INDO AMERICAN JOURNAL OF PHARMACEUTICAL RESEARCH

# ANTIBACTERIAL AND ANTIOXIDANT POTENTIAL OF METHANOLIC LEAF EXTRACT OF *PUTRANJIVA ROXBURGHII* WALL

**Dr.Narendra Kumar**

*Senior Lecturer, Amity Institute of Biotechnology, Amity University Haryana, Manaser-122413, Gurgaon, Haryana, India.*

**ARTICLE INFO**

**Article history**
Received 21/07/2015
Available online
31/07/2015

**Keywords**
Antibacterial Activity,
Antioxidant Potential,
*Putranjiva* Methanolic
Leaf Extract.

**ABSTRACT**

For antibacterial activity the methanolic extracts of 12 plants leaves were extracted. The antibacterial testing *in vitro* was done following disc diffusion method against *Bacillus subtilis*, *Erwinia herbicola*, *Escherichia coli*, *Salmonella typhi*, *Pseudomonas putida Staphylococcus aureus Klebsiella pnemoniae* ,*Vibrio cholera.*Amongst the leaf methanolic extracts tested, the extracts of *Aegle marmelos* (L.) Corre, *Anethum graveolens* L and *Eupatorium capillifolium* (Lam.) Small showed significant antibacterial activity against the bacterial pathogen. *Putranjiva roxburghii* Wall showed highest antibacterial activity followed by *Artabotrys hexapetalus* (L.f.) Bhandari. The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was used to determine the antioxidant activity of potent extract. *Putranjiva roxburghii* Wall showed the appreciable antioxidant activity. This showed highest antioxidant activity at 40 mg/ml concentration with a percent inhibition of 65.54 and IC50 value 7.29 mg/ml. The reducing power of the extracts was found to increase with increasing concentration of the extract .The results provide evidence that the methanolic leaf extract extract of *Putranjiva roxburghii* can be further recommended in the treatment of the infections caused by the bacterial pathogens .The result indicates that *Putranjiva roxburghii* is a potential source of antibacterial and natural antioxidants.

***Corresponding author***
**Dr.Narendra Kumar**
Senior Lecturer,
Amity Institute of Biotechnology,
Amity University Haryana, Manaser-122413,
Gurgaon,Haryana,India
narendra.microbiology@rediffmail.com

*Please cite this article in press as **Dr.Narendra Kumar** et al. Antibacterial and antioxidant potential of methanolic leaf extract of Putranjiva roxburghii Wall. Indo American Journal of Pharmaceutical Research.2015:5(07).*

## INTRODUCTION

From antiquity the Medicinal plants have been an effective source of both traditional and modern medicine. These plants have been shown to have genuine utility and about 80% of the rural population depends on them as primary health care [1].They have antioxidant activities, so investigated throughout the world. As antioxidants to prevent oxidative damage caused by free radical, it can interfere with the oxidation process by reacting with free radicals, chelating, catalytic metals and also by acting as oxygen scavengers[2].

The use of plants for treating diseases is as old as the human species. Plants produce a wide variety of secondary metabolites such as vitamins, terpenoids, tannins, flavonoids, alkaloids and other metabolites, which are rich in antimicrobial and antioxidant activities[3,4].

Popular observations on the use and efficacy of medicinal plants significantly contribute to the disclosure of their therapeutic properties, so that they are frequently prescribed, even if their chemical constituents are not always completely known. A number of plants have been documented for their biological[5,6] and antimicrobial properties[7,8]. Review of literature revealed that although the bulk of traditional antibiotics can still manage drug-resistant bacteria but many commonly used antibiotics are no longer effective [9,10].

More strains of pathogens have become antibiotic resistant while some have become resistant to several antibiotics and chemotherapeutic agents giving rise the phenomenon of multidrug resistance. The objective is to search antibacterial and antioxidant.In order to search a broad spectrum antibacterial agents from natural resources, 12 medicinal plants were selected to assess their antibacterial potential and antioxidant potential of dominant ones.

## MATERIALS AND METHODS

### Plant material preparation:

The fresh leaves of 12 plants were washed with tap water and then with 90 per cent alcohol and chopped into smaller pieces with a knife .They were shade dried for 15 days and then crushed using pestle and mortar and further reduced to powder using electric blender and then stored in airtight closed bottles until tested and analyzed.

### Extraction of methanolic extract:

20 g of the powdered sample of the plant was soaked in 100 ml of methanol in a 250 ml conical flask at room temperature and shaked upto 10 times upto 24 h. The extract was filtered using muslin cloth and then Whatman no.1 filter paper. The filtrates were then evaporated to dryness in a rotary evaporator to remove residual solvents and then stored in screw capped bottles for further use.

### Preparation of Microbial strain:

The bacterial species viz.,*Bacillus subtilis* ,*Erwinia herbicola*, *Escherichia coli* ,*Salmonella typhi* ,*Pseudomonas putida Staphylococcus aureus Klebsiella pnemoniae* ,*Vibrio cholera* were used for evaluation of antibacterial assay. The stock cultures were maintained in nutrient agar (NA) slant at 4°C and sub-cultured monthly. Working cultures were prepared by inoculating a loopful of each test microorganism in 10 ml of nutrient broth (NB) from NA slants. Broths were incubated at 37°C for 18-20 hours. The suspension was diluted with sterile distilled water to obtain approximately $10^6$ CFU/ml.

### Antibacterial activity determination

The antibacterial activity of metanolic leaf extracts of plants were evaluated using disc diffusion technique[11] with slight modification. 10ml of sterilized nutrient agar medium was poured in 80 mm × 15 mm size Petridishes and was allowed to solidify. The plates were seeded by spreading 0.1 ml of overnight inoculum and allowed to set for 30 mins. For, testing, sterile, 7mm diameter filter paper discs were soaked in metanolic plant leaf extracts at 300 µg/ml concentration and placed on the surface of inoculated media agar plates using sterile forceps and then gently pressed down onto the agar surface. Disk soaked with the solvent was used as control. The positive control plates were inoculated with test organism. All the plates were incubated at 35-37ºC for 24h. Clear inhibition zones around the discs indicated the presence of antibacterial activity. Diameter of inhibition zones were measured in millimeters.

### MIC (Minimum inhibitory concentration)determination:

For minimum inhibitory concentration value for bacterial pathogen a series of twofolds dilution of methanolic extract concentrations (25 µg/ml- 3200 µg /ml) was prepared in Petridishes. 10ml of sterilized and molten nutrient agar medium was poured in each dish already containing 100µl amount of methanolic leaf extracts. Plates were dried at 35ºC for 30minutes prior to spot inoculation with 5µl of overnight bacterial culture (adjusted to 0.5 MacFarland standard) containing approximately $10^6$ CFU/spot using an sterilized inoculating loop. Nutrient agar with solvent was used as positive control. The inoculum spots were allowed to dry at room temperature and plates were incubated at 35-37ºC for 24h. MICs were determined as the lowest concentration of oil inhibiting the visible growth of microorganisms on agar plate .

**MBC (Minimum bactericidal concentration) Determination**

The MBC of the extracts was determined as described by Mishra *et al.*,[12]. Fresh nutrient agar medium was poured into Petriplates and allowed to solidify. Inoculum from various poisoned plates of MIC experiment showing no growth was submitted to subculture on freshly prepared plates. The lowest concentration of antimicrobial agent from which bacteria do not recover on fresh medium was treated as MBC.

**DPPH free radical scavenging activity**:

Effect of methanolic leaf extracts on DPPH radical was studied following Güllüce *et al.*[13] with slight modifications. 0.003% of DPPH (Hi Media) was prepared in methanol and 2ml of this solution was mixed with different concentrations of extracts (5, 10, 15, 20, 25, 30, 35and 40 mg/ml) dissolved in methanol. Reaction mixture was vortexed thoroughly and left for 30mins. After 30mins absorbance of the mixture was measured at 517nm in an UV spectrophotometer (Hitachi) against a blank (pure methanol). Control sample was also prepared as above without any methanolic extractl. Ascorbic acid, BHT (Butylated hydroxytoluene) and BHA (Butylated hydroxyanisole) was taken as reference standards. Experiments were performed in five times and average was calculated. IC50 value was determined from percent inhibition versus concentration graph.

**Reducing Power method**

This was studied following Tirtha *et al* [14] with slight modification.The Accurately weighed 10 mg of the extract in 1 ml of distilled water were mixed in to the mixture of 2.5 ml of 0.2M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. This mixture was incubated at $40^0$C for 20 min. and then 2.5 ml of 10% trichloro acetic acid was added to the mixture.They were centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5ml) was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml, 0.1 %) and the absorbance was measured at 700 nm.

**RESULTS AND DISCUSSION**

It is evident Table 1, that some of the methanolic leaf extracts showed moderate to high inhibiting activity while most of the extracts did not found effective against tested the tested bacterial pathogen. The zones of inhibition ranged from 10-29mm. Results showed that extracts of *Aegle marmelos* (L.) Correa, *Anethum graveolens* L and *Eupatorium capillifolium* (Lam.) Small showed significant antibacterial activity against the bacterial pathogen. Additionally, the extracts of *Anisomeles ovata* R.Br *Callistemon lanceolatus* (Sm.) Sweet, *Citrus medica* var. limonia (L.) Hiroe, *Eucalyptus citriodora* Hook, *Feronia limonia* (L.) Swingle also showed moderated inhibitory activity .*Putranjiva roxburghii* Wall showed highest antibacterial activity (29mm) against both *Salmonella typhi* and *Erwinia herbicola* followed by Artabotrys hexapetalus (L.f.) Bhandari.25mm for *Salmonella typhi* and 24mm for *Erwinia herbicola* respectively.The zone of inhibition formed by *Cannabis sativa* metanolic extracts was negligible.

**Table 1.Antibacterial activity of different plant extracts against *Salmonella typhi* and *Erwinia herbicola* by Disc Diffusion technique at 300 µg/ml concentration.**

| SN | Plant extracts | Family | Zone of Inhibition (mm) *Salmonella typhi* | Zone of Inhibition (mm) *Erwinia herbicola* |
|---|---|---|---|---|
| 1. | *Aegle marmelos* (L.) Correa | Rutaceae | 15.31±0.56 | 16±0.56 |
| 2. | *Anethum graveolens* L. | Umbelliferae | 17.31±0.56 | 18.30±0.56 |
| 3. | *Anisomeles ovata* R.Br | Lamiaceae | 7.12±0.54 | 7.22±0.52 |
| 4. | *Artabotrys hexapetalus* (L.f.) Bhandari | Annonaceae | 25±0.71 | 24±0.71 |
| 5. | *Callistemon lanceolatus* (Sm.) Sweet | Myrtaceae | 7.32±0.52 | 7.21±0.57 |
| 6. | *Citrus medica* var. limonia (L.) Hiroe | Rutaceae | 9.31±0.46 | 8.31±0.76 |
| 7. | *Cannabis sativa* L | Cannabaceae | - | - |
| 8. | *Eupatorium capillifolium* (Lam.) Small | Asteraceae | 19.57±0.91 | 18.41±0.84 |
| 9. | *Eucalyptus citriodora* Hook | Myrtaceae | 8.25±0.56 | 6.35±0.64 |
| 10. | *Feronia limonia* (L.) Swingle | Rutaceae | 5.33±0.47 | 5.21±0.42 |
| 11. | *Lantana camara* L | Verbenaceae | 10.21±0.66 | 9.01±0.50 |
| 12. | *Putranjiva roxburghi* Wall | Euphorbiaceae | 29.33±0.41 | 29.33±0.31 |

*Putranjiva roxburghi* Wall exhibited strong action against *Erwinia herbicola* and *Salmonella typhi* with MIC value of 300 µg/ml and mbc valus 600 µg/ml respectively (Table,2). It is evident from table,2 that methanolic leaf extract of *Putranjiva roxburghii* have broad spectrum of antibacterial action against *Bacillus subtilis, Escherichia coli, Pseudomonas putida, Staphylococcus aureus, Klebsiella pnemoniae, Vibrio cholerae.*

**Table 2-MIC,MBC of most potent plant extract *Putranjiva roxburghi.***

| SN | Name of bacterial species | Zone of inhibition in mm at 300 µg/ml concentration | MIC | MBC |
|---|---|---|---|---|
| 1. | *Bacillus subtilis* | 15.31±0.45 | 500 µg/ml | 1000 µg/ml |
| 2. | *Erwinia herbicola* | 29.33±0.31 | 300 µg/ml | 600 µg/ml |
| 3. | *Escherichia coli* | 14.61±0.40 | 300 µg/ml | 600 µg/ml |
| 4. | *Salmonella typhi* | 29.33±0.41 | 300 µg/ml | 600 µg/ml |
| 5. | *Pseudomonas putida* | 13.60±0.43 | 500 µg/ml | 1000 µg/ml |
| 6. | *Staphylococcus aureus* | 18.66±0.35 | 400 µg/ml | 800 µg/ml |
| 7. | *Klebsiella pnemoniae* | 17.60±0.40 | 400 µg/ml | 800 µg/ml |
| 8. | *Vibrio cholerae* | 16.33±0.42 | 500 µg/ml | 1000 µg/ml |

**Mic: minimum inhibitory concentration; mbc:minimum bactericidal concentration.**

The DPPH radical scavenging activity of most potent methanolic extract, *Putranjiva roxburghi* notably reduced the concentration of DPPH free radical, with an efficacy lower than that of reference BHA (Butylated hydroxyl anisole) and BHT (Butylated hydroxytoluene). The results showed significant decrease in the concentration of DPPH free radical due to the scavenging ability of extract and reference. Decrease in concentration of DPPH was observed with the increase in concentration of extract. The highest antioxidant activity was observed at 40µg/ml concentration (65.54%). IC50 value of extract was found to be 7.29 mg/ml.

It is evident from Table ,3 that the reducing power of the extracts was found to increase with increasing concentration of the extract.Increased absorbance of the reaction mixture indicated the increased reducing power.

**Table 3: Reducing power of methanolic leaf extract of *Putranjiva.***

| S .N | Concentration(µg/ml) | Absorbance at 700nm |
|---|---|---|
| 1. | 5 | 2.01 |
| 2. | 10 | 2.13 |
| 3. | 15 | 2.19 |
| 4. | 20 | 2.203 |

The present study was designed to obtain preliminary information on the antibacterial activity of 12 methanolic plant leaf extracts. Disc diffusion method was used in this study. Out of 12 extracts tested, only methanolic extracts of *Putranjiva roxburghi* Wall and *Artabotrys hexapetalus* exhibited good antibacterial activity and gave zone of inhibition followed by the methanolic extract of *Aegle marmelos* (L.) Correa, *Anethum graveolens* L and *Eupatorium capillifolium* (Lam.) Small against against *Erwinia herbicola* and *Salmonella typhi*.Eloff [15] found that methanol was the most effective solvent for plant extraction than any other solvents. Soniya *et al* [16] also found methanol as the most effective solvent. *Putranjiva roxburghi* Wall showed highest inhibitory activity against bacterial pathogen. Shahwar *et al*[17]reported that extracts of *P. roxburghii* contains antioxidant activities .The present investigation also revealed antioxidant potential.

## CONCLUSION

*P. roxburghii methanolic leaf* extract was found to have good antioxidant activity as well promising antibacterial activity. Further research is necessary for elucidating the active principles and their mode of action.

## ACKNOWLEDGEMENTS

The author is thankful to Director Prof.S.M Paul Khurana,Amity Institute of Biotechnology,Amity University Haryana for providing Laboratory facilities.

**REFERENCES**


1. Israel O, Auguster O and Edith OA(2010) Antioxidant and antimicrobial activities of polyphenols from ethnomedicinal plants of Nigeria. African Journal of Biotechnology 9 (20)**:** 2989-2993.
2. Vinay RP, Prakash RPand Sushil SK(2010) Antioxidant Activity of Some Selected Medicinal Plants in Western Region of India. Advances in Biological Research, 4 (1): 23-26.
3. Wong CC, HB Li, KW Cheng, and F Chen (2006). "A systematic survey of antioxidant acitivity of 30 chinese medicinal plants using the ferric reducing antioxidant power assay". Food Chem., 97: 705-711.
4. Baker JC, RA Owens, BD Whitaker, NM Mock, DP Roberts, KL Deahl and AA Aver'yanov (2010)."Effect of viroid infection on the dynamics of phenolic metabolites in the apoplast of tomato leaves". Physiol. Mol. Plant Pat., 74**:** 214-220.
5. Grover JK, S Yadav and V Vats (2002)."Medicinal plants of India with anti-diabetic potential". *J. Ethnopharmacol*., 81: 81–100.
6. Gajera HP, SV Patel and BA Golakiya (2005). "Antioxidant properties of some therapeutically active medicinal plants— an overview". JMAPS., 27: 91–100.
7. Arora DS (1998). "Antimicrobial activity of tea (*Camellia sinensis*)". Antibiot. Chemother., 2: 4–5.
8. Polambo E S and SJ Semple (2001). "Antibacterial activity of traditional Australian medicinal plants". J. Ethnopharmacol., 77: 151–157.
9. Levy SB (1998) "The challenge of antibiotics resistance". Sci. Am., 278: 46-53.
10. Wright GD (2010), "Antibiotic resistance in the environment: a link to the clinic?". Curr. Opin. Microbiol., 13: 589-594.
11. NCCLS (National Committee for Clinical Laboratory Standards) (1997), "Performance Standards for Antimicrobial Disk Susceptibility Test". Approved Standard M2-A6: Wayne PA
12. Mishra AK , A Mishra, S Tripathi and NN Tripathi (2008). "Susceptibility of Enterococcus faecalis to plant volatiles oils". J. Microb. World, 10:108-112.
13. Güllüce M., M. Sökmen, D. Daferera, G. Ağar, H. Ozkan, N. Kartal, M. Polissiou, A Sökmen and F. Sahin (2003), "In vitro antibacterial, antifungal, and antioxidant activities of the essential oil and methanol extracts of herbal parts and callus cultures of *Satureja hortensis* L". J. Agric. Food Chem., 51 :3958-65.
14. Tirtha G, Tapan KM, Mrinmay das, Anindya bose, Deepak KD(2007) In *Vitro* Antioxidant and Hepatoprotective Activity of Ethanolic Extract of *Bacopa monnieri* Linn. Aerial Parts .Iranian journal of pharmacology & therapeutics,6:77-85.
15. Eloff JN (1998). "Which extractant should be used for the screening and isolation of antimicrobial components from plants?" J. Ethnopharmacol., 60: 1-8.
16. Soniya M, T Kuberan, S Anitha and P Sankareswari (2013). "*In vitro* antibacterial activity of plant extracts against Gram positive and Gram negative pathogenic bacteria". International Journal of Microbiology and Immunology Research. 2: 001-005.
17. Shahwar D, Raza MA, Saeed A, Riasat M, Chattha FI, Javaid M(2012) Antioxidant potential of the extracts of *Putranjiva roxburghii*, *Conyza bonariensis*, *Wood fordia fruiticosa* and *Senecio chrysanthemoids*. Afr J Biotechnol 11(18)**:**4288-95.