INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 10, Issue 10, 2018

Original Article

ANTICANCER AND ANTIMICROBIAL POTENTIAL OF *BARLERIA PRIONITIS* LEAVES ETHANOL EXTRACT

PRIYANKA KUMARI PANCHAL[a*], SANJEEV KUMAR MEENA[b], KAMAL SINGH[c], NISHI SHARMA[a]

[a]IBMER, Department of Biotechnology and Life Science, Mangalayatan University, Aligarh, India, [b]Biochemistry Division, CSIR-Central Drug Research Institute (CDRI), Lucknow, India, [c]Vallabhbhai Patel Chest Institute, University of Delhi, Delhi, India
Email: drpriyanka.biotech@gmail.com

Received: 14 Jun 2018 Revised and Accepted: 03 Sep 2018

ABSTRACT

Objective: The present study was focused to screen traditionally used *Barleria prionitis* for anticancer effects against various cell lines and antimicrobial effect against various pathogenic strains of bacteria and fungi.

Methods: Extraction of *Barleria prionitis* leaves in ethanol was done by the Soxhlet method. After extraction, phytochemical estimation of these seven secondary metabolites like alkaloids, flavonoids, anthraquinones, saponins, terpenoids, tannins, and cardiac glycosides was done as per the protocols of Kokate. Minimum Inhibitory Concentration (MIC) effect of *Barleria* leaf ethanol (BLE) was done by the dilution method on five bacterial and five fungal strains. Further analysis (anticancer activity) was done with SRB (Sulphorhodamine B) assay. Statistical analysis of antimicrobial and anticancer activity was done by using MS Excel 2007 to±standard deviation and student t-test.

Results: *Barleria* leaf extract with ethanol is a non-polar solvent extract and considered as the best solvent to extract the maximum number of secondary metabolites like alkaloids, saponins, flavonoids, and tannins. BLE extract gave excellent MIC (Minimum Inhibitory Concentration) effects against pathogenic bacteria and pathogenic fungal strains. BLE had highly effective activity against *Pseudomonas aeruginosa* with 1.25 mg MIC, the OD value of the sample was 0.02±0.0005 (±SD) with 0.0211-0.0245 range. MIC against fungal strains had effective activity against *Candida vaginitis* with 6.25 mg, the OD value of the sample was 0.02±0.0003 (±SD) with 0.0213-0.0232 range. BLE extract had given more than 70% inhibition against breast cell lines (MCF-7) and 75.16% inhibition of DLD1 cell lines; it was near to Doxorubicin antibiotic (81%). Breast metastatic cell line (MDMAMB-468) was found 60% inhibited with BLE extract and there was a great difference in the results of Doxorubicin. Out of six experimented cell lines, BLE gave very good inhibition for two cell lines, i.e. Breast (MCF-7) and Colon cell lines (DLD-1).

Conclusion: BLE extract had shown the best antimicrobial and antifungal effect, against *Pseudomonas aeruginosa* and *Candida vaginitis* respectively. BLE also showed an anticancer effect against Lung cell lines (A549), Breast cancer cell line (MCF-7), Breast metastatic cell line (MDMAMB-468), Colon cell line (DLD-1) and lung metastatic cell line (NCIH358) at a statistically significant level ($p=<0.05$). It did not give any significant results against the colon metastatic cell line (SW620).

Keywords: *Barleria prionitis*, Anticancer, Antimicrobial, Phytochemical constituents, BLE(*Barleria* leaf ethanol), Leaf

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DOI: http://dx.doi.org/10.22159/ijpps.2018v10i10.27967

INTRODUCTION

According to all India ethnobiological survey carried out by the ministry of environment and forest, Government of India, there are over 8000 species of medicinal plants being used by people of India. Medicinal plants are used as a natural source to combat against various diseases like diabetes, asthma, diarrhea, cancer, etc. U. S National Cancer Institute (NCI) in 1957 launched a step to find out anticancer agents from higher plants. Today, most of the useful and creative drugs can be derived from higher altitude plants as natural products [1]. Global scenario indicated that breast cancer and colorectal cancer is the most prominent cancer in case of both women and men [2].

Barleria prionitis is an Ayurvedic herb distributed in the tropical Asia, Africa, and Yemen. The whole plant or its specific parts (leaf, stem, bark, and flower) have been used for the treatment of toothache, jaundice, fever, gastrointestinal disorder, etc. [3]. Phytochemical constituents and extracts of this plant have infatuated wide range of pharmacological include antimicrobial, anti-arthritic cytoprotective, hepatoprotective, diuretic, antidiarrheal, enzyme inhibitory and antinociceptive activities without any toxic effects. It was observed that the leaves showed higher degree antioxidant potential and phenolic content in comparison to flower and stem [4]. *Barleria prionitis* occupy a significant place in the Ayurvedic medicine in India; it has been used as traditionally due to its Phytochemistry, pharmacology, and toxicity. In a recent study, *Barleria prionitis* has also shown good antimicrobial activity against various strains by using the gradient plate technique method [12].

The present study was focused to screen traditionally used *Barleria prionitis* for anticancer effects against various cell lines and antimicrobial effect against various bacterial and fungal strains.

MATERIALS AND METHODS

Chemicals and reagents

Ethanol (LC-MS grade), Distilled water (Millipore, Milford, MA, USA), broth (Mueller Hinton broth), culture medium for cell lines (RPMI-1640 with 5% FBS), spectrophotometer (API 4000 QTRAP™MS/MS system from AB Sciex, Concord, ON, Canada), Plate reader for cell lines (Epoch Microplate reader, Biotek, USA).

Procurement of plant material

The plant was collected from the government nursery of Modinagar, Dist. Ghaziabad, U. P, India and authenticated by NBRI, Lucknow, India (Voucher/Specimen no. is NBRI-SOP-202), a voucher specimen was deposited in the department.

Extraction

The plant's part (Leaves) was separated from the plant and washed carefully under running tap water followed by distilled water. These

were dried in 45 °C for one week and pulverized to a fine powder using a sterilized mixer grinder. Then the 50 g powder of the leaves was extracted by the Soxhlet method in ethanol, which was taken on the basis of their polarity 19.4.

Qualitative phytochemical investigation of extract

A qualitative chemical test was conducted, to check the presence of the various phytochemical constituents. The presence of various phytochemicals found in the extract is given in table 1 for a qualitative screening known amount of 20 mg [5].

Minimum inhibitory concentration against bacterial and fungal strains

Collection of strains

The bacterial pathogens strains *Cornybacterium diphtheria*: MTCC 116, *Pseudomonas aeruginosa*: MTCC 10462, *Bacillus thruengiensis:* MTCC 10484, *Bacillus anthracis*: MTCC 10095, *Salmonella typhi*: MTCC3231, *Chlamydia pneumonia*: MTCC 7162 and fungal strains like *Aspergillus fumigatus*: MTCC 4163, *Cryptococcus neoformans*: MTCC 63333, *Candida albicans*: MTCC 7253 were collected from innovative life sciences, Lucknow and *Candida vaginitis*: Clinical isolate, *Blastomyces dermatitidis*: Clinical isolate, Gaurang Homeo Clinic, Aliganj, Sector-1, Lucknow 226010, Uttar Pradesh.

Screening of bioactive compounds against various microbes

The method used to screen plant extracts before determination of MIC was agar well diffusion method, in which 25 ml of nutrient agar media poured in a sterile Petri dish, 100µl of test organisms were spread on the surface of media, wells were prepared with the help of sterile borer and wells were aseptically filled with 50µl of plant extract along with positive (antibacterial compound tetracycline and antifungal compound fluconazole at 50µg/ml and 100µg/ml respectively) and negative control (autoclaved DMSO). Plates were incubated aerobically at 37 °C/25 °C as bacteria/fungus culture for 72 h. The diameter of zones of inhibition was measured, and I found the initial concentration of MIC was 10 mg/ml for bacterial strains and 20 mg/ml for fungal strains

Preparation of bacterial/fungal strains and culture conditions

All the cultures were obtained from Lucknow, India. Cultures of *Salmonella typhi, Chlamydia pneumoniae, Bacillus thruengiensis, Bacillus anthracis, and Pseudomonas aeruginosa* were maintained on the nutrient agar medium and fungal strains of *Aspergillus fumigatus, Candida neoformans, Candida albicans, Candida vaginitis, Blastomyces dermatitidis* on Potato Dextrose agar medium by making slants, and the stock cultures were sub cultured at monthly intervals.

Dilution method

3 ml of nutrient broth (for bacterial cultures) and Potato Dextrose (for fungal strains) were taken in 5 test tubes, sterilized by autoclave. Plant extracts with a final concentration of 100 mg/ml (for bacterial pathogens) and Plant extracts with a final concentration of 200 mg/ml (for fungal pathogens) were mixed with another extra 3 ml of liquid media (Potato Dextrose, PD) and liquid media (Nutrient Broth). 1 ml culture was added into the first tube, mixed properly, and after mixing; 3 ml of the solution was taken from the first test tube and transferred to the second test tube. The same was repeated up to the 5th test tube, followed by removal of an extra 3 ml solution from last test tube to keep the media volume constant.

30 µl of fresh culture was added to all of the test tubes and were incubated at 37 °C for overnight. The inhibition of growth of pathogens was recorded in terms of optical density at 600 nm. The concentration of plant extract just before the test tube showing growth was recorded as MIC [6].

Statistical analysis

Statistical analysis was done in triplicate, and the data were expressed as the mean±standard deviation with range. Analysis of this study was done using the Statistical Package for the Social sciences (SPSS) Version 20.0 and MS Excel 2007. One way ANOVA followed by Turkey's honestly significant difference post hoc test was used to compare the data, in which *p values* were considered significant at a 95% confidence interval ($p<0.05$).

Total activity

Total activity is the volume at which the test extract can be diluted with the ability to kill microorganisms. It was calculated by dividing the amount of extract from 1g plant material by the MIC of the same extract or compound isolated and is expressed in ml/g [7].

$$\text{Total activity} = \frac{\text{Extract per gram dried plant part}}{\text{MIC of extract}}$$

Anticancer activity

The cell lines were collected from the Biochemistry Division, CSIR-Central Drug Research Institute (CDRI), Lucknow and also preceded at CDRI under the supervision of Dr. Dipak Datta and his Technician Mr. Sanjeev Meena.

Preparation of test sample

The test samples/molecules were weighed in microcentrifuge tubes and stock solutions of 50 mg/ml made by dissolving the samples in DMSO. Stocks were stored at-20 °C. A working solution of 200 µg/ml concentration was made by diluting the stock solution in culture medium prior to the assay.

Sulforhodamine B (SRB) assay (Addition of cells)

The human cancer cell lines Breast (MCF-7), Colon (DLD-1), Lung (A549), Breast Metastatic (MDMAMB-468) and Lung Metastatic (NCIH358) were maintained in RPMI-1640 medium. Colon Metastatic (SW620) cell lines were maintained in DMEM medium. The enumerated cells were dispensed in a 96-well tissue culture plate; each well receives 100 µl.

Addition of test sample

100 µl of working solution of the test sample was added to the cell monolayer to give a final concentration of 100 µg/ml for each sample; duplicate wells were included.

Addition of SRB and calorimetric reading

After 48 h incubation, cells attached to the substratum of the plate were fixed by adding cold 50% trichloroacetic acid (TCA, 50 µl/well) on top of the medium and incubated at 4 °C for 1h. After that, the plate was gently washed 5 times with slow running tap water via plastic tubing to remove TCA, culture medium and dead cells. After washing, the plates were allowed to dry in air. To dry plates, 50 µl/well of SRB solution was added and left at room temperature for 30 min. At the end of the staining period, unbound SRB was removed by quickly rinsing plates 4-5 times with 1% (v/v) acetic acid.

Plates were allowed to air-dry at room temperature. 150 µl of 10 mmol Tris base solution was added to each well and the plate was shaken for 15 min on a gyratory shaker to solubilize the protein-bound dye. Absorbance was measured at 510 nm in a microplate spectrophotometer.

Data analysis

Percentage of cell growth inhibition in the presence of the test sample was calculated as follows:

$$\textbf{\% of cells killed} = \textbf{100} - \left[\frac{\text{Mean OD}_{test}}{\text{Mean OD}_{control}} \times 100\right]$$

Statistical analysis

Statistical analysis was done using IBM SPSS statistics 20.0 and MS Excel 2007 with a range (±standard deviation).

RESULTS

Table 1: Phytochemical estimation of *Barleria prionitis* leaf ethanol extract

S. No.	Secondary metabolites	Method	Results
1	Alkaloids	Hager's method and Tannic acid	Positive
2	Saponins	Foam test	Positive
3	Steroids	Salkowaski reaction	Negative
4	Flavonoids	Shindona test and Sodium hydroxide test	Positive
5	Tannins	Lead Acetate test	Positive
6	Cd. Glycosides	KellarKillani test and Baljet's test	Negative
7	Anthroquinone	Confirmation test from kokate.(Pharmacology Hand Book)	Negative

Table 2: MIC of *Barleria prionitis* leaf ethanol extracts against bacteria strains

S. No.	Name of strains	20 mg/ml	10 mg/ml	5 mg/ml	2.5 mg/ml	1.25 mg/ml	Negative control	Positive control	MIC
1	*Salmonella typhi*	0.0±0.0002 0.0006-0.002	0.02±0.0005 0.0213-0.0241	0.01±0.0004 0.0102-0.0129	0.01±0.0002 0.0112-0.0128	0.12±0.0003 0.1207-0.1229	0.65	NG NG	2.5
2	*Chlamydia pneumoniae*	0±0.0003 0.0003-0.0022	0.01±0.0002 0.0118-0.0128	0.0±0.0004 0.0004-0.0025	0.13±0.0005 0.1298-0.1329	0.24±0.0061 0.0003-0.2428	0.61	NG NG	5
3	*Bacillus thruengiensis*	0±0.0002 0.0003-0.001	0.0±0.0001 0.0003-0.0012	0.02±0.0001 0.0210-0.0219	0.02±0.0003 0.0201-0.0218	0.18±0.0004 0.1803-0.1828	0.68	NG NG	2.5
4	*Pseudomonas aeruginosa*	0±0.0002 0.0003-0.002	0.0±0.0001 0.0006-0.0014	0.02±0.0005 0.0215-0.0246	0.01±0.0003 0.0109-0.0128	0.02±0.0005 0.0211-0.0245	0.61	NG NG	1.25
5	*Chlamydia diphtheriae*	0.02±0.0004 0.0204-0.0233	0.01±0.0007 0.0091-0.0129	0.01±0.0003 0.0111-0.0129	0.04±0.0001 0.0421-0.0432	0.21±0.0004 0.2110-0.2132	0.72	NG NG	2.5

*±Standard Deviation with range, n= 5 NG= No growth, MIC=Minimum Inhibitory Concentration, Negative control=DMSO

Table 3: MIC of *Barleria prionitis* leaf ethanol extract against fungal strains

S. No.	Name of strains	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	3.13 mg/ml	Negative control	Positive control	MIC
1	*Aspergillusfumigatus*	0±0.0001 0.0002-0.0008	0.02±0.0004 0.0203-0.0225	0.02±0.0006 0.0198-0.0238	0.22±0.0002 0.2202-0.2218	0.36±0.0003 0.3610-0.3631	0.43	NG	12.5
2	*Cryptococcus neoformans*	0.01±0.0008 0.0107-0.0157	0.01±0.0003 0.0108-0.0125	0.04±0.0005 0.0398-0.0432	0.25±0.0003 0.2502-0.2521	0.43±0.0002 0.4309-0.4325	0.48	NG	12.5
3	*Candida albicans*	0±0.0004 0.0003-0.0032	0.02±0.0002 0.0209-0.0222	0.05±0.0002 0.0518-0.0532	0.19±0.0002 0.1919-0.1932	0.28±0.0005 0.0002-0.2831	0.45	NG	12.5
4	*Candida vaginitis*	0±0.0003 0.0002-0.0021	0±0.0001 0.0003-0.0013	0.02±0.0006 0.0210-0.0244	0.02±0.0003 0.0213-0.0232	0.19±0.0003 0.1905-0.1928	0.42	NG	6.25
5	*Bacillus dermatitidis*	0.02±0.0003 0.0215-0.0232	0.02±0.0003 0.0211-0.0231	0.01±0.0006 0.0102-0.0143	0.18±0.0003 0.1802-0.1822	0.29±0.0003 0.2914-0.2931	0.48	NG	12.5

*±Standard Deviation with range, n=5, NG= No growth, MIC=Minimum Inhibitory Concentration, Negative control=DMSO

Table 4: Total activity of BLE for bacteria

Pathogen details		Total activity	
S. No.	**Strains**	**T A**	**MIC**
1	*S. typhi*	214.4	2.5
2	*C. pneumoniae*	107.2	5
3	*B. thruengiensis*	214.4	2.5
4	*P. aeruginosa*	428.8	1.25
5	*C. diphtheria*	214.4	2.5

Table 5: Total activity of BLE for fungi

Pathogen details		Total activity	
S. No.	**Strains**	**T A**	**MIC**
1	*A. fumigatus*	42.88	12.5
2	*C. neoformans*	42.88	12.5
3	*C. albicans*	42.88	12.5
4	*C. vaginitis*	85.76	6.25
5	*B. dermatitidis*	42.88	12.5

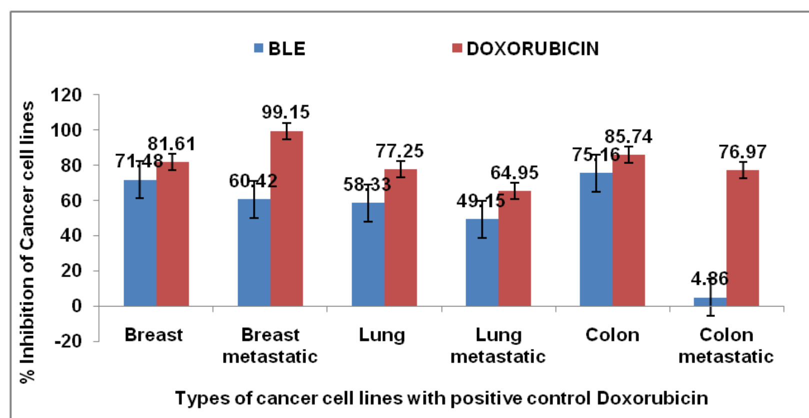

Fig. 1: Percent Inhibition activity for anticancer effects of BLE and doxorubicin drug against cancer cell lines and metastatic cancer cell lines, *±standard deviation with mean, n= 3

DISCUSSION

Barleria leaf extract with ethanol is a non-polar solvent extract and considered as the best solvent to extract a maximum number of secondary metabolites (4/7) i.e. alkaloids, saponins, flavonoids, and tannins. In various other studies, phytochemicals such as alkaloids, saponins, flavonoids, sterols, tannins, and anthraquinone were extracted as a secondary metabolite from BLE [8-10].

BLE extract showed good MIC effects against pathogenic bacterial and pathogenic fungal strains. This effect occurred due to the presence of alkaloids, saponins, flavonoids, and tannins (individually or in combination). Table 2 shows the results of MIC against bacterial strains. In which, BLE had high antimicrobial activity against *Pseudomonas aeruginosa* with 1.25 mg MIC, the OD value of the sample was 0.02±0.0005 (±SD) with 0.0211-0.0245 range. Table 3 shows the results of MIC against fungal strains. In which, BLE had high antifungal activity against *Candida vaginitis* with 6.25 mg MIC, OD value of the sample is 0.02±0.0003 (±SD) with 0.0213-0.0232 range. BLE was very effective against *C. Vaginitis,* and *A. fumigates* but showed the moderate effect against *B. dermatitidis, C. albicans,* and *C. neoformans.*

Barleria leaf ethanol extract gave more than 70% inhibition against Breast cell lines (MCF-7) comparable to Doxorubicin antibiotic (81%). Breast metastatic (MDMAMB-468) was 60% inhibited with *Barleria* leaf ethanol extract, and there was a significant difference in the results of Doxorubicin. *Barleria* leaf with ethanol (BLE) gave very good inhibition for two cell lines i.e. Breast (MCF-7) and Colon cell lines (DLD-1) out of six cell lines. *Barleria prionitis* was found inactive for anticancer effects in reported study methanol extracts of leaf [11].

CONCLUSION

Barleria leaf ethanol (BLE) extract had given best inhibitory effects against *Pseudomonas aeruginosa* in the study included bacterial strains. *Candida vaginitis* has been found to be best sensitive strain than other experimental fungal strain included in our study. BLE was found to be the best anti-cancer activity against most experimented cancer cell lines included in our study like Lung cell line (A549), Breast cell line (MCF-7), Breast metastatic cell line (MDMAMB-468), Colon cell line (DLD-1) and lung metastatic cell line (NCIH358) with ($p<0.05$) significance. It did not give any significant results against colon metastatic cell lines (SW620). The study would provide further insight in the development of herbal as well as other drugs and therapies for the treatment of various types of cancer and other diseases.

ACKNOWLEDGMENT

I considered it my right to communicate profound and overwhelmed instinct of gratitude and indebtedness to my esteemed Supervisor Dr. Nishi Sharma, Assistant Professor-II, Department of Biotechnology and Life Sciences for her insight sagacity. I sincerely thanks to Mr. R. P Mishra, Director of Innovative Life Sciences for providing me laboratory to complete my research work mainly antimicrobial activity and Dr. DipakDutta, Senior Scientist and Mr. Sanjeev Meena, Senior Technician, Biochemistry Division, CDRI, Lucknow for complete my anticancer activity study.

AUTHORS CONTRIBUTIONS

Mrs. Priyanka Kumari Panchal, a Ph. D. Student, the main author for this present study, Managalayatan University, Aligarh, Mr. Sanjeev Meena, Senior Technician, Biochemistry Division, CDRI, Lucknow, completes my anticancer activity study. Mr. Kamal Singh, Ph. D. Student, VPCI, University of Delhi, help to provide statistical analysis and language, Dr. Nishi Sharma, Assistant professor, Managalayatan University, Aligarh, work was completed under her supervision.

CONFLICT OF INTERESTS

The author(s) declare(s) that they have no conflict of interest to disclose

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