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Research Article

Evaluation of antibacterial, antioxidant and anticancer activity of *Beloperone plumbaginifolia* (jacq.) Nees leaves extract

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Abstract

In an attempt to search for new antimicrobials, anticancer compounds from plant sources, *Beloperone plumbaginifolia* leaves were selected for its rich traditional background. The bioactive compounds were extracted from its leaves by soxhlet extraction with chloroform and methanol solvent. The methanol extract has performed well when compared to chloroform extract; the 11 mm zone of inhibition for methanol extract was very closer against the standard with 12 mm zone of inhibition. Moreover the methanol extract had demonstrated excellent anticancer activity at IC_{50} of 62.5 µg/ml whereas IC_{50} value for chloroform extract was 125 µg/ml. overall the results reveals the pharmacological potential of Beloperone plumbaginifolia plant.

Key Words: Antimicorbial, Beloperone plumbaginigfolia, $IC_{50,}$ Zone of inhibition.

Introduction

Medicinal plants have been used in our traditional system in the form of Siddha, Ayurveda, Unani and Homeopathy in different formulation for different types of diseases. These kinds of medicinal plants have been investigated by scientists all over the world to produce novel chemotherapeutics for number of diseases including cancers. Over 60 % of cancer therapeutics existing are derived from plants. Drugs from natural products alone yields revenue of US $ 14 billion a year in US and US $ 40 billion world wide.

Medicinal plants are an excellent source of medicinal agents such as novel chemotypes and pharmacophores and provide novel chemical scaffolds for elaboration by combinatorial approaches[1]. The search from medicinal plants is not only for anticancer agents but also for antimicrobial compounds since the microorganisms are becoming increasingly resistant to antibiotics due to biological, social and environmental factors and its becoming a threat to deal with infectious diseases.

Beloperone plumbaginifolia is a traditional herb used in Indian system of traditional medicine and folklore medicine. It has been widely used as an antidote for snake and scorpion bite. Besides this, the extracts of the plants were used for the treatment of Psoriasis. Hence in this study Beloperone plumbaginifolia is investigated for the first time in the world for evaluating its antibacterial, antioxidant and anticancer activities.

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Grasslands are important for *in situ* conservation of genetic resources. Of a total of 10,000 species, only 100 to 150 forage species have been cultivated, but many more hold potential for sustainable agriculture. The grasses assume importance not only as livestock feed, but also as soil builders and binders and aid in soil conservation. In their principal role, the tropical grasses stand as the highest potential yielder of starch and proteins equivalent to any other crop plants and further being the dominant component of tropical pastures, as the cheapest sources of animal feed (M.P.Rajora, 2002). Perennial grasses are major components of tropical pastures providing bulk of herbage to animals. The grass and grazing are important constituents of fodder resources in India. Out of the total land area of 3.2 million sq. km of this country, about one-third falls under arid and semiarid zone. (Vora & Bhatnagar, 2003).

Cenchrus ciliaris also known as buffel grass or African foxtail is a palatable nutritious and warm season grass naturally occurring in the drier parts of the world. It is a valuable tufted perennial grass in arid and semi arid areas characterised by severe drought, high temperature, low rainfall and sandy soil. It is an excellent grazing perennial suited to pasture and rangelands. Its high soil binding capacity is due to its clustered root system in the upper 8 to 10 cm layer of soil. It survives extreme and prolonged drought but grows vigorously when favourable conditions set in. Buffel grass (*Cenchrus ciliaris* Linn.), a perennial pasture grass species, has wider adaptability in varied edaphic habitats all over the country. It is one of the prominent species of *Dichanthium-Cenchrus-Lasiurus* grass cover type of tropical India.

Material and Methods

Collection of plant material:

Fresh leaves of Beloperone plumbaginifolia (Jacq.) were collected form Herbal Garden, National Institute of Siddha, Tambaram Sanatorium, Chennai- 600 047. The leaves were shade dried for 3 days and powdered well.

Extraction

15 gm of powdered leaf was weighed and subjected to soxhlet extraction. The powdered leaf was simultaneously extracted with methanol and chloroform and the respective solvents were allowed to evaporate under vacuum. The resultant dry powder extract was collected and stored at – 20 degree Celsius until further analysis.

Test samples

Throughout the study sample 1 represents methanol extract and sample 2 represents chloroform extract of *Beloperone plumbaginifolia* leaves.

PHYTOCHEMICAL ANALYSIS

The qualitative tests for tannins, saponins, flavonoids, alkaloids, proteins, steroids, and anthraquinones were carried out in sample 1 and 2 leaves using standard procedures described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973) [2-4].

ANTIBACTERIAL ACTIVITY

Both sample 1 and 2 were tested against microorganisms such as *Staphylococcus aureus, Bacillus subtilis, Escherichia.coli, Salmonella typhi and Shigella boydi.* The Ampicillin *(10 mcg/disc)* was used as a standard antibiotic, and Dimethyl sulphoixde was used as a negative control.

Preparation of inoculum

Stock cultures were maintained at 4°c on slant of nutrient agar. Active cultures for experiments were prepared by transferring a loop full of cells from the stock cultures to test tubes of nutrient broth for bacteria that were incubated at 24 h at 37ºC. The Assay was performed by agar disc diffusion method.

Agar disc diffusion method

Antibacterial activity of plant extracts was determined by disc diffusion method on Muller Hinton agar (MHA) medium. The Muller Hinton Agar medium is poured in to the petriplate. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistered with the bacterial suspension. The disc were placed in MHA plate and add 20 µl of [sample concentration: 1000µg] each samples were placed in the disc. The plates were incubated for 24 h at 37ºC. Then the microbial growth was determined by measuring the diameter of zone of inhibition.

ANTIOXIDANT ACTIVITY

DPPH free radical scavenging activity

DPPH free radical scavenging activities of both the samples were evaluated by the method of Brand-Williams[5]. According to this procedure, 3 ml of absolute methanol was added in all test tubes along with blank, followed by adding of 100µl of absolute methanol to blank. 100 µl of BHT to the standard and 100 µl of respective samples to all other tubes marked as tests were added. Finally, 200 µl of DPPH reagent was added to all the test tubes including blank. All the test tubes were incubated at room temperature and dark condition for minimum of 30 minutes. The absorbances of all samples were checked at 517nm.

Percentage of Inhibition of DPPH Activity=

$$\frac{\text{Abs control-Abs Sample}}{\text{Abs Control}} \times 100$$

Where, Abs Control= Optical density of Control, Abs Sample = Optical density of sample extract.

ANTICANCER ACTIVITY

Cell line and culture

HT-29 cell lines were obtained from National Centre for Cell Sciences, Pune (NCCS). The cells were maintained in Minimal Essential Media supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO_2 at 37 °C.

Reagents

MEM was purchased from Hi Media Laboratories, Fetal bovine serum (FBS) was purchased from Cistron laboratories, Trypsin, methylthiazolyl diphenyl-tetrazolium bromide (MTT), and Dimethyl sulfoxide (DMSO) were purchased from (Sisco research laboratory chemicals Mumbai). All of other chemicals and reagents were obtained from Sigma Aldrich Mumbai.

In vitro assay for Cytotoxicity activity (MTT assay)

The Cytotoxicity of samples on HT-29 was determined by the MTT assay[6]. Cells (1×10^5/well) were plated in 1ml of medium/well in 24-well plates (Costar Corning, Rochester, NY). After 48 hours incubation the cell reaches the confluence. Then, cells were incubated in the presence of various concentrations of the samples 1 & 2 in 0.1% DMSO for 48h at 37°C. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 200µl/well (5mg/ml) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide cells(MTT) phosphate- buffered saline solution was added. After 4h incubation, 0.04M HCl/isopropanol were added. Viable cells were determined by the absorbance at 570nm. Measurements were performed and the concentration required for a 50% inhibition of viability (IC50) was determined graphically. The absorbance at 570 nm was measured with a UV- Spectrophotometer using wells without sample containing cells as blanks. The effect of the samples on the proliferation of HT-29 was expressed as the % cell viability, using the following formula:

% cell viability = A570 of treated cells / A570 of control cells × 100%.

Results

Phytochemical analysis

Proteins and steroids were present in both the extracts. However methanol extract contains alkaloids and saponins, whereas alkaloids were present only in chloroform extract. Tannins and anthraquinones were not found in both the extracts (Table 1).

Antibacterial assay

In antibacterial assay, when compared to standard antibiotic, the activities of both the extracts were slightly lower. Their zone of inhibition was 8 – 12 mm against the entire microorganisms tested. The methanol extract had

significantly inhibited the growth of *Shigella boydi* with 11 mm zone of inhibition which is very close to the standard which had 12 mm zone of inhibition (Table 2).

Antioxidant assay

In antioxidant assay using DPPH free radical scavenging activity, methanol had shown a greater antioxidant activity of 72. 2 % than the chloroform extract of 61.1 % but considering the standard antioxidant (Butylated hydroxyl toluene) BHT activity of 99.4 % both are lower (Table 3).

Anticancer assay

The results of the anticancer activity of methanol and chloroform extract tested against the HT- 29 colon cancer cell lines determined by MTT assay and percentage viability calculated by spectrophotometer at A570 nm were listed in table 3. In 48 hours of incubation with samples of varying concentration diluted with DMSO, both the extract has shown remarkable anticancer activity (table 4 & 5). The extracts from methanol and chloroform had induced cell death in a confluence cancer cell population to a varying percentage according to their varying concentration. For instance, 96 % of the HT 29 cells were died at the concentration of 1000 µg/ml of methanol extract whereas 89.8 % cell died at the concentration of 1000 µg/ml of chloroform extract. The IC_{50} values for both the extracts were calculated by the graph 1 and 2. In this calculation it was found that the methanol extract had induced apoptosis in 51.1 % of HT-29 colon cancer cells at the IC 50 of 62.5µg/ml, whereas the chloroform extract had induced 49 % of cell death at IC50 of 125 µg/ml concentration. It should be noted that at 125 µg/ml concentration methanol extract had killed 59.2 % of HT- 29 colon cancer cell lines.

Discussion

The presence of flavonoids, saponins, alkaloids, steroids reveals the pharmacological potential of this plant. For instance, the flavonoids, saponins present in the methanol extract may be the reason for its overall better performance than chloroform extract, and its excellence in anticancer assays.

Flavonoids are polyphenolic compounds found in numerous plants and are promising candidates for cancer prevention and they also have antioxidant, iron chelating activity, etc, Flavonoids from apple has shown to inhibit HT 29 colon cancer cell growth by significantly modulating the gene expression related to xenobiotic metabolism[7]. Induction of cancer cell apoptosis by flavonoids is associated with their ability to inhibit fatty acid synthesis activity[8]. Saponins on the on the other hand is a steroid or triterpenoids has immunostimulant, hypocholesteromic and also have remarkable cytotoxicity and causes apoptosis in a dose dependent manner. Avicins are a class of natural saponins with selective proapoptotic activity in cancer cells[9]. Alkaloids have strong lipid peroxidative properties has been used in the treatment of psoriasis. They also induce cancer cell death by inhibition of topoisomerases I, DNA interaction, mitochondrial damage, etc.

The antibacterial activity of the methanol extract against *Shigella boydii* is promising when compared the results against *Escherichia coli, Staphylococcus aureus, Bacillus subtilis* and *Salmonella typhi*. Genus *Shigella* affects 164.7 million people a year and 1.1 millions die from developing countries and 1.5 millions die in industrialized countries. They are increasingly becoming resistant to antibiotic due to plasmid encoding functions. Among the genus *Shigella*, *Shigella boydii* accounts for 6 % of infections[11]. Hence the future studies has been planned to evaluate the effect of methanol extract against most dominant species of *Shigella* such as *S. flexneri, S. sonnei,* etc,

The antioxidant though present is not efficient as that of the Butylated hydroxyl toluene (BHT). This may be due to the low levels of antioxidant compounds present in the extract. Surprisingly, from the results of the anticancer investigations, it was revealed that both the extract had higher activity against HT 29 colon cancer cell lines. The higher activity of methanol extract will be partly explained by different bioactive compounds extracted in methanol than the chloroform (Table 1), and mainly by the presence of 3, 5-Dihydroxy-6-methyl-2, 3-dihydro-4H-pyran-4-one, (DDMP) in methanol fractions observed in our previous unpublished work by GC/MS analysis. DDMP has earlier reported to have an excellent anti colon cancer activity[12].

CONCLUSION

It can be concluded that the methanol fractions of the plant extract had better activity than the chloroform extract. It had higher cancer activity and selectively active against *Shigella boydii.* Hence the methanol fractions have to be investigated in detail to selectively indentify the compound responsible for anticancer and specific antibacterial activity.

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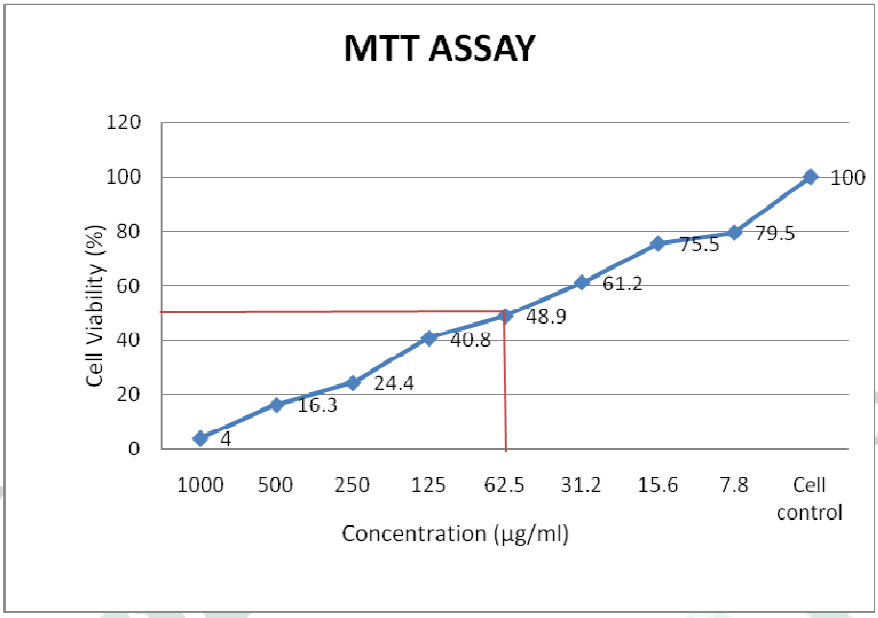
Graph 1: IC_{50} calculation for anticancer effect of methanol extract.



Graph 2: IC_{50} calculation for anticancer effect of chloroform extract

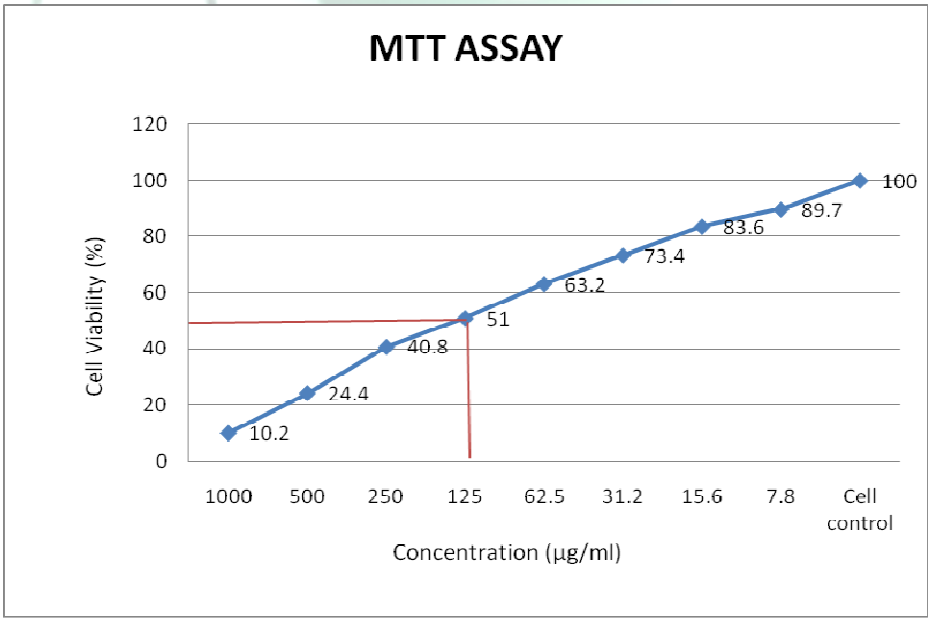


Table 1: Phytochemical screening of methanol and chloroform extract of *Beloperone plumbaginifolia* (Jacq.) leaves. + indicates presence of compound and – indicates absence of compound. Sample 1: methanol extract, Sample 2 – Chloroform extract.

S.No	Name of the Test	Result in sample 1	Result in sample 2
1	Tannin	-	-
2	Flavonoids	+	-
3	Saponins	+	-
4	Alkaloids	-	+
5	Protein	+	+
6	Steroid	+	+
7	Anthraquinones	-	-

Table 2: Antibacterial effect of methanol and chloroform extract of *Beloperone plumbaginifolia* (Jacq.) leaves. mm - indicates milli meter. Sample 1: methanol extract, Sample 2 – Chloroform extract.

S.No	Microorganisms	Zone of inhibition in mm			
		Sample1	Sample 2	DMSO	Std
1.	*S .aureus*	12mm	8mm	-	16mm
2.	*Bacillus subtilis*	9mm	9mm	-	14mm
3.	*E. coli*	9mm	8mm	-	14mm
4.	*Salmonella typhi*	9mm	-	-	13mm
5.	*Shigella boydi*	11mm	9mm	-	12mm

Table 3: Quantitative Antioxidant Analysis of Chloroform and Methanol Extract Using DPPH Free Radical Scavenging Activity.

Extracts	Blank	Absorbance	%
Chloroform Sample	0.18	0.07	61.1
Methanol Sample	0.18	0.05	72.2
BHT(Butylated hydroxyl toluene)	0.18	0.001	99.4

Table 4: Anti Cancer effect of *Sample-1 (Methanol extract)* on HT-29 cell line

S.No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1	1000	Neat	0.02	4.0
2	500	1:1	0.08	16.3
3	250	1:2	0.12	24.4
4	125	1:4	0.20	40.8
5	62.5	1:8	0.24	48.9
6	31.2	1:16	0.30	61.2
7	15.6	1:32	0.37	75.5
8	7.8	1:64	0.39	79.5
9	Cell control	-	0.49	100

Table 5: Anti Cancer effect of *Sample-2 (Chloroform extract)* on HT-29 cell line

S.No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1	1000	Neat	0.05	10.2
2	500	1:1	0.12	24.4
3	250	1:2	0.20	40.8
4	125	1:4	0.25	51.0
5	62.5	1:8	0.31	63.2
6	31.2	1:16	0.36	73.4
7	15.6	1:32	0.41	83.6
8	7.8	1:64	0.44	89.7
9	Cell control	-	0.49	100

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