International Research Journal of Biological Sciences _____ ISSN 2278-3202
Vol. **8(10),** 31-37, October **(2019)** *Int. Res. J. Biological Sci.*

Phytochemical analysis, antioxidant, antimicrobial, and antiurolithiasis activities of endophytic *Penicillium notatum* isolated from *Aloe vera* roots

Rohit S. Mane[1,2*], Priyanka R. Jagtap[3], Manoj A. Vyawahare[3], Amarja H. Bhosale[4], Vishrut V. Ghare[5], and Bidhayak Chakraborty[6]

[1]Department of Microbiology and Biotechnology, Karnatak University, Dharwad, Karnataka, India
[2]Department of Life Sciences, Mandsaur University, Mandsaur, M.P. India
[3]Department of Microbiology, New arts, Commerce and Science College, Ahmednagar, Maharashtra, India
[4]Department of Microbiology, Dr. Babasaheb Ambedkar Marathawada University, Sub-Campus Osmanabad, Maharashtra, India
[5]Department of Microbiology, S.B.B Alias Appasaheb Jedhe College of Arts, Commerce & Science, Pune, India
[6]Department of Botany, Karnatak University, Dharwad, Karnataka
rohitmane@kud.ac.in

Available online at: www.isca.in, www.isca.me
Received 29th April 2019, revised 15th August 2019, accepted 28th September 2019

Abstract

The objective of present investigation was to assess phytochemical analysis, antioxidant, antimicrobial, and antiurolithiasis activities of endophytic fungi derived bioactive compounds obtained from Aloe vera roots. Fungal endophytes were isolated by means of potato dextrose agar media from Aloe vera roots and grown in Potato dextrose broth for the production of the bioactive compounds. The qualitative phytochemical screening was performed for the aqueous crude extract of the fungus. Phytochemicals like phenols and flavonoids were quantitatively estimated and further purified by thin layer chromatography and FTIR. The antiurolithiasis and antimicrobial activity of crude extracts was analyzed. A whole of six fungal endophytes were isolated and Penicillium notatum were identified as core endophytic fungus by 18S rRNA analysis. The fungus revealed total 1.86g and 0.15g of wet and dried biomass in PDB/100ml with 2.60g/100ml of the aqueous crude extract. The phytochemical screening showed terpenoids, flavonoids, saponins, phenols and tannins, alkaloid, cardiac glycosides, fixed oils and fats, proteins, carbohydrates with 2.26g of phenol and 0.22g of flavonoids/100g of gallic acid equivalent quantitatively. TLC revealed intermediate polar basic compounds, polar basic compounds, alkaloids, flavonoids, saponins, and terpenoids while FTIR analysis documented different functional groups such as alkanes, amides, alkenes, alcohol and phenols, alkyl halides, ether, amines, and nitrile groups. Ferric ions reducing power was reported at the rate of 1.2407±0.00702 while PM assays reported 0.8983±0.00351. The crude extract showed highest inhibition zone at the 100% concentration against Escherichia coli (16.3±0.57) and Aspergillus niger (20.5± 0.24). The maximum antiurolithiasis activity showed at 100% concentration at the rate of 67.5±1.49 of crude extract.

Keywords: Endophytic *Penicillium notatum*, Bioactive compounds, antiurolithiasis and antimicrobial activity.

Introduction

Urolithiasis is a third prevalent urological disorder of humans formed usually within the kidney in the form of stones from ancient times[1]. There are quite a few types of kidney stones but the most common are calcium oxalate which represents 80% of analyzed stones. At present, kidney stone affects extra in industrialized countries owing to the change in lifestyle[1]. Formation of the stone is a multifaceted process and involves several physicochemical actions which start with crystal nucleation, supersaturation, aggregation, and ending with retention inside the urinary tract[2]. Another focused area is microbial infections[3].

Infection is harass of an organism's body by pathogens, their multiplication, and the response of host tissues to the communicable agents and the toxins they create. Infections are caused by pathogens and these pathogens are bacteria, fungi, viruses, actinomycetes, and protists. No promising drugs are available in the market to dissolve or to remove the kidney stones due to which natural resources have gained awareness among citizens due to their established clinical efficacy and better patient compliance[4]. Plants are chief contributors in the drug designing and the development against different human and animal diseases[6]. Fungi are also measured in traditional and folk medicines, almost certainly since pre-history, the aptitude to identify helpful properties. Plant and fungal based drugs played a vital role in the sickness pathology, target identification, assay development, mechanism of action, and clinical development[6]. Therefore present research is highlighting the endophytic fungi area, combination of plants and fungi[6].

Endophytes are the plant microbes which exist in inside the plant tissues and show major uniqueness such as ubiquitous nature, symbiotic relationships with their hosts, indirect defense

against herbivorous, bioactive compounds production, Fitness, and competitive ability, and nutrient-exchange ability[7]. They produce different bioactive compounds by mimicking their host plant. These all bioactive compounds are medically active that have been identified include antibiotics, anticancer drugs, immunosuppressant's, psychotropic drugs, fungicides, and cholesterol inhibitors[8]. Therefore the present research work was focused on i. Isolation and characterization of fungal endophytes from *Aloe vera* roots ii. production, purification, and characterization of endophytic fungi derived bioactive compounds iii. bioassays.

Materials and methods

Plant material, Authentication and Isolation of fungal endophytes: We selected the *Aloe vera* plant for the study of fungal endophytes on the basis of ethnobotany and pharmacology[9]. Plant samples were collected from Shrirampur, Ahmednagar District, Maharashtra. The plant samples were authenticated and a voucher specimen was deposited with Dr. Kotresha K., Department of Botany, Karnatak Science College, Dharwad under the number N0-01/2018.

Samples of *Aloe vera* roots were selected and washed with the running tap water to remove dust and soil particles. Then engrossed with ethanol (70%) for 2 minutes and sodium hypochlorite (4%) for 2 minutes and 1 minute in $HgCl_2$ (0.1%). Lastly washed with distilled water, cut, and implanted on Potato Dextrose Agar plates containing ampicillin (50mg/L). All plates were incubated at 25°C ± 2°C for 15 to 20 days.

Identification, Characterization, and Preservation of fungal endophytes: Identification and Characterization of fungal endophytes were performed by morphological methods[9] and the selected fungus was identified by 18S rRNA analysis[13]. Pure culture of fungus was obtained by Subculturing on Potato Dextrose Agar plates without the addition of antibiotics. Preservation was performed by transferring pure cultures on Potato Dextrose Agar slants and preserved at 15°C up to further use.

Production, Extraction, and Phytochemical screening of endophytic fungus derived bioactive compounds: The selected endophytic fungus was cultivated in Potato Dextrose Broth at 25°C ± 2℃ for 15 to 20 days at static condition. 100ml of aqueous solvent was added and the crude extract was obtained by filtration, solvent extraction method and Rota evaporator at 40℃ under controlled conditions. At the same time separated biomass was collected, weighed, and stored at 15°C for further use.

Qualitative phytochemical analysis of an aqueous crude extract of endophytic fungus was performed for different bioactive compounds by following standard protocols[10] while quantitative analysis of flavonoids and phenol content was performed as per standard protocols[10].

Thin layer chromatography and FTIR of endophytic fungi derived bioactive compounds: Thin layer chromatography was performed to detect the bioactive compounds present in crude extract[3]. TLC was performed on precoated TLC plates with silica gel 60 F254 Merck KGaA, Darmstadt, USA. The crude extract was dissolved in 50% DMSO. The drop of extract was spotted on TLC aluminum sheets with the help of capillary tube, dried, and process was repeated 3 times.

The TLC sheet was developed with different solvent systems such as, i. Intermediate polar basic compounds- Chloroform: Ethyl acetate: Formic acid (5:4:1), ii. Polar basic compounds – Ethyl acetate: Ethanol: Water (8:1.2:0.8), iii. Alkaloids- Chloroform: methanol: Ethanol (18:1:1), iv. Flavonoids – Chloroform: Methanol (18:2), v. Saponins – Chloroform: glacial acetic acid: Methanol: Water (6:2:1:1), vi. Terpenoids- Benzene: Ethyl acetate (1:1).

After drying, the TLC sheets were visualized under a Ultraviolet transilluminator and retention factor (Rf) of different spots were intended by using the following formula,

Rf = [Distance traveled by solute / Distance traveled by solvent]

FTIR was used for the detection of the different functional groups present in the crude extract of endophytic fungi[3]. The FTIR was handled by the diffuse reflectance technique in which the dried fraction sample was assorted with potassium bromide to form a very well powder and then compressed into a thin pellet. The pellet was used for the analysis of different functional groups. The samples were irradiated by a broad spectrum of infrared light and the stage of absorbance at a meticulous incidence was plotted after. The absorbance was calculated between 400-600nm for the recognition and quantification of natural groups.

Bioassays: Antioxidant Activity: Antioxidant activity was performed with the help of ferric ion reducing power assay[12] and phosphomolybdenum assay[8].

Antimicrobial activity: Microorganisms: The crude extract was screened against different pathogens by using well diffusion method. The bacteria such as *E. coli, S. Typhi, S. aureus, Bacillus spp.* and *Pseudomonas aeruginosa* and fungi such as *A. niger, A. fumigatus*, and *A. flavus* were used.

Well Diffusion Method: Muller-Hinton agar medium plates were prepared and microbial cultures were spread to the concentration of 1.0×10^4CFU/ml attuned with saline. The Fluconazole and Telithromycin (10mg/mL) were used as optimistic control and DMSO was used as the unenthusiastic control. Extracts were added in the preapared wells with controls and all plates were incubated 37℃ for 24hrs or 72hrs and the inhibition zone was recorded with the assist of zone reader.

Antiurolithiasis activity: Antiurolithisis activity was performed for the inhibition of oxalate crystals because of its satisfactory results simplicity and reproducibility in order to study inhibitory capacity of crude extract. The solutions of $CaCl_2.2H_2O$ (9mM) and $Na_2C_2O_4$ (3mM) were prepared using a buffer containing 0.15 M NaCl and 0.05 M Tris HCl at pH 6.5. The inhibitory solution was prepared by adding 0.25 gm of crude extracts in 100mL of 0.15M NaCl solution. 1mL of $CaCl_2$ solution was assorted with 1 mL of inhibitory solution of crude extract with different concentration (25%, 50%, 75%, 100%). Blank reading were reported and sodium oxalate (1mL) was added. The absorbance was measured at 620nm with the help of UV spectrophotometer at different time intervals.

Results and discussion

The plant specimen was identified and authenticated by Dr. Kotresha K., at the Department of Botany, Karnatak Science College, Dharwad. The herbaria of the collected samples were deposited at the same department. The roots collected were surface sterilized and implanted on PDA for the isolation of fungal endophytes. All collected samples showed growth of fungal endophytes after 15 days of incubation period under the dark condition.

Total of six fungal endophytes was isolated which mainly belonging to *Penicillium* species. Further 18S rRNA analysis confirmed as *Penicillium notatum*[13]. The fungi sequence was submitted in the NCBI with accession number[13]. On media, they showed variations in the color and morphology as compared with epiphytic Penicillium species (Figure-1). Hyphae showed severe changes in morphology when observed under different objectives containing a liquid medium. Hyphae were with abridged width, a disintegration of the cytoplasm, and accretion of nuclei at broken hyphal tips were detected. The mycelia were branched, multinucleated on a septum. Conidiophores were at the end of each branch.

These individual units play a significant role in reproduction; conidiophores were the main dispersal route of the fungi. Pure culture of fungus was obtained by Subculturing on Potato Dextrose Agar plates without addition of antibiotics. Preservation was performed by transferring pure cultures on Potato Dextrose Agar slants and preserved at 15°C up to further use. Total wet biomass of endophytic *Penicillium notatum* was recorded as 1.86g/100ml of PDB and the dried biomass was 0.15g/100ml of PDB (Figure-2).

Obtained 40 ml of the filtrate were concentrated into 2.60 g/100ml of the aqueous solvent by using Rota evaporator at 40℃ after 6-8 hrs rotation at 90 rpm and used for further processes. Phytochemical screening of the crude extracts showed the presence of terpenoids, flavonoids, saponins, phenols and tannins, alkaloid, cardiac glycosides, fats, proteins, fixed oils, and carbohydrates.

It was found that the fungus crude extract controlled 2.26g of phenol/100g of gallic acid equivalent with the flavonoids content at the rate of 0.225g. The fungus extract was subjected to TLC analysis for the detection of intermediate polar basic compound, polar basic compound, alkaloid, flavonoids, saponins, and terpenoids (Figure-3). The R_f value was calculated are shown in Table-1.

The Rf values were ranging from 0.16 to 0.91. FTIR analysis of aqueous crude extract of *Penicillium notatum* revealed different functional groups such as amides, alkanes, alkenes, alcohol and phenols, alkyl halides, ether, amines and nitrile groups. The results are showed in Table-2 and Figure-4.

In ferric ion reducing power assay, aqueous crude extract showed effective antioxidant activity at the rate of 1.2407±0.00702. PM assay of aqueous crude extract was performed with ascorbic acid as a standard. The results exposed that the aqueous crude extract showed 0.7983±0.00351 senior antioxidant capacity and was compared to the standard ascorbic acid. Aqueous crude extract of *Penicillium notatum* were utilized to evaluate their antimicrobial activity against food pathogens including *Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus species, Aspergillus fumigatus, Aspergillus flavus,* and *Aspergillus niger* using disc diffusion method.

The results revealed that fungus crude extract were potentially efficient in suppressing food poisoning bacteria with variable potency. The fungus crude extract was most effective against *Escherichia coli* to retard their growth at concentration of 100mg/ml with the rate of 16.3±0.57 while in the case of fungal pathogens; extract was effective against *Aspergillus niger* at the concentration of 100mg/ml at the rate of 20.5 ± 0.24. The results are shown in Table-3. Antiurolithiasis activity of the fungus extract was effective e at the concentration of 100% with 67.5±1.49 (Figure-5).

Table-1: Thin Layer Chromatography of aqueous crude extract with their Rf values.

Components	Crude extract (R_f value)	Standard (R_f value)
Intermediate polar basic compound	0.36	0.36
Polar basic compounds	0.78	0.82
Alkaloids	0.32	0.29
Flavonoids	0.43	0.41
Saponins	0.91	0.91
Terpenoids	0.16	0.15

Table-2: FTIR analysis of aqueous crude extract of *Penicillium notatum.*

Frequency	Type of vibration	Type of bond	Functional Groups	Intensity
3463.97	Stretch	O-H	Alcohols, Phenols	Strong, broad
2960.92	Stretch	C –H	Alkanes	Strong
2925.36	Stretch	C –H	Alkanes	Strong
2852.00	Stretch	C –H	Alkanes	Strong
2100.48	Stretch	-C ≡ C-	Alkynes	No variable
1636.26	Stretch	- C= C	Alkenes	Variable
1577.43	Stretch	C = C	Aromatics	Medium-weak, multiple bands
1459.69	Bending	C –H	Alkanes	Variable
1424.37	Stretch	C = C	Aromatic	Medium-weak, multiple bands
1384.92	Stretch	N – O	Nitro compound	Strong
1355.38	Stretch	N – O	Nitro compound	Strong
1327.23	Stretch	C – N	Amines	Medium-weak
1164.65	Stretch	C –F	Alkyl halides	Strong
1088.19	Stretch	C – F	Alkyl halides	Strong
546.56	Stretch	C – Br	Alkyl halides	Strong

Table-3: Antimicrobial activity of aqueous crude extract.

Crude Extract	Zone of Inhibition (mm)				
	Escherichia coli	*Salmonella typhi*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Bacillus spp.*
	16.3±0.57	14..6±0.37	0.0±0.0	8.3±0.66	17.6±0.31
Telithromycin	16.8 ± 0.37	20.5 ± 0.24	16.8 ± 0.37	15.6 ± 0.53	18.7 ± 0.61
	Aspergillus fumigatus		*Aspergillus flavus*	*Aspergillus niger*	
Crude Extract	8.4±0.33		18.7 ± 0.61	20.5 ± 0.24	
Fluconazole	12±0.12		19.7 ± 0.61	22.5 ± 0.24	

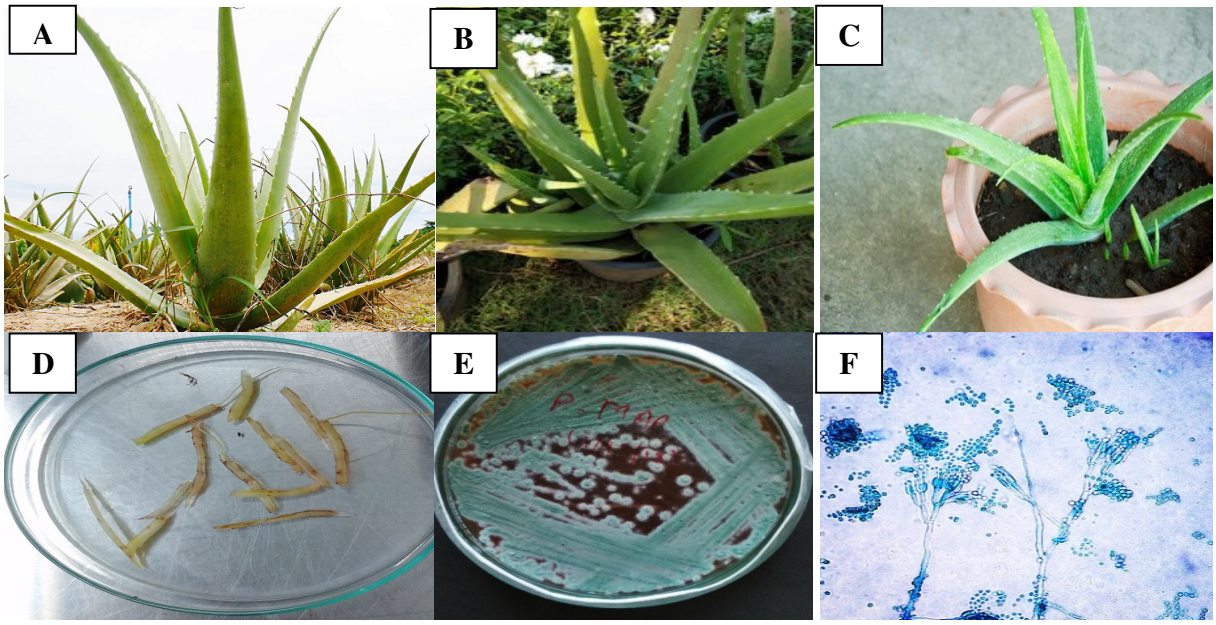

Figure-1: Plant material and Isolation of fungal endophytes. Farmhouse of *Aloe vera* from Shrirampur study area [A], *Aloe vera* grown in natural place [B], Grown *Aloe vera* in pot for study purpose [C], *Aloe vera* roots isolated from plant [D], Isolation of endophytic *Penicillium notatum* [E] and Microscopic morphology of *Penicillium notatum* [F].

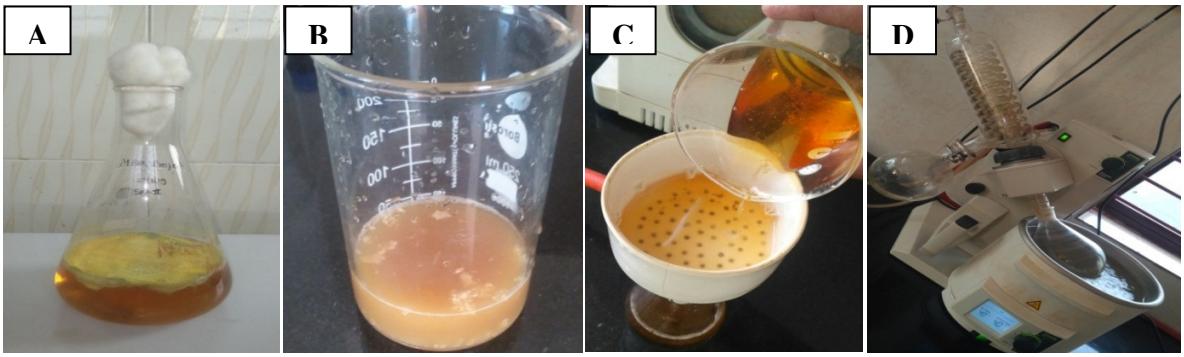

Figure-2: Production, Extraction and concentration of bioactive compounds. [A] Fermented Potato dextrose broth, [B] Aqueous fermented broth, [C] Vacuum Filtration of aqueous fermented broth, [D] Concentration of aqueous fermented broth into crude extract by Rotary evaporator.

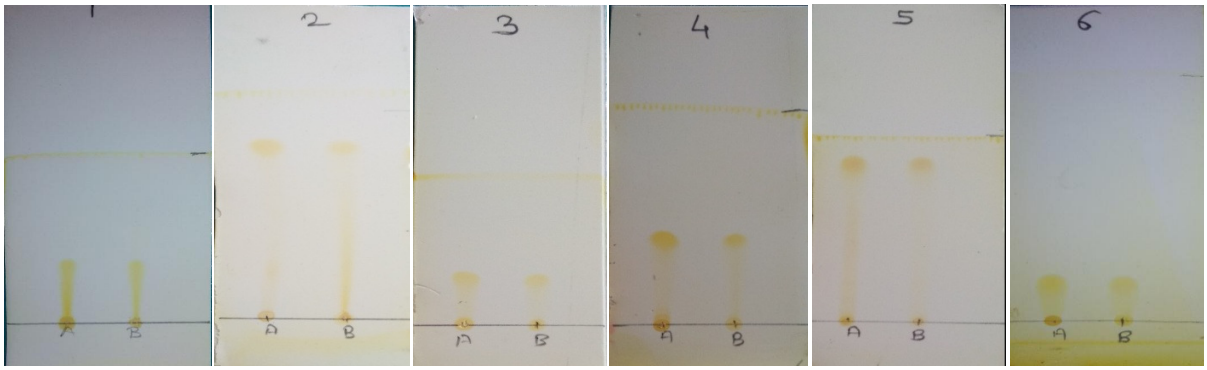

Figure-3: Plates of thin layer chromatography of crude extract (A= Crude extract spot, B= Standard solution), [1] Intermediate polar basic compounds, [2] Polar basic compounds, [3] Alkaloids, [4] Flavonoids, [5] Saponins, [6] Terpenoids.

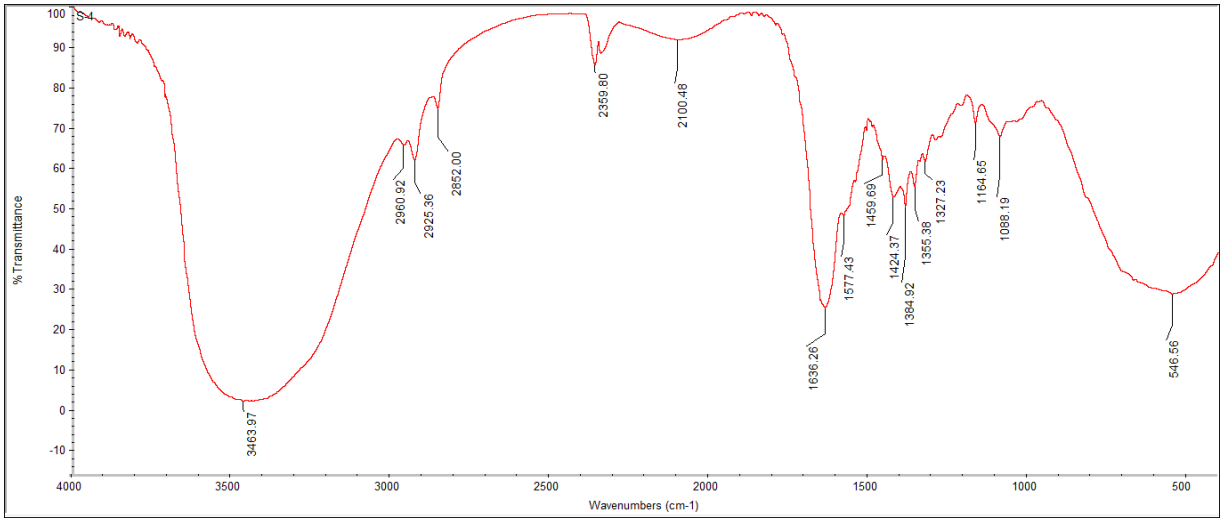

Figure-4: FTIR graph of aqueous crude extract obtained by *Penicillium notatum*

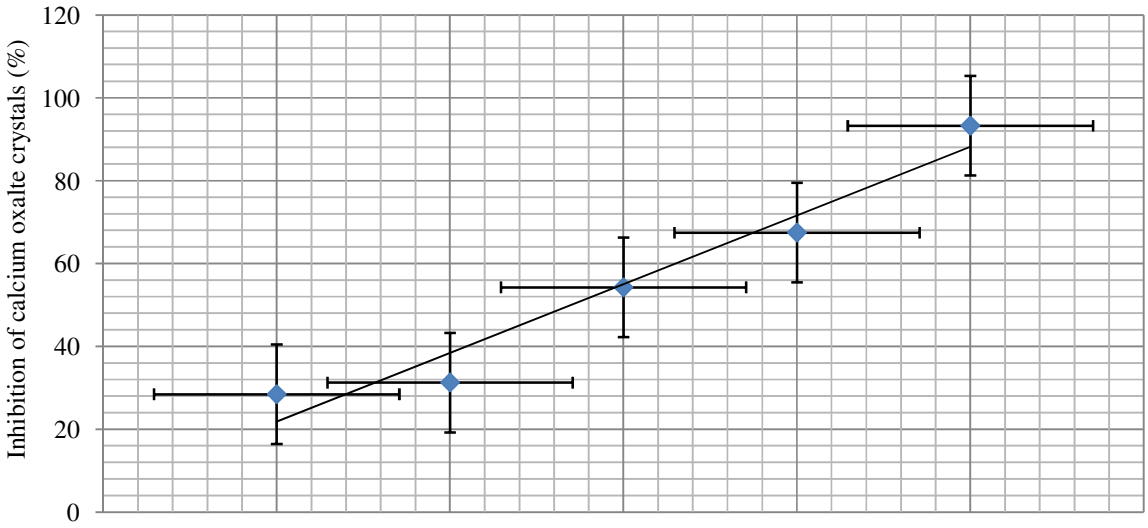

Figure-5: Antiurolithiasis Activity.

Discussion: *Aloe vera* is the succulent evergreen plant, mainly found in tropical climates around world. It is the medicinal, short-stemed herb[9]. *Aloe vera* leaves contains phytochemicals which are mostly active against different pathogens. The plant tissues, especially roots are excellent reservoirs for endophytic microorganism isolated from up till uncharted areas and from extreme environment is the choice for development of potential novel metabolite[11]. Due to their wide ethnobotany and pharmacology *Aloe* vera was selected from Shrirampur, Ahmednagar District, Maharashtra. Total of the six endophytic fungi were isolated and most of them belonging to *Penicillium* species. 18S rRNA analysis confirmed as *Penicillium notatum*[13]. This result supports the findings that fungal endophytes from grass, belonging to *Penicillium* species[5]. Submerged state fermentation successfully produced 2.60 g/100ml of the aqueous crude extract under controlled conditions by the liberation of fungus biomass. The phytochemical analysis showed terpenoids, flavonoids, saponins, phenols and tannins, alkaloid, cardiac glycosides, fixed oils and fats, proteins, carbohydrates with 2.26g of phenol/100g and 0.225g of flavonoids of gallic acid equivalent. Different bioactive compounds were analyzed from the aqueous crude extract of *Penicillium* Species[10]. Our results are coincidently similar to the available reports from other plants not to *Aloe vera*. Many reports related to bioactive compounds production are presented as phenolic compounds[11]. Phenolic and flavonoids compounds mainly observed as an antibacterial agents while terpenoids and alkaloid; cardiac glycosides have been reported to possess antifungal and antiurolithiasis activity[8,12]. Till date no reports were available on bioactive compounds from endophytic *Penicillium notatum* in

urolithiasis hence this study revealed new approach in urolithiasis. Crude extract showed effective inhibition of CaOx under controlled conditions. An endophytic *Penicillium notatum* has ability to produce various bioactive compounds which will be used for various human diseases so it would be reported as an organism of pharmaceutical importance[4,9]. However, further studies will need to undertaken to reveal the chemical structure of bioactive compounds and their toxic profile.

Conclusion

Plants contain endophytic microorganisms and they are able to produce secondary metabolites. The present study suggest that the endophytic *Penicillium notatum* purified from *Aloe vera* has great variations and production of phytochemicals and these may be useful in the drug discovery and development against different pathogens and in kidney stone.

References

1. Nerli R.B., Patil S., Hiremath M.B. and Patil R.A. (2014). Renal stone disease in the border regions of Karnataka, Maharashtra and Goa: Role of diet, urinary pH and body mass index. *Indian Journal of Health Sciences and Biomedical Research (KLEU)*, 7(2), 83.

2. Jayaraman U.C. and Gurusamy A. (2018). Review on urolithiasis pathophysiology and aesulapian discussion. *Journal of Pharmacy*, 8(2), 30-42.

3. Mane R.S., Paarakh P.M. and Vedamurthy A.B. (2018). A brief review on fungal endophytes. *International Journal of Secondary Metabolites*, 5(4), 288-303.

4. Ved P.V. (2015). Endophytic fungi as resource of bioactive compounds. *International Journal of Pharma and Biosciences*, 6(1), 887-898.

5. Mane R.S. and Choradiya B.R. (2018). Studies on endophytic bacteria isolated from grass against dysentery causing Shigella species. *International Journal of Chemistry Studies*, 2(1), 30-32.

6. Tiwari K. (2015). The future Products: Endophytic Fungal Metabolites. *Biodiversity, Bioprospecting and Development*, 2(1), 1-7.

7. Mathan S., Subramanian V. and Nagamony S. (2013). Optimization and antimicrobial metabolites production from endophytic fungi *Aspergillus terreus* KC 582297. *European Journal of experimental biology*, 3(4), 138-144.

8. Mane R.S., Jagtap P.R. and Vyawahare M.A. (2019). Production, purification and evaluation of different functional groups from endophytic *Penicillium* species derived bioactive compounds isolated from *Aloe vera*. *International Journal of Chemistry Studies*, 3(2), 35-38.

9. Devi N.N., Prabakaran J.J. and Wahab F. (2012). Phytochemical analysis and enzyme analysis of endophytic fungi from *Centella asiatica*. *Asian Journal of Tropical Biomedicine*, 2(3), S1280-S1284.

10. Wink M. (2015). Modes of action of Herbal medicines and plant secondary metabolites. *Medicines*, 2, 251-286.

11. Jena S.K. and Tayung K. (2013). Endophytic fungal communities associated with two ethno-medicinal plants of Smilipal biosphere reserve, India and their antimicrobial prospective. *Journal of Applied Pharmaceutical Science*, 3, S7-S12.

12. Walaa K.M. and Manish N.R. (2013). The diversity of anti-microbial secondary metabolites produced by fungal endophytes: an interdisciplinary perspective. *Fronteries in Microbiology,* 4, 1-12.

13. Mane R.S. (2019). Fungal endophytes (Unpublished doctoral research). Karnatak University, Dharwad, India.