WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES

Volume 3, Issue 3, 1862-1874. Research Article ISSN 2278 – 4357

CHEMICAL PROFILING AND EVALUATION OF BIOACTIVITY OF SOLVENT EXTRACTS OF *PTEROSPERMUM ACERIFOLIUM* LINN: AN ETHNOMEDICINAL PLANT OF SIMILIPAL BIOSPHERE RESERVE

[1]S.K. Rath, [2]J.K. Patra, [2]S. Gouda, [3]S.K. Dutta and [4]H.N. Thatoi*

[1]School of Life Sciences, Ravenshaw University, Cuttack, INDIA

[2]Environmental Laboratory, Department of Biotechnology & Medical Engineering, National Institute of Technology, Rourkela, INDIA

[3]North Orissa University, Baripada, INDIA

[4]Department of Biotechnology, College of Engineering and Technology, Biju Patnaik University of Technology, Bhubaneswar, INDIA

Article Received on 13 February 2014, Revised on 27 February 2014, Accepted on 16 March 2014

***Correspondence for Author**
H.N. Thatoi
Department of Biotechnology, College of Engineering and Technology, Biju Patnaik University of Technology, Bhubaneswar, INDIA

ABSTRACT

Pterospermum acerifolium Linn (Sterculiaceae) commonly known as Muchukunda in Odisha is a deciduous tree widely used in traditional medicine. Flowers of *Pterospermum acerifolium* is reported to be used for different medicinal purposes like antimicrobial and other health disorders. However, few theories are available on the use of its bark. The present study aims at phytochemical profiling and screening of different solvent extracts for itsantioxidant and antibacterial potential. The study reveals that bark extracts of *P. acerifolium* are a rich source of bioactive constituents like phenolic compounds (2.36%), alkaloids (2.10%), flavonoids (1.84%) and tannin (2.16%) per dry weight. The antioxidant evaluation of the extracts showed that acetone extract are high in total phenol, total ascorbic and total antioxidant content whereas, the DPPH free radical scavenging, ABTS and the Metal chelating activity assay were recorded to be higher in the ethanol extract, followed by acetone in comparison to standard antioxidant BHT. It was also found that the acetone extract have strong antibacterial properties against all the nine human pathogenic strains with MIC and MBC values of 1.25 to 2.5 mg/ml. The results of the present study validate the medicinal potential of the plant which can be further utilized in pharmaceutical industries for drug discovery.

KEYWORDS: antioxidant, antibacterial, bioactive compounds, free radicals, *Pterospermum acerifolium* and therapeutic agents

INTRODUCTION

In the last few decades numerous biologically active compounds have been discovered by evaluation of ethno-pharmacological data and a number of new antibiotic and antioxidant potentials have been produced. Although a large number of synthetic drugs are available for the purpose of antioxidant and infectious diseases, their clinical efficacy is being threatened by the development of stressful living condition and emergency of multidrug-resistant pathogens. Naturally extracted substances are presumed to be safe since they occur in plant foods, and are seen as more desirable than their synthetic counter parts [1]. Many bioactive compounds like anthrocyanins, flavonoids and phenolics are plant based and play an important role in protecting plants against strong sunlight, severe oxygen stress and also in human health care measures [2]. Antioxidant and other antimicrobial properties of these plants may be due to the presence of wide spectrum of bioactive compounds. The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes[3].

Pterospermum acerifolium Linn (Sterculiaceae) commonly known as 'Kanakchampa' or "Dinner plate tree" is a deciduous tree distributed in tropical Asia and has a wide application in traditional system of medicinal treatment such as ayurvedic anticancer treatment and treatment of small pox eruptions [4]. Flowers of *Pterospermum acerifolium* is being commonly used in various ailments like anthelmintic, abdominal pain, blood troubles, ascites, anti-ulcer, urinary discharges anti-inflammatory analgesic and anti-oxidant activity [5-7]. Stem/bark of the plant is also reported to have antimicrobial activity and a possible source of bioactive secondary metabolites[8]. Thus, the present study was undertaken to evaluate the activity of various extracts of bark of *Pterospermum acerifolium* as a potential source of antioxidant and antibacterial compounds along with the presence of phytochemicals responsible for such activities.

MATERIALS AND METHODS

Collection of plant material

Barks of *Pterospermum acerifolium* Linn (Sterculiaceae) were collected from forests of Similpal Biosphere Reserve (20° 17′- 22° 10′ North Latitude and 85° 57′- 85° 47′ East Longitude), Mayurbhanj, Odisha. Plant was identified by Dr. A. K. Biswal, Department of

Botany, North Orissa University, Baripada, Odisha. The samples were initially rinsed with 1% mercuric chloride and again washed with distilled water, shade dried, homogenized to powder and were subjected to extraction with different solvents.

Preparation of plant extracts

Crude extracts of acetone (99 %), ethanol (99 %), methanol (99 %) and aqueous were obtained by extracting 25 g of fine powder from shade dried barks of *P. acerifolium* with 250 ml of respective solvents in a air tight conical flask on a rotator shaker for 48 hr. Extracts were filtered through Whatman no.1 filter paper and evaporated to dryness by vacuum distillation. The percentage yields of each extract were calculated and were stored at 4°C for further analysis.

Phytochemical Analysis

Aqualitative phytochemical test to detect the presence of alkaloid, tannin, saponin, flavonoid, cardiacglycosides, sterols, anthroquinone glycosides, carbohydrates and protein was carried out using standard procedures[9] and Quantitative phytochemical analysis of dry bark powders was carried out using standard protocols [9-11]. Dry bark powder was screened for the presence of alkaloids, phenol, flavonoids, tannin, thiamine, riboflavin, nitrogen, phosphorus, potassium, P_2O_5, K_2O, carbohydrate, protein and total amino acid content.

Screening of antioxidant activity

Total phenolic content was estimated according to the methods of Slinkard and Singleton[12] using catechol as standard phenolic compound. The phenol content was expressed in terms of % dry weight. The ascorbic acid content was estimated following the methods of Barros et al. [13] with slight modifications. Ascorbic acid content was expressed in % dry weight. Total antioxidant capacity of plant extracts was determined by Prieto et al. [14]. The absorbance was measured at 695 nm against blank and the results were expressed as mg catechol equivalent/gm dry weight (DW). Reducing power of solvent extracts was determined by the method of Oyaizu [15]. Absorbance was measured at 700 nm in a spectrophotometer. DPPH (2, 2-diphenyl-1-picryl hydrazyl) radical scavenging effect of the plant extract was determined by following modified methods of Patra et al. [16]. Briefly, 2.0 ml of 0.1 mM DPPH (2, 2- diphenyl-1-picrylhydrazyl) solution (in methanol) was added to the test tubes containing 0.1 ml aliquot of solvent extracts of the plant and standard BHT (Buthylated hydroxytoluene) at 50, 75 and 100 µg/ml concentration. The mixture was vortexed for 1

minute and kept at room temperature for 30 min in the dark. The absorbance of all the sample solution was measured at 517 nm by using spectrophotometer (Systronics 114). Percentage scavenging effect of the plant extract against DPPH free radical was calculated from the following equation.

% Scavenging = $(A_0 - A_1) / A_0 X 100$

Where A_0 is the absorbance of control. A_1 is the absorbance of test sample.

Ferrous ion chelating activity was assessed as described by Patra et al. [11]. Different concentrations (50, 75 and 100 µg/ml) of plant extracts were added to 0.5 ml ferrous chloride (2 mM) and left for incubation at room temperature for 5 min. Then the reaction was initiated by adding 0.1ml of ferrozine (5 mM), and the mixture was adjusted to 3 ml with deionized water, shaken vigorously and incubated at room temperature for 10 min. Absorbance was measured at 562 nm. BHT was used as a positive control. Metal chelating effect of the plant extract was calculated from the following equation.

Metal chelating effect (%) = $(A_0 - A_1) / A_0 X 100$

Where A_0 is the absorbance of control. A_1 is the absorbance of test sample.

ABTS radical scavenging assay was carried out by the method of Thaipong et al. [17]. The ABTS radicals pre-generated by mixing 7mM ABTS solution with 2.25 mM potassium persulphate on incubation for 12-16 hr in dark was equilibrated by diluting with ethanol to which 100Mm of different solvent extracts was added respectively. Absorbance was taken at 734 nm for 5 min. BHT was used as standard antioxidant.

Radical scavenging activity (%) = $(A_0 - A_1)/ A_0$ X 100

Where $A_{0=}$ Absorbance of control, $A_{1=}$ Absorbance of test sample

Separation/ purification of bioactive compounds

Screening of antibacterial activity

In vitro antibacterial activity of bark extracts (in acetone and ethanol solvent) of *P. acerifolium* was carried out against nine human pathogenic bacteria obtained from Institute of Microbial Technology, Chandigarh. *Staphylococcus aureus* (MTCC 1144), *Shigella flexneri* (Lab isolate), *Bacillus licheniformis* (MTCC 7425), *Bacillus brevis*

(MTCC 7404), *Vibrio cholera* (MTCC 3904), *Pseudomonas aeruginosa* (MTCC 1034), *Staphylococcus epidermidis* (MTCC 3615), *Bacillus subtilis* (MTCC 7164) and *E. coli* (MTCC 1089)). Antibacterial evaluation of the solvent extracts was undertaken by agar cup plate method of Patra et al. (2011) and minimal inhibitory concentration (MIC) was determined by two fold micro dilution method. Chloramphenicol (30µg/disc) was used as the standard antibiotics.

Statistical Analysis

Experiments were carried out in triplicates and the data was expressed as mean value ± standard deviation. Where applicable, the means of all the parameters were examined for significance by two way analysis of variance (ANOVA) and the differences between samples were determined by Duncan's Multiple Range test using GenStat discovery (edition3) statistical software package. Differences were considered significant at a probability level of $p<0.05$.

RESULTS AND DISCUSSION

The preliminary phytochemical screening of different solvent extracts showed the presence of bioactive compounds such as alkaloids, steroid, flavonoids and tannin and phenolic compounds (Table 1). The finding are also in accordance with Malik et al. and Chatterjee et al., [4,18,] in which the presence of polyphenolic content, saponin, tannin and other bioactive were reported in the methanol and aqueous extract of bark of the plant. High content of primary and secondary metabolites were measured using standard protocols in the bark extract of *Pterospermum acerifolium* with phenol (2.36±0.026), tannin (2.16±0.026), and alkaloids (2.10±0.026) in term of percentage dry weight (Table 2). Plants rich in various secondary metabolites serves as defense mechanism for the plant and have also been found *invitro* to have antimicrobial properties and other biological activities such as oxidative damage on gastric tissues, anti-inflammatory, anthelmintic, hepatoprotective and many more [4, 6, 19, 20,21,22].

The chemical complexity of different fractions and mixture of compounds present in the bark of the plant could lead to scattered results, depending on the test employed. Therefore, an approach with multiple assays for evaluating the antioxidant potential of extracts would be more informative and even necessary. Different assays were used for the determination of antioxidants activity of the four different solvent extracts. All the solvent extracts of *P.*

acerifolium showed significant antioxidant potential in all the assays and the results were comparable with the standard Butylated hydroxyl toluene (BHT). Acetone extract showed highest phenol content and ascorbic acid contentof 11.40±0.24%and1.85±0.06% dry weight (DW) respectively (Table 3). The Total antioxidant capacity was found out to be highest in the ethanol extract (175.25±1.03mg Catechol equivalent/ DW followed by methanol, acetone and aqueous extract (Table 3). The DPPH free radical scavenging potential of all the four solvent extracts were carried out in three different concentrations (50µg/ml, 75µg/ml and 100µg/ml) and the result was presented in the figure 1. It is found that the ethanol, acetone and methanol extracts of the bark of the plant showed potent DPPH radical scavenging activity with about 77 % scavenging which increased with increase in the concentration of the sample. The results are comparable with that of the standard BHT, which showed less DPPH activity (51-61 %). The results of the ABTS free radical scavenging potential of the solvent extracts also highlights same trend with scavenging potential ranging between 22-98 % (Figure 1). Similarly the Metal chelating activity assay was recorded to be higher in the ethanol extract, followed by acetone, aqueous and methanol extracts. However the standard BHT, showed less metal chelating activity (Figure 1). Reducing assay performed on extracts of *P. acerifolium* was found to be low as compared to the standard BHT (Figure 1). Antioxidant property of the ethanol extracts may be a associating factor due to the presence of a diverse array of secondary metabolites such as tannins, terpenoids, alkaloids, vitamins in the plant extracts [20,23] and high contents of phenolic compounds that react differently with oxidants or may be because of their synergistic effect [24,25].The effective antioxidant mechanism of the solvent extracts may be due to any of the functions such as prevention of chain initiation, binding of transition-metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging [6,26]. The results of all the experiments are analyzedstatistically and the significant differences among the results are interpreted by Duncan's Multiple Range test basing on the least significant difference values (LSD).

The antibacterial activity of solvent extracts (acetone and ethanol) of bark of *P. acerifolium* was evaluated against nine human pathogenic bacterial strains using Chloramphenicol as standard antibiotic and the result is presented in Table 4. Acetone extract of *P. acerifolium* was found to possess higher antibacterial activity against most of the tested bacterial strains compared to ethanol extract with zone of inhibition ranging between 8-25 mm. The MIC and

MBC values of the acetone extract ranges from 1.25 to 5 mg/ml except for *S. flexneri* and *V. cholerae* against which it was 10 mg/ml (Table 4).

Table 1.Qualitative phytochemical screening of different solvent extracts of bark of *P. acerifolium*

Phytochemicals	Solvent extracts			
	Acetone	Ethanol	Methanol	Aqueous
Steroid	+	+	+	-
Alkaloid	+	+	+	-
Cardiac glycoside	+	+	-	+
Protein and amino acid	+	+	+	+
Tannin and phenolic compounds	+	+	+	+
Ascorbic acid	+	+	-	+
Fixed oil and fats	+	+	-	+
Anthraquinone	-	-	-	-
Terpenoid	-	-	-	-
Carbohydrate	+	+	+	+
Saponin	-	+	+	+
Gums and mucilage	+	+	+	-

Table 2.Chemical content of crude powders of bark of *P.acerifolium*

Parameters	Values in Percentage dry weight
Primary metabolites	
Carbohydrate content	10.56±0.020
Protein content	10.67±0.020
Total amino acid	0.085±0.0026
Secondary metabolites	
Phenol	2.36±0.026
Flavonoid	1.84±0.026
Tannin	2.16±0.026
Alkaloid	2.10±0.026
Vitamins	
Thiamine	0.281±0.002
Riboflavin	0.112±0.002
Minerals	
Nitrogen content	1.713±0.026
Phosphorous content	0.1±0.023
Potassium content	0.57±0.026
P_2O_5	0.23±0.053
K_2O	0.688±0.031

Table 3. Different Antioxidant assays of solvent extracts of bark of*P. acerifolium*

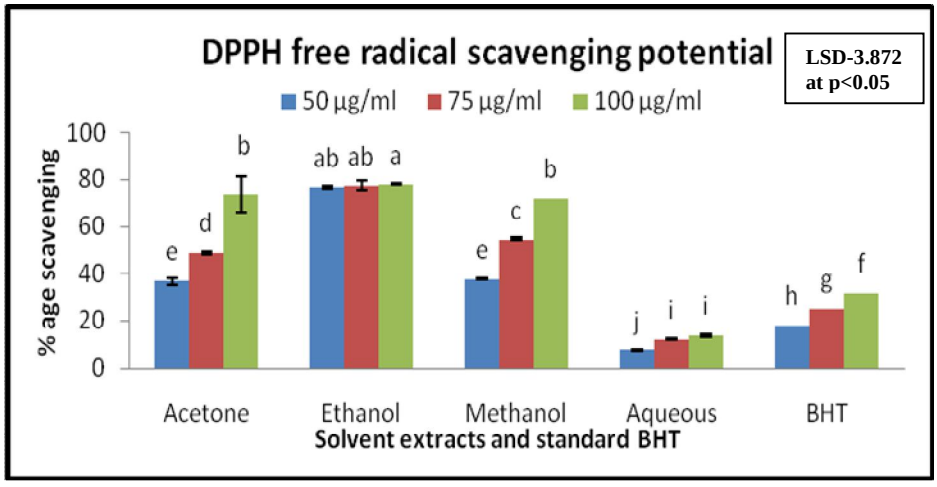
Extracts	Total Phenol content (% DW)	Total ascorbic acid content (% DW)	Total antioxidant capacity (mg Catechol equivalent / gm dry weight)
Acetone	11.40^{a}±0.24	1.85^{m}±0.06	46.75^{y}±0.66
Ethanol	0.96^{d}± 0.12	1.14^{n}±0.05	175.25^{w}±1.03
Methanol	1.46^{c}±0.10	1.17^{n}±0.00	91.34^{x}±0.90
Aqueous	5.18^{b}±0.14	1.29^{n}±0.10	37.51^{z}±0.98
LSD at *p<0.05*	0.45	0.576	2.007

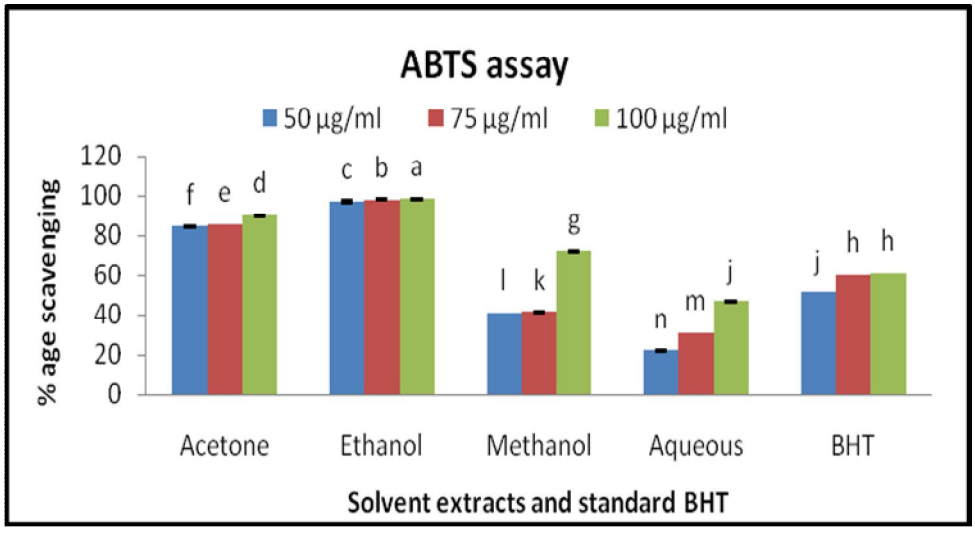
LSD- Least significant difference; Difference in superscript letters indicate significance level at *p<0.05*

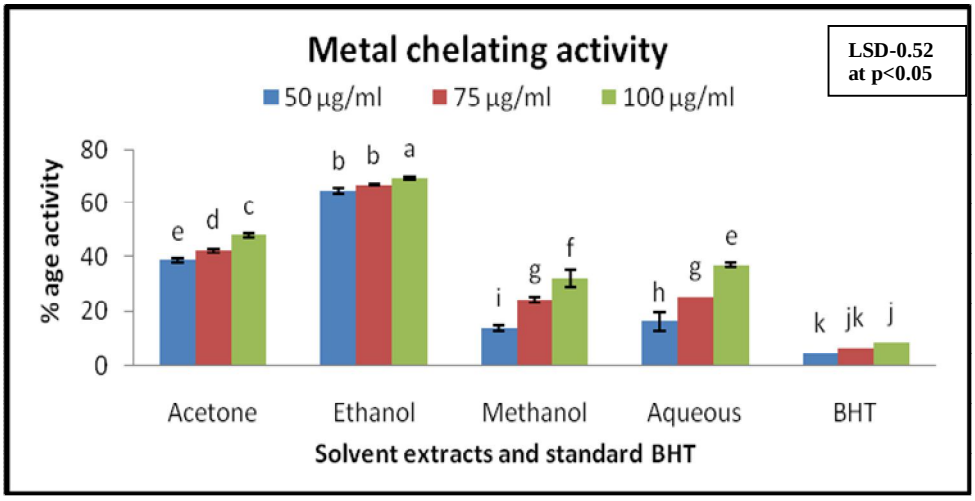
Table 4. Antibacterial activity of bark extracts of *P. acerifolium* against human pathogenic bacterial strains

Strains	Acetone extract			Ethanol extract			Standard antibiotics
	Antibacterial activity (50 mg/ml)	MIC	MBC	Antibacterial activity (50 mg/ml)	MIC	MBC	Chloramphenicol
	Inhibition zones in mm	Values in mg/ml		Inhibition zones in mm	Values in mg/ml		Inhibition zones in mm
S.aureus	25.33^{c} ± 0.57	1.25	2.5	18.33^{f} ± 1.52	>10.0	>10.0	27 ± 0
S. flexneri	10.00^{i} ± 00	10.0	>10.0	10.33^{i} ± 0.57	5.0	10.0	22 ± 0
B.licheniformis	20.66^{e} ± 1.52	2.5	5.0	20.33^{e} ± 0.57	5.0	10.0	21± 0
B.brevis	28.33^{b}± 1.15	1.25	2.5	30.33^{d} ± 0.57	1.25	2.5	NA
V.cholerae	23.66^{d} ± 1.15	10.0	>10.0	13.00^{h} ± 00	1.25	2.5	28± 0
P.aeruginosa	20.33^{e} ± 0.57	5.0	10.0	NA	ND	ND	24± 0
S.epidermidis	11.00^{i} ± 1.00	2.5	5.0	16.00^{g} ± 00	5.0	10.0	37± 0
B.subtilis	20.33^{e} ± 0.57	2.5	5.0	18.00^{f} ± 1.00	10.0	>10.0	32± 0
E. coli	08.66^{j} ± 0.57	2.5	5.0	14.00^{h} ± 1.00	5.0	10.0	40± 0
LSD at *p<0.05*	1.02			1.02			

'NA' – No activity, '>'- value is greater than, Difference in superscript letters indicate significance level at *p<0.05*







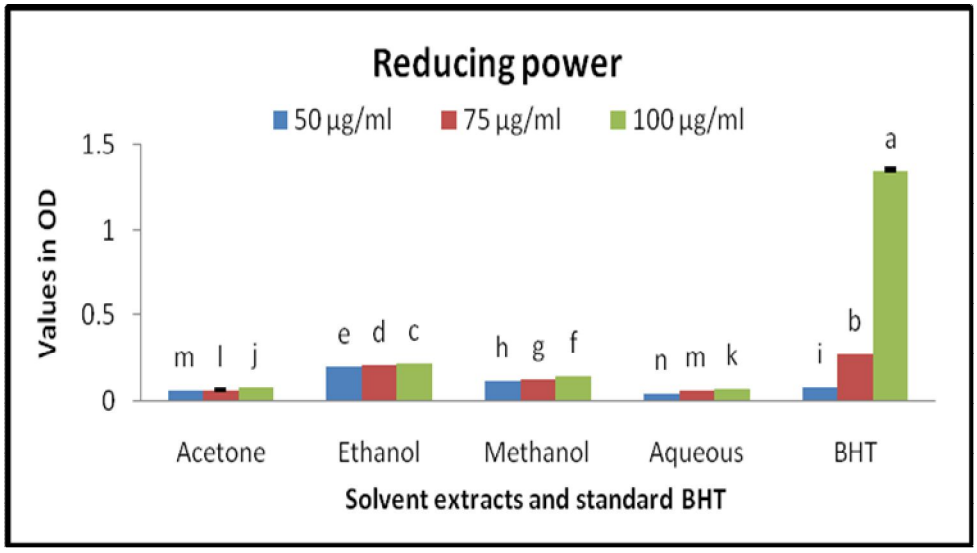


Figure1. Different Antioxidant assay of solvent extracts of bark of *P. acerifolium*

LSD- Least significant difference; Difference in superscript letters indicate significance level at $p<0.05$ Similar results was also recorded by Nandy et al [27] where the bark extract of *P. acerifolium* showed the lowest inhibition zone against *S. flexneri* and *V. cholera* in comparison to other gram positive and gram negative bacteria.The variability in the activity of different extracts may be due to the solvent used for extraction or facts that materials of plant origin are subjected to contamination and deterioration with time and other photochemical interaction. The finding are also supported by documentation of Panda and Dutta [8] in which the antibacterial properties was concentration dependent and non-polar extracts (petroleum ether, hexane and butanol) showed higher antimicrobial properties compared to polar extracts (methanol and aqueous). The antibacterial activity of the plant has been attributed to the presence of polyphenolic, alkaloids, anthraxquinone and other secondary metabolites and could explain the rationale for the use of the plant in the treatment of the various conditions in traditional medical practice [28,29].

CONCLUSION

Traditional healing systems around the world that utilize herbal remedies is an important source for the discovery of new therapeutic agents and *Pterospermum acerifolium* has an important place among such plants. The present study suggest that, bark extracts of *Pterospermum acerifolium* can be used as a source of therapeutic agents in treatment for various types of bacterial infections and also as a source of potential antioxidant which can be further exploited in pharmaceutical industries. Further evaluation and trails are required for the search of other active constituents and theirwide exploration.

ACKNOWLEDGEMENT

The authors are grateful to help and cooperation of field staff and Director, Similipal Biosphere Reserve. We are grateful to the authorities of Ravenshaw University and North Orissa University for providing necessary facilities to carry out this research.

REFERENCES

1. Pratt DE. Natural antioxidant from plant material. In: IMT, Huang, CT. HO & CY Lee (Eds). Phenolic compounds in food and their effects on health. New York American Chemical Society. 1992; pp 54-72.
2. Santhakumari P, Prakasam A, Pugalendi KV. Modulation of oxidative stress parameters by treatment with Piper betle leaf in streptozotocin induced diabetic rats. Indian J pharmacol, 2003; 35: 373.

3. Basile A, Sorbo S, Giordano S, Ricciardi L, Ferrara S, Montesano D, et al. Antibacterial and allelopathic activity of extract from *Castanea sativa* leaves. Fitoterapia ,2000; 71:110-116.
4. Chatterjee P, Chakraborty B, Nandy S, Dwivedi A, Datta R. *Pterospermum acerifolium* Linn. : A comprehensive review with significant pharmacological activities. International Journal of Pharmacy & Life Sciences, 2012; 3 (2):1453-1458.
5. Mazumdar PM, Sasmal D, Arulmozhi A, Kumar PM. Comparative study of in vitro Antioxidant activity of the methanolic extracts of *Salvia splendens* and *Pterospermum acerifolium*. Pharmacologia, 2012; 3 (9): 444- 449.
6. Manna AK, Manna S, Behera AK, Kar S. In vitro antioxidant activity of P. acerifolium barks. Journal Pharmacy Research, 2009; 2(6): 1042-4.
7. Manna AK, Jena J. Anti-inflammatory and Analgesic activity of bark extract of *Pterospermum acerifolium*. International Journal of Current Pharmaceutical Research, 2009; 1(1):32-37.
8. Panda SK, Dutta SK. Antibacterial activity from bark extracts of *Pterospermum acerifolium* (L.) Wild. International Journal of Pharmaceutical Sciences and Research, 2011; 2(3): 584-595.
9. Edeoga HO, Okwa DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr J Biotechnol, 2005; 4: 685-688.
10. Sadasivam S, Manickam A. Biochemical methods. New Age, International (P) Ltd. Publication: 2005, New Delhi.
11. Patra JK, Dhal NK, Thatoi HN. *In vitro* bioactivity and phytochemical screening of *Suaeda maritime* (Dumort): A mangrove associate from Bhitarkanika, India. Asian Pac J Trop Med, 2011; 4:727-734.
12. Slinkard K, Singleton VL Total phenol analysis: Automation and comparison with manual methods. Am J Enology and Viticulture; 1977; 28: 49-55.
13. Barros L, Ferreira MJ, Queiros B, Ferreira IC, Baptista P. Total phenol, ascorbic acid, β-carotene and lycopene in Portuguese wild edible mushroom and their antioxidant acitivities. Food Chem, 2007; 103: 413-419.
14. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phodphomolybdenum complex: specific application to the determination of Vitamin E. Anal Biochemi, 1999; 269: 337-341.

15. Oyaizu M. Studied on product of brown ingreaction, prepared from glucose amine. Jpn. J.Nutr, 1986; 44: 307-315.
16. Patra JK, DasMohapatra A, Rath SK, Dhal NK, Thatoi HN. Screening of antioxidant and anti filarial activity of leaf extracts of *Excoecaria agallocha* L. International Journal of Integrative Biology, 2009; 7(1): 9-15.
17. Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, Byrne DH. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. J Food Comp Anal. 2006; 19: 669-675.
18. Malik KS, Khan Z, Ajaib M. Investigation of in-vitro antioxidant potential of ethnobotanically important tree, *Pterospermum acerifolium*. Pak. J. Bot, 2012; 44: 105-109.
19. Pattanaik P, Parida S. Antioxidant and Anti-Inflammatory Screening of Barks of *Pterospermum a cerifolium* (Sterculiaceae). International Journal of Pharma World Research, 2010; l1(3): 1-19.
20. Sannigrahi S, Parida S, Patro VJ, Mishra MS, Pathak A. Antioxidant and anti-inflammatory potential of *Pterospermum acerifolium.* International Journal of Pharmaceutical Sciences Review and Research, 2010; 2(1): 1-5.
21. Shetty SG, Hidayathulla VS, Chandrashekar KR. Antimicrobial activity and phytochemical screening of *Pterospermum reticulatum.* International Journal of Pharmacy and Pharmaceutical Sciences, 2011; l 3(5): 35-37.
22. Mazumder PM, Ghosh S, Paramaguru R. Evaluation of atihyperlipidemic and anttioxidant activity of *Pterospermum acerifolium* (l.) Willd. Pharmacology online, 2011; 3: 128-146
23. Head, KA. Vitamin C: Antioxidant or pro-oxidant in vivo. Free Radical Res, 2008; 25: 439.
24. Akond ASMGM, Khandaker L, Hossain KG, Furuta Y. Total Polyphenol, Polyphenol Oxidase, Antioxidant Activity and Color Profiles of Some Wheat Varieties from Bangladesh. Research Journal of Agriculture and Biological Sciences, 2010; 6(2): 186-190.
25. Mir AM, Sawhney SS, Kumar S. Comparative study upon the antioxidant potential of *Saracaindica* and *Pterospermum acerifolium.* Journal of Chemical and Pharmaceutical Research, 2012; 4(11):4716-4720.

26. Pattanaik P, Ravi J, Srinivas B, Mohanty C, Panda OP, Nanda SS. Comparative study of antioxidant activity of alcoholic and ethyl acetate extract of the bark of *Pterospermum acerifolium*. International Journal of Pharmacy &Technology, 2011; 3(3): 3335-3344.
27. Nandy S, Datta R, Hossain K, Samanta A. Antimicrobial activity of methanolic leaves extract of *Pterospermum acerifolium* (L) Willd. Life sciences Leaflets, 2012; 6: 4-12.
28. Erah PO, Osuide GE, Omogbai EKI. Hypoglycaemic effect of the extract of *Solenostemon monostachys* (P. Beauv) leaves. J. West Afr Pharm, 1996; 10(2):21-7
29. Chandrakar V, Pandey B, Bhattacharya C. Antibacterial activity of crude extract of leaves of *Pterospermum acerifolium.* Indian Journal of Science, 2013; 5(14): 1-4.