UB, Shaikh HM, Goyal SN, Patil CR. Membrane stabilization assay for anti-inflammatory activity yields false positive results for samples containing traces of ethanol and methanol. *World J Pharm Pharm Sci*, 5(3), 2016, 493-497.
12. Leelaprakash G, Dass SM. In vitro anti-inflammatory activity of methanol extract of Enicostemma axillare. *Int J Drug Dev Res*, 3(3), 2011, 189-196.
13. Tiselius HG. Epidemiology and medical management of stone disease. *BJU Int*, 91, 2003, 758-767.
14. Hadjzadeh M, Khoei A, Hadjzadeh Z, Parizady M. Ethanolic extract of Nigella sativa L. seeds on ethylene glycol-induced kidney calculi in rats. *Urol J*, 4, 2007, 86-90.
15. Raats ICJ, van den Born J, Berden JHM. Glomerular heparin sulfate alterations: mechanisms and relevance for proteinuria. *Kidney Int*, 57, 2000, 385-400.
16. Obligado SH, Goldfarb DS. The association of nephrolithiasis with hypertension and obesity: a review. *Am J Hypertens*, 21, 2008, 257-264.
17. Taylor EN, Stampfer MJ, Curhan GC. Obesity, weight gain, and the risk of kidney stones. *JAMA*, 293, 2005, 455-462.
18. Momoh MA, Adikwu MU. Evaluation of the effect of colloidal silver on the antibacterial activity of ethanolic extract of the lichen Parmelia perlata. *Afr J Pharm Pharmacol*, 2(6), 2008, 106-109.
19. Sharma AK, Sharma KK, Sharma MC, Dobhal MP. Two dibenzofurans identified as heterocyclic natural compounds from lichen Parmelia perlata. *J Pharmacog Phytochem*, 2(5), 2014, 95-97.
20. Lakshmi V, Ameta K, Mishra V, Srivastava A, Agarwal SK, Palit G, et al. Gastroprotective effect of ethanolic extract of Parmelia perlata in rats. *J Phytopharmacol*, 2(6), 2013, 19-25.
21. Bhattarai T, Subba D, Subba R. Nutritional value of some edible lichens of east Nepal. *J Appl Bot*, 73, 1999, 11-14.
22. Paul S, Singh ARJ, Sasikumar CS. An antioxidant and bioactive compound study of Parmelia perlata, Ganoderma lucidum, and Phellinus igniarius supplementary drug. *Asian J Pharm Tech Innov*, 2(7), 2014, 1-5.
23. Rahman H, Vijaya B, Ghosh S, Pant G, Sibi G. In vitro studies on antioxidant, hypolipidemic, and cytotoxic potential of Parmelia perlata. *Am J Life Sci*, 2(6-1), 2014, 7-10.
24. Jothi G, Brindha P. Antidiabetic and antihyperlipidemic effect of Parmelia perlata Ach. in alloxan-induced diabetic rats. *Int J Pharm Pharm Sci*, 6(1), 2013, 43-46.
25. Patil SB, Ghadyale VA, Taklikar SS, Kulkarni CR, Arvindekar AU. Insulin secretagogue, alpha-glucosidase, and antioxidant activity of some selected spices in streptozotocin-induced diabetic rats. *Plant Foods Hum Nutr*, 66, 2011, 85-90.
26. Shailajan S, Joshi M, Tiwari B. Hepatoprotective activity of Parmelia perlata (Huds.) Ach. against CCl_4-induced liver toxicity in Albino Wistar rats. *J Appl Pharm Sci*, 4(2), 2014, 70-74.
27. Abdur R, Abdul L, Sumbul R, Afaq SH. Study on extracts of Parmelia perlata Ach. for its antimicrobial potential against certain microorganisms. *Int Res J Pharm*, 4(11), 2013, 102-106.
28. Hussain M, Raza SM, Farooq U, Bakhsh H, Majeed A, Aziz A. In vitro antimicrobial potential of lichen (Parmelia perlata) against different pathogenic microbes. *Int J Pharm Sci*, 4(4), 2014, 666-670.
29. Charles OE, Kenneth CO, Michael UA, Emmanuel CI, Dominic OA, Georgina NO, et al. In vitro evaluation of the antiviral activity of extracts from the lichen Parmelia perlata (L.) Ach. against three RNA viruses. *J Infect Dev Ctries*, 1(3), 2007, 315-320.
30. Goyal PK, Verma SK, Sharma AK. Antiurolithiatic potential of Parmelia perlata extract against AMPH crystals. CT *Int J Pharm Integr Life Sci*, 1(1), 2015, 40-44.
31. Kumar K, Upreti DK. Parmelia spp. (Lichens) in ancient medicinal plant lore of India. Econ Bot, 55(3), 2001, 458-459. doi: 10.1007/BF0286656.
32. Nayaka S, Upreti DK, Khare R. Medicinal lichens of India. In: Trivedi PC, ed. Drugs from Plants. Avishkar Publishers, *Jaipur, India*, 2010
33. Gayathri D, Swamy CT. Lichens: A novel and potential source as antimicrobials for human use. *J Phytol*, 4, 2012, 38-43.
34. Vidyalakshmi A, Kruthika K. Antibacterial activity of Parmelia perlata. *Asian Pac J Trop Biomed*, 2012, S892-S894.
35. Karunaratne V, Bombuwela K, Kathirgamanathar S, Thadhani VM. Lichens: A chemically important biota. *J Natl Sci Found Sri Lanka*, 33(3), 2005, 169-186.
36. Kosanic MM, Rankovic BR, Stanojkovic TP. Antioxidant, antimicrobial and anticancer activities of three Parmelia species. J Sci Food Agric, 92, 2012, 1909-1916.
37. Rankovic BR, Kosanic MM, Stanojkovic TP. Antioxidant, antimicrobial and anticancer activities of the lichens Cladonia furcata, Lecanora atra, and Lecanora muralis. BMC Complement *Altern Med*, 11, 2011, 97.
38. Goswami PK, Samant M, Srivastava RS. Multi-faceted Saxifraga ligulata: A mini review. Int J Res Ayurveda Pharm, 4(4), 2013, 608-611.
39. Goyal PK, Verma SK, Sharma I, Sharma A, Sharma AK. Ethnobotanical and phytopharmacological updates on Didymocarpus pedicellata. *Indo Am J Pharm Res*, 5(8), 2015, 2747-2755.
40. Kosanić M, Šeklić D, Marković S, Ranković B. Evaluation of antioxidant, antimicrobial, and anticancer properties of selected lichens from Serbia. *Dig J Nanomater Biostruct*, 9, 2014, 273-287.
41. Seung KJ, Keshavjee S, Rich ML. Multidrug-resistant antiurolithic activity and extensively drug-resistant antiurolithic activity. *Cold Spring Harb Perspect Med*, 5(9), 2015, a017863.
42. Kumar V, Robbins SL. Robbins Basic Pathology. 8th ed. Philadelphia: Elsevier, 2007. ISBN: 978-1-4160-2973-1.

43. Thippeswamy B, Sushma NR, Naveenkumar KJ. Antimicrobial property of bioactive factor isolated from Parmelia perlata. *Int Multidiscip Res J*, 2(2), 2012, 1-5.
44. Tanwar K, Mathur J, Kachhawa JBS. Phytochemical investigation and anti-fertility activity of lichen Parmelia perlata. Int J Recent Res Rev, 8(4), 2015, 1-5.
45. Diwakar Y, Chitra V, Evelyn SS. Study of Parmelia perlata for its potential as an anti-inflammatory and antiarthritic agent using in vitro model. *Asian J Pharm Clin Res*, 12(1), 2019, 95-97.
46. Goyal PK, Verma SK, Sharma AK. Pharmacological and phytochemical aspects of lichen Parmelia perlata: A review. Int J Res Ayurveda Pharm, 7(1), 2017, 102-107.
47. Kosanić M, Šeklić D, Marković S, Ranković B. Evaluation of antioxidant, antimicrobial, and anticancer properties of selected lichens from Serbia. *Dig J Nanomater Biostruct*, 9, 2014, 273-287.
48. Goyal PK, Verma SK, Sharma I, Sharma A, Sharma AK. Ethnobotanical and phytopharmacological updates on Didymocarpus pedicellata. *Indo Am J Pharm Res*, 5(8), 2015, 2747-2755.
49. Priyanka KG, Samant M, Srivastava RS. Multi-faceted Saxifraga ligulata: A mini review. *Int J Res Ayurveda Pharm*, 4(4), 2013, 608-611.
50. Kosanic MM, Rankovic BR, Stanojkovic TP. Antioxidant, antimicrobial, and anticancer activities of three Parmelia species. *J Sci Food Agric*, 92, 2012, 1909-1916.