**International Journal of Current Advanced Research**
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: SJIF: 5.995
Available Online at www.journalijcar.org
Volume 6; Issue 8; August 2017; Page No. 5269-5275
DOI: http://dx.doi.org/10.24327/ijcar.2017.5275.0688

Research Article

# ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF *BUTEA MONOSPERMA* LINN.IN *VIVO* AND *IN VITRO*

**Renu Sarin* and Chand Kor Kumari**

Laboratory of Bioactive Natural Products, Department of Botany, University of Rajasthan, Jaipur-302004, India

**A R T I C L E I N F O**

***Article History:***

Received 19th May, 2017
Received in revised form 12th June, 2017 Accepted 6th July, 2017
Published online 28th August, 2017

***Key words:***

Antimicrobial; FRAP; LPO; Peroxidase; *Butea monosperma.*

**A B S T R A C T**

The present research deals with antimicrobial and antioxidant activity in parts and callus cultures of *Butea monosperma*. The ethanolic extracts exhibited antimicrobial activities with zones of inhibition ranging from 4 to 16 to mm. All the extracts exhibited appreciable activity against all the clinically important bacterial and fungal species Maximum Inhibition zone (14mm) was observed in callus (14mm) against *E.coli* and minimum in seeds (4mm) against *Bacillus subtilis.* In case of fungus,maximum activity was observed in callus (16mm) against *A. niger* and minimum against *T. reesei* The antimicrobial activity of the extract was compared with the standard drugs. The ability of the crude extracts of *B.* to inhibit the growth of various bacteria and fungi showed its broad spectrum antimicrobial potential, which may be employed in the management of microbial infections. Further antioxidant activity was done using FRAP, Lipid Peroxidation Assay and Peroxidase assay. In the present investigation maximum (240 µm/l/gdw) antioxidant activity was found in methanol extract of flowers and minimum in hexane fraction of Seeds (34 µm/l/g dw) using FRAP Assay.Here maximum (61.3 µm/l/g dw) LPO activity was observed in callus cultures and minimum (40.3 µm/l/g dw) in flowers.Peroxidase assay showed maximum (8.18 µm/l/g dw) antioxidant activity in callus cultures and minimum (2.87µm/l/g dw ) in seeds of *B.monosperma.* Hence this study offers a base of using as herbal alternative for the synthesis of antimicrobial and antioxidant activity.

*Copyright©2017* ***Renu Sarin and Chand Kor Kumari.*** *This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.*

## INTRODUCTION

Using plants as medicines to cure many diseases predates written human history. Many of the herbs and spices used by human being to season food also produce useful medicinal compounds (Lai and Roy, 2004). The use of herbs and spices in cuisine developed in part as a response to threat many pathogensresponsible for various diseases. Studies show that in tropical climates where pathogens are the most abundant, recipes are the most highly spiced. Further, the spices with the most potent antimicrobial activity tend to be selected (Solecki, 1975).

Plant tissue culture has brought a revolutionary break through because of its several advantages in micro propagation and production of secondary metabolites. Plant tissue culture has facilitated in producing and raising the yield of commercially important biosynthetic compounds.Plant tissue culture techniques have become especially important in the agricultural community over the past ten years. The therapeutic efficacies of many indigenous plants for several disorders have been described by practitioners of traditional herbal medicines (Natarajan *et al*., 2003).

**Corresponding author:* **Renu Sarin**
Laboratory of Bioactive Natural Products, Department of Botany, University of Rajasthan, Jaipur-302004, India

Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions (Velioglu *et al*, 1998). Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism (Tiwari, 2008). The most common reactive oxygen species (ROS) include superoxide (O2) anion, hydrogen peroxide (H2O2), peroxyl (ROO-) radicals, and reactive hydroxyl (OH.) radicals. The nitrogen derived free radicals are nitric oxide (NO.) and peroxynitrite anion (ONOO.). ROS have been implicated in over a hundreds of diseases states which range from arthritis and connective tissue disorders to carcinogenesis, aging, physical injury, infection and acquired immunodeficiency syndrome (Joyce, 1987). Peroxidases are associated with such biochemical and physiological processes as in growth, cell formation, fruit development, ethylene biosynthesis as well as various stresses (Matamoremoros *et al*, 2003). Both aerobic and anaerobic organisms possess superoxide dismutase enzyme, which catalyze the breakdown of superoxide radical (Shirwaiar *et al*, 2007).

Recently, multiple drug resistance has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases

(Service, 1995) making it a global growing-problem. Isolation of microbial agents less susceptible to regular antibiotics and recovery of increasing resistant isolates during antibacterial therapy is rising throughout the world which highlights the needs of new principles. Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanism of actions (Sanchez *et al*., 2016).

*Butea monosperma* is commonly known as Flame of forest, belonging to the family Fabaceae. It is locally called as palas, palash, mutthuga, bijasneha, dhak, khakara, chichra, Bastard Teak, Bengal Kino, Nourouc and is common throughout India, Burma and Ceylon except in very acrid parts.The seeds of B. monosperma administered as crude powder at doses of 1, 2 and 3 g/kg to sheep naturally infected with mixed species of gastrointestinal nematodes exhibited a dose and a time-dependent anthelmintic effect..An extract from the flowers of *B. monosperma*, a plant drug used in India for the treatment of liver disorders, showed significant activity in different models of liver damage (Wagner *et al*., 1986).

## MATERIALS AND METHODS

***Plant Material***

Various plant parts of *B.monosperma* (flowers, leaves and sseeds) were collected from the fields at Jaipur and authenticated. The voucher specimen of experimental plant was Deposited in the Herbarium of Department of Botany, University of Rajasthan, Jaipur. Plant parts were separated, cleaned and Oven dried at 35ºC for 30 min till constant Weight was achieved and powdered.

***Tissue culture studies for callus cultures***

In the present study following plant parts were used as explants:-

Seeds- Seeds and in vitro seedlings used for culture work were inoculated to MS medium without hormone and MS medium supplemented with various concentrations and combinations of growth hormones.

Nodal segments- Both nodal, internodal segments were taken from young juvenile tree.

***Culture Medium***

MS medium was used for all tissue culture studies.

***Stock preparation***

Stock solutions of organic and inorganic nutrients were prepared in sterile distilled water, stored in a refrigerator at 40C. Main growth regulators used were auxins viz 2, 4-Dichloro-phenoxy Acetic Acid (2,4 –D), Indole Acetic Acid (IAA) , Naphthalene Acetic Acid (NAA), Indole Butyric Acid (IBA) and Cytokininsviz Kinetin (Kn) , Benzyl Amino Purine (BAP). The auxins were dissolved in small quantity of absolute alcohol and cytokinins in 1N HCl. All stocks were prepared using sterile double distilled water. Double glass – distilled water was used only up to 2 days of preparation and 200 ml of each stock solution was made.

***Media preparation***

Medium was prepared by dissolving required amount of stock solutions (Table-1). Sucrose (w/v 30 gL-1) was dissolved, filtered, mixed in stock solutions measured for the preparation of media and was made to final volume. The required growth hormones were added to the medium and for solidifying the medium 0.8% of bacteriological grade agar was used. The pH of the medium was adjusted to 5.8 with the help of 1N NaOH and 1N HCl. In each flask (100mL Borosil) approximately 30 mL and in a test tube 20 ml medium was dispensed. Slants were made to provide the larger surface area for inoculation. All the culture vessels which contained medium were plugged with non –absorbent cotton and mouth of flask/ test tube was wrapped with aluminum foil or paper and these vessels were autoclaved at 15 lbs pressure for 15-20 min.

***Surface Sterilization and inoculation***

Seeds were surface sterilized with mercuric chloride (HgCl2) solution (0.1%; w/v) for 2 min and subsequently rinsed thrice with sterile distilled water where as nodal segments were treated with antibiotic (Ciprofloxacin, 250mgL-1) prior to inoculation in order to remove any kind of microbial interactions. Surface sterilization and inoculation of seeds and nodal segments were done in a Laminar flow hood fitted with ultraviolet light. Before starting the inoculation work, slab of Laminar flow was cleaned with rectified spirit and culture vessels containing autoclaved media, petri dishes, and spirit lamps, cotton and other things required were kept on the slab of transfer chamber. A day before inoculation of work, transfer chamber was fumigated with fumes obtained by heating formic acid and potassium permanganate (KMnO4). The forceps, scalpels, needles; scissors were kept in a glass tube column containing rectified spirit. UV light was used for an hour to sterilize the chamber. Seeds as well as nodal segments were inoculated in the flasks containing culture medium aseptically.

Cultured flasks were incubated in culture chamber. The temperature of chamber was maintained at 25± $1^0$ C using air conditioner and light intensity (1200 lux) was provided from fluorescent tubes (40 watt) and incandescent bulbs (40 watts). A photoperiod of 16h light was provided. The cultures were observed and examined every week and final morphogenetic data were recorded.

***Maintenance of Callus***

Callus initiated from *in vitro* grown seedlings when media was supplemented with various growth hormones

***Antimicrobial Activity***

Ethanolic extracts were used for determination of antimicrobial activity of *B.monosperma.* Four bacterial sps. and four fungal sps. were selected for the antimicrobial screening.

***Microorganisms Used***

Clinical laboratory isolates of bacteria viz. *Streptomyces grisveus, Staphylococcus aureus, Escherichia coli, Bacillus subtilis*and fungi viz. *Tricodermaressie* , *Fusariumoxysporum , Penicilliumfuniculosum*and *Aspergillus niger*were procured from the Microbiology Laboratory, SMS Medical College, Jaipur.

***Preparation of Extract***

The ethanolic extracts were obtained by macerating 100 g of dried powder of different plant parts and 10 g of callus in 95% ethanol and kept on a rotary shaker for 24 h, separately. Each of the extract was filtered, centrifuged at

5000rpm for 15 min, dried under reduced pressure and stored at 4 ºC in airtight bottles.

***Culture and Maintenance of Microbes***

Above mentioned pure cultures of *Streptomyces grisveus, Staphylococcus aureus, Escherichia coli* and *Bacillus subtilis* and fungal isolates *Tricodermaressie, F. oxysporum, Penicilliumfuniculosum* and *A. Niger* used as indicator organisms. These bacteria were grown in nutrient agar medium prepared by autoclaving 8% Nutrient Agar (Difco-Laboratories, Detroit, USA) in distilled water at 15 lbs psi for 25-30 min and incubating at 37°C for 48 h. Each bacterial culture was maintained on the same medium after every 48 h of sub-culturing. A fresh suspension of test organism in saline solution was prepared from a freshly grown agar slant before every antimicrobial assay.

***Determination of Antibacterial Assay***

*In vitro* antibacterial activity of the crude ethanol extracts were studied against gram +ve and --ve bacterial strains by the agar well diffusion(Bonjar *et al*, 2005). Mueller Hinton Agar No.2 (Hi Media, India) was used as the bacteriological medium. The extracts were diluted in 100% dimethylsulphoxide at the concentrations of 5 mg mL-1. The Mueller Hinton agar was melted and cooled to 48-50 ºC and a standardized inoculum (1.5×108 CFU mL-1, 0.5 McFarland) was then added aseptically to the molten agar and poured into sterile petridishes to give a solid plate. Wells were prepared in the seeded agar plates. The test compound (40 µl) was introduced in the well (6 mm). The plates were incubated overnight at 37$^{o}$C. The antimicrobial spectrum of the extract was determined for the bacterial species in terms of zone sizes around each well. The diameters of zone of inhibition produced by the agent were compared with those produced by the commercial control antibiotic streptomycin and ampicillin. For each bacterial and fungal strain, controls were maintained where pure solvents were used instead of the extract. The control zones were subtracted from the test zones and the resulting zone diameter was measured with antibiotic zone reader to nearest mm. The experiment was performed in triplicate to minimize the error and the mean values are presented.

***Determination of Antifungal Assay***

Antifungal activity of the experimental plant was investigated by agar well diffusion method (Perez *et al*.,1990).The yeasts and saprophytic fungi weresubculturedon Sabouraud's Dextrose Agar (SDA; Merck, Germany) medium and respectively incubated at 37 °C for 24 h and 25 °C for 2 - 5 days. Suspensions of fungal spores were prepared in sterile PBS (phosphate buffered saline) and adjusted to a concentration of 106 cells mL-1. Dipping a sterile swab into the fungal suspension was rolled on the surface of the agar medium. The plates were dried at room temperature for 15 min. Wells of 10 mm in diameter and about 7 mm apart were punctured in the culture media using sterile glass tube. 0.1 mL of several dilutions of fresh extracts was administered to fullness for each well. Plateswere incubated at 37 °C. After incubation of 24 h, bioactivities were determined by measuring the diameter of inhibition zone (mm). The diameters of zone of inhibition produced were with those of standard clotrimazole used as standard antifungal agent. All the experiments were performed in triplicate and mean values were taken.

Various plant parts of *B. monosperma*were taken for detailed antioxidant activity and antioxidant enzyme study.

***FRAP Assay (Ferric reducing ability of Plasma)***

The FRAP assay depends upon the reduction of ferric tripyridyltriazine (Fe (III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe (II)-TPTZ) by a reductant at low pH. The method was performed by the protocol of Varga*et al*, 1998.

***Reagents***

- Acetate buffer, 300mM/L pH 3.6 (3.1 g sodium acetate x $H_2O$ and 16 mL conc. Acetic acid per 1 mL of buffer solution).
- 10mM 2, 4, 6-tripyridyl –s- triazine (TPTZ) in 40 mM 1 HCl.
- 20mM $FeCl_3$ x 6 $H_2O$ in distilled water

FRAP working solution: 25 mL acetate buffer (1), 2.5mL TPTZ solution and 2.5mL $FeCl_3$ x 6 $H_2O$ solutions. The working solution must be always freshly prepared. Aqueous solution of known Fe (II) concentration was used for calibration (in a range of 100-1000µ mol/L).
Assay; - Blank: FRAP reagent
Sample: FRAP reagent -1.5mL, plant extract- 50mL

***Procedure***

Plant sample (1g) were cut into small pieces and mashed with a cool mortar and pestle using quartz sand and 9 mL cool 0.1M phosphate buffer was added. (pH 7.6, containing 0.1mM EDTA). This mixture was filtered through a filter paper and centrifuged at 15,000 rpm for 10 min. The supernatant was used for the measurements. The volume was made up to 5mL and O.D. was taken at 593nm.

Calculation: The relative activities of samples were assessed by comparing their activities standard curve of ferrous sulphate.

***Lipid Peroxidation (LPO) Assay***
***Reagents and Test sample solutions***

- Ethanol
- Trichloroacetic acid (20% w/v in water)
- Butylated hidroxytoluene (0.01% w/v in ethanol)
- Thiobarbituric acid (0.5% w/v in 20% TCA)

***Procedure***

The level of lipid peroxidation was measured in terms of malondialdehyde content of a product of lipid peroxidation described by Hodges *et al* (1999). 1g plant sample was homogenized with 25 mL of ethanol in pre chilled mortar and pestle and refrigerated centrifuged at 10000rpm for 20 minutes at 4ºC. The clear supernatant was taken as the enzyme extract. In one test tube 1mL of enzyme extract was added in 0.8 mL trichloroacetic acid (TCA) and 0.2 mL of butylated hidroxytoluene. In second test tube 1mL of enzyme extract was added in 0.8 mL thiobarbituric acid (TBA) and 0.2 mL of butylated hidroxytoluene. The mixtures were incubated at 95ºC for 25 min. The reaction was stopped by cooling in ice bath for 15 min. Reaction tubes were centrifuged at 10,000g for 10 min and supernatants were used

to determine the absorbance at 532 nm and 400nm. The value for non-specific absorption at 600 nm was subtracted.

***Peroxidase Assay (POXA)***

The method of assay measures the oxidation of pyrogallol to purpurogallin by peroxidase when catalyzed by peroxidase at 420 nm and at 20°C.

Pyrogallol+ $H_2O_2$ $\xrightarrow{\text{Peroxidase}}$ Purpurogallin

***Reagents and Test sample solutions***

- Phosphate buffer (0.1M, pH- 6.8)
- Phosphate buffer (125µmol, pH- 6.8)
- Pyrogallol (50µmol)
- $H_2O_2$ (30%)

***Procedure***

Plant sample (200mg) was homogenized with 10mL of phosphate buffer and refrigerated centrifuged at 10000 rpm for 20 minutes. The clear supernatant was taken as the enzyme extract.

The activity was assayed after the method of Chance &Maehly (1955) with the following modifications. 2.4mL of phosphate buffer, 0.3mL of pyrogallol and 0.2mL of $H_2O_2$ was added. The amount of purpurogallin formed was determined by taking the absorbency at 420nm immediately after adding 0.1mL enzyme extract.

## RESULTS

***Tissue culture***

***Effect of auxins (2,4-D and NAA) in combination with cytokinin (BAP/Kn) on callus induction***

It was observed that BAP (2.5 mg/L) in combination with NAA (0.5 mg/L) was best for callus induction and establishment (Fig 1 and 2) The callus so produced was compact and blackish in color. It grew profusely and possessed high capacity of growth. This callus when sub cultured show vigorous growth. Kn did not proved to be beneficial for producing callus.

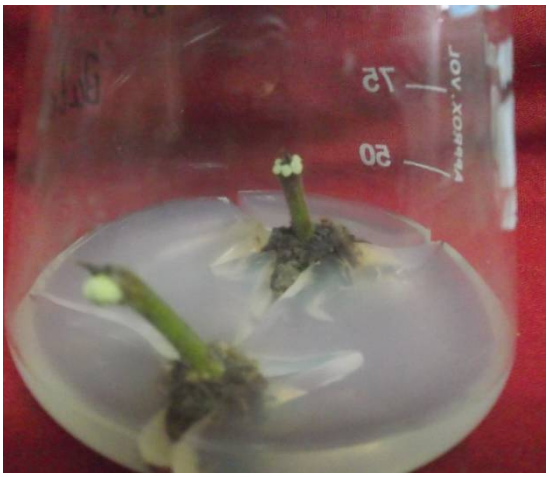

**Fig 1** Initiation of callus from nodal segments of *B. monosperma* in BAP+NAA (2.0+0.5 mg/L).

***Antimicrobial activity***

In the present investigation, *in vitro* antimicrobial activity of crude extract of *B. monosperma*isolated from plant part and calli was quantitatively evaluated. The crude extract was tested against some pathogenic microbial strains like *Bacillus subtilis, Staphylococcus aureus, , Streptomyces grisveus* and *Escherichia coli* and fungal isolates viz. *Aspergillus niger, Trichodermareesei, Fusariumoxysporium and Penicilliumnotatum* were collected from the stock cultures of Microbiology Laboratory, SMS Medical College Jaipur, India.

**Fig 2** Blackish and compact callus induction from nodal segments of B. monosperma in BAP+NAA (2.5+0.5 mg/L)

The extracts gave varying degree of inhibitory effect against all tested pathogenic strains. (Table 1and 2).

**Table 1** Antibacterial activity of *B. monosperma* against various bacterial strains

| Serial no. | Name of bacterial strain | Flowers (zone in mm) | Leaves (zone in mm) | Seed (zone in mm) | Callus (zone in mm) | Standard as Ciprofloxacin |
|---|---|---|---|---|---|---|
| 1 | *Escherichia coli* | 10 AI= 0.5 | 12 AI= 0.6 | 8 AI= 0.4 | 14 AI= 0.7 | 20 |
| 2 | *Staphylococcus aureus* | Nil | Nil | Nil | nil | 20 |
| 3 | *Streptomyces grisveus* | Nil | 10 AI= 0.5 | Nil | 12 AI= 0.6 | 20 |
| 4 | *Bacillus subtilis* | 8 AI= 0.4 | 6 AI= 0.3 | 4 AI= 0.2 | 10 AI= 0.5 | 20 |

AI- Activity index = $\frac{\text{Sample zone}}{\text{Standard Zone}}$

**Table 2** Antifungal activity of *B. monosperma* against various fungal strains

| Serial no. | Name of fungus strain | Flowers (zone in mm) | Leaves (zone in mm) | Seed (zone in mm) | Callus (zone in mm) | Standard as ketokenazole |
|---|---|---|---|---|---|---|
| 1 | *Trichodermaressei* | Nil | Nil | Nil | 4 AI= 0.18 | 22 |
| 2 | *Aspergillus niger* | nil | nil | 8 AI= 0.36 | 16 AI= 0.72 | 22 |
| 3 | *Penicillium-funiculosum* | 12 AI= 0.54 | 14 AI= 0.63 | 8 AI= 0.36 | 10 AI= 0.45 | 22 |
| 4 | *Fusariumoxysporium* | nil | 6 AI= 0.27 | 8 AI= 0.36 | 10 AI= 0.45 | 22 |

AI- Activity index = $\frac{\text{Sample zone}}{\text{Standard Zone}}$

It was observed that against various bacterial strains maximum activity was observed in callus against *E. coli* while minimum in seed against *Bacillus subtilis* while *Staphylococcusaureus* was found to be resistant. Against various fungal strains maximum activity was found in callus against *Aspergillus niger* while minimum in leaves against *Fusariumoxysporium* and *Trichodermaressei* was found to be resistant.

***Antioxidant activity***

***FRAP Assay***

Where; MF – Methanol Fraction; HF – Hexane Fraction; EAF – Ethyl Acetate Fraction

In the present investigation maximum (240µm/l/gdw) antioxidant activity was found in methanol extract of flowers and minimum in hexane fraction of Seeds (34 µm/l/g dw) using FRAP Assay (Table 3)

**Table 3** Antioxidant activity of *B. monosperma* in various plant parts and callus cultures using FRAP Assay (in µm/l/g dw)

| Fractions | Various Fractions of Plant Parts i.e. Leaves, Flower, Seeds and Callus | | | |
|---|---|---|---|---|
| **MF** | 200 | 240 | 65 | 187 |
| **HF** | 220 | 80 | 34 | 97 |
| **EAF** | 35 | 74 | 56 | 66 |

***Lipid Peroxidation Assay***

LPO (Lipid Peroxidation) is oxidative deterioration of polyunsaturated lipids and it involves ROS and transition metal ions. The level of lipid peroxidation was measured in terms of malondialdehyde content as a product of lipid peroxidation. Here maximum (61.3 µm/l/g dw) LPO activity was observed in callus cultures and minimum (40.3 µm/l/g dw) in flowers(Table 4).

**Table 4** Antioxidant activity of *B. monosperma* in various plant parts and callus cultures using Lipid Peroxidation Assay(in µm/l/g dw)

| | Various Fractions Plant Parts | | | |
|---|---|---|---|---|
| | **Leaves** | **Flower** | **Callus** | **Seeds** |
| **LPO Activity** | 55.8 | 40.3 | 49.6 | 61.3 |

***Peroxidase activity***

Peroxidase assay showed maximum (8.18 µm/l/gdw) antioxidant activity in callus cultures and minimum (2.87 µm/l/g dw) in seeds of *B.monosperma* (Table 5).

**Table 5** Antioxidant activity of *B. monosperma* in various plant parts and callus cultures using Peroxidase Assay(in µm/l/g dw)

| | Various Fractions Plant Parts | | | |
|---|---|---|---|---|
| | **Leaves** | **Flower** | **Callus** | **Seeds** |
| **Peroxidase activity** | 7.68 | 5.07 | 2.87 | 8.18 |

## DISCUSSION

Awareness of medicinal plants usage is a result of the many years of struggles against illnesses due to which man learned to pursue drugs in barks, seeds, fruit bodies, and other parts of the plants. Plant tissue culture technique is now a well-established technology to improve the quality and quantity of useful plants. Like many other technologies, it has gone through different stages of evolution; scientific curiosity, research tool, novel applications and mass exploitation. Initially, plant tissue culture was exploited as a research tool and focused on attempts to culture and study the development of small, isolated segments of plant tissues or isolated cells. Around the mid twentieth century, the notion that plants could be regenerated or multiplied from either callus or organ culture was widely accepted and practical application in the plant propagation industry ensued. The technique was heralded as the universal mass clonal plant propagation system for the future and the term 'micropropagation' was introduced to describe more accurately the processes.

Internal hormonal levels, blocked direct regeneration in the experimental plant. Hussey (1975) has improved differences in callus production between different types of explants of Hyacinth. It seems that one of the important reasons why the rate of produced callus in scale explants was higher than other of plant was because the existence of meristemoid-like cell is in basal plate of bulb that is in better situation for callus. The effects of different types of auxin on tissue culture of hyacinth have been compared and the existences of differences affecting of IAA, NAA and 2.4-D (Hussey, 1975) IBA and NAA and IBA (Hussey, 1975) have been reported. Now Plant tissue culture techniques has been successfully used for the micro propagation of members of useful plants *(*Kalidass and Mohan, 2009); (Soni *et al*., 2015); (Veraplakorn, 2016); (Bianchetti *et al*., 2017).The antioxidant assays used in this study measured the oxidative products at the early and final stages of oxidation. However, the components responsible for the antioxidative activities of the wetland medicinal plants are still unclear. Further work must be performed to isolate and identify these compounds.

Free radicals are the cause for several major disorders. So, evaluation of antioxidant activity in plants could result in the discovery of natural antioxidants with pharmacological and food value. The importance of phenol compounds in plants as natural antioxidants and their use as substitutes to synthetic antioxidants in food additives is well known (Paramapojn and Gritsanapan, 2009). Therefore, these observations could help in developing new drugs for the therapeutic use in human-beings. Therefore, the present work was aimed to analyze the antioxidant potential of *B. monosperma.*

The ferric ion reducing antioxidant power (FRAP) assay is based on the electron transfer mechanism. Antioxidant compounds from the plant extract are capable of donating single electron to the ferric-TPTZ complex which is the main component in the assay. This reaction will cause a reduction of Fe3+ -TPTZ into blue ferrous-TPTZ complex, with absorption at 593 nm. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.

The body posses defense mechanisms against free radical-induced oxidative stress, which involve preventative mechanisms, repair mechanisms, physical defenses and antioxidant defenses. Enzymatic antioxidant defenses include superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) etc., while non-enzymatic antioxidants are ascorbic acid (vitamin C), α-tocopherol (vitamin E), glutathione (GSH), carotenoids, flavonoids and etc.

The present study was undertaken to assess the free radical scavenging capacity of *B. monosperma*. From the results, it was observed that all the parts of plant along with callusshowed promising activities of catalase, SOD and peroxidase enzyme activity. From our experiment it was deducted that *B. monosperma* possessed high enzyme activity levels. Since antioxidant enzymes altogether work in a network, therefore this plant, possessing high enzymatic

activities of catalase, peroxidase and SOD can efficiently serve as potential antioxidant additives in human diet thereby preventing oxidative damage by reactive oxygen species.

Infectious diseases are the major cause of morbidity and mortality worldwide. The number of multidrug resistant microbial strains and the appearance of strains which reduced susceptibility to antibiotics are continuously increasing. Such increase has been attributed to indiscriminate use of broad spectrum antibiotics, immunosuppressive agents, intravenous catheters organ transplantation and ongoing epidermis of human immunodeficiency virus (HIV) infections. The emergence of multidrug resistant strain of many pathogens is a serious threat and makes chemotherapy more difficult. Moreover, the current cost of most of the chemotherapeutic agents is unbearable to the public especially in developing countries like India (Sarala *et al.*, 2010).Therefore, attempts must be directed towards the development of effective natural, non-toxic drug for treatment and provided the impetus to the search for new antimicrobial substances from various source like medicinal plants.

In the present investigation we tested our extracts against *Escherichia coli, Streptomyces grisveus, Bacillus subtilis, Staphylococcus aureus* and in fungus *Aspergillus niger, Trichoderma reesei, Fusarium oxysporium and Penicillium notatum*

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay (Tona*et al.*, 1998). Latest reports are available on the antiviral, antibacterial, and antifungal, properties of plants (Malik and Bhatia, 2017).

Antimicrobial activity of ethanol and water extracts of *B. monosperma* was evaluated against various microbes. The antimicrobial properties exhibited by the extracts may be associated with the presence of flavonoids, tannins, saponins, sterols, cardiac glycosides and other secondary metabolites and alkaloids found in the plant extracts.

The screening of plants usually involves various approaches; ethno botanical approach is one of the common methods that are employed in choosing the plant for pharmacological study. Traditionally plant parts, extracts, infusions etc were used for treatment of various diseases. The data shows that each plant extract shows different degree of ZOI (Zone of inhibition) against different microorganisms (bacterial & fungal isolates).

Extrinsic and intrinsic parameters mainly affect the ZOI. The extrinsic parameters (like pH of the medium, period and temperature of incubation, volume of the well, concentration of plant extracts and size of inoculums) pose no much error in the results as they were fixed and standardized during experiment, However, intrinsic factors might be responsible for variability in diffusion of extract which result in variable ZOI (Prasai*et al.*, 2004). Thus, the study ascertains the value of plants used in ayurveda, which could be of considerable interest to the development of new drugs. Results obtained from this study, indicated that, the plant extracts showed the strongest antimicrobial activity than the commercially available antibiotics. Further research has to be conducted to find out the possibility of this medicinally important plant as a potent anti microbial drug and for other pharmacological properties to develop as cost effective formulation.

**Acknowledgements**

The authors would like to acknowledge with thanks to UGC, New Delhi for granting SRF to one of the authors (Chandkor Kumari) and Head, Department of Botany, University of Rajasthan, Jaipur, for providing all the facilities in the Department to carry out research work.

## References

Lai, P.K. and Roy, J. 2004. Antimicrobial and chemo preventive properties of herbs and spices. *Curr. Med. Chem.* 11 (11): 1451–60.

Solecki, R.S. 1975. Shanidar IV, a Neanderthal Flower Burial in Northern Iraq. *Sci.* 190 (4217): 880–881.

Natarajan, V., Venugopal, P.V., and Menon, T. 2003. Effect of *Azadarichta indica* (neem) on the growth pattern of dermatophtyes. *Ind. J. Med. Microbiol*. 21:98-101

Tiwari S. 2008. Plants: A Rich Source of Herbal Medicine. *Ind. J. Nat. Prod.* Vol 1: 27-35

Velioglu YS, Mazza G, GAO L and Oomah BD. 1998. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. J. *Agri. Food. Chem.* 46: 4113-4117.

Joyce DA.1987. Oxygen radicals in disease. *Adv. Drug. Reac. Bull.* 127:476-79.

Matamoremoros MA, Dalton DA, Ramos J, Clement MR, Rubio MC and Becana M. 2003. Biochemistry and molecular biology of antioxidants in the rhizobia-legume symbiosis . *Plant. Physiol*. 133: 499-509.

Shirwaiar A, Shirwaikar A and Punitha ISR. 2007. Antioxidant studies on the methanol stem extract of *Coscinium fenestratum*. *Nat. Prod. Sci*. 13(1): 40-45.

Service RF. 1995. Antibiotics that resist resistance. Science, 270: 724-727.

Sánchez duardo, Catalina RM, Sandra C, Catalina LR,Ledy GB and David O M.2016. Antibacterial and Antibiofilm Activity of Methanolic PlantExtracts against Nosocomial Microorganisms. *Evidence-Based Complementary and Alternative Medicine*. 2016, Article ID 1572697: 1-8

Bonjar Shadidi, Aghighi S and Karimi NA. 2005. Antibacterial and anti fungal survey in plants used in indigenous herbal-medicine of South East Regions of *Iran. J. Biol. Sci.* 4: 405-412

Perez C, Paul M and Bazerque P. 1990. An antibiotic assay by the agar-well diffusion method. *Acta. Biol. Med. Exp*. 15:113-115

Varga I SZ, Matkovics B, Sasvári M and Salgó L .1998. Comparative study of plasma antioxidant status in normal and pathological cases. *Curr. Topics. Biophys.* 22: 219-224

Hodges DM, DeLong JM, Forney CF and Prange RK. 1999. Improving the thiobarbituric acid reactive substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta. Med*. 207: 604-611.

Chance B and Maehly AC. 1955. Assay of catalase and peroxidases. *Methods. Enzymol*. 2: 764 -775.

Hussey, G. 1975. Propagation of Hyacinths by tissue culture. Scientia Horti. 3: 21–28.

Kalidass, C. and Mohan, V.R. 2009. In vitro rapid clonal propagation of *Phyllanthus urinaria* Linn. (Euphorbiaceae) - A medicinal plant. Researcher. 1: 56-61

Soni, Sangeeta Soni , Tulika Kangsabanik , Shagufta Khan and U. N. Soni. 2015. *In vitro* micropropagation and callus culture of *Spilanthes acmella. World J of pharmacy and pharmaceutical sciences.* 4, (11): 1081-1091

Veraplakorn, V. 2016. Micro propagation and callus induction of *Lantana camara* L. - A medicinal plant. Agriculture and Natural Resources. 50, 5:338-344.

Bianchetti,Ricardo Ernesto Cristiano Ferrara de Resende, Vinícius Sacramento Pacheco, Felipe Floriano Dornellas, Aline Mystica Silva de Oliveira, Jober Condé Evangelista Freitas and Paulo Henrique Pereira Peixoto. 2017. An improved protocol for *in vitro* propagation of the medicinal plant *Mimosa pudica L. African Journal of Biotechnology*. 16(9): . 418-428.

Paramapojn, S. and Gritsanapan, W. 2009. Free radical scavenging activity determination and quantitative analysis of curcuminoids in *Curcuma zedoaria* rhizome extract by HPLC method. *Curr. Sci.* 97(7): 1069-1073.

Sarala, G.; Shibumon, G. and Benny, P.J. 2010. Antimicrobial effect of *Punica grantum* on pyogenic bacteria*. J. Pharma. Biomed. Sci.* 3(6).

Tona, L.; Kambu, K., Ngimbi, N., Cimanga, K. and Vlietinck, A.J. 1998. Antiamoebic and phytochemical screening of some Congolese medicinal plants. *J. Ethnopharmacol.* 61: 57-65

Malik P and Bhatia V. 2017. Comparative testing of antibacterial activity of aqueous extract of *Bergenia ligulata* Rhizome and ethanolic extract of *Butea monosperma* flowers for herbal gel formulation. *Journal of Pharma and Bio. Sci.* 12 (1): 89-94.

Prasai, T.; Lekhak, B. and Baral, M.P. 2004. Antibacterial property of medicinal plants against gram negative bacteria isolated from water. *Proceedings of IV National Conf. Sci. Technol.* 2: 410.

**How to cite this article:**

Renu Sarin and Chand Kor Kumari (2017) 'Antimicrobial and Antioxidant Activity of Butea Monosperma Linn.in Vivo And in Vitro', *International Journal of Current Advanced Research*, 06(08), pp. 5269-5275.
DOI: http://dx.doi.org/10.24327/ijcar.2017.5275.0688

*******