Antimicrobial and Antioxidant activity of Ethanolic extract of *Euphorbia prostrata* Ait. leaves.

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***Abstract*- The secondary metabolites available in the plants have been targeted as the lead compounds to develop new drug in the recent times. Testing the plants for their medicinal properties can give the glimpses of their potentiality to be used as the resource for the new drugs. For this purpose, many such plants which have been used as the constituents in many medicinal formulations have been scientifically analysed for their medicinal properties. One of such medicinal plants which has been used traditionally is Euphorbia prostrata Ait., This plant was analysed for its antioxidant and antimicrobial activities. The plants were shade dried and extracted with ethanol using fine powder of the plant. The analysis showed the presence of antioxidant and antimicrobial activity with the ethanolic extracts of the leaves. Further studies on the biological activity of these compounds may shed more light on the medicinal importance of this plant.**

INTRODUCTION

Herbs are being used for the promotion of health, prevention and treatment of diseases in India from ancient times. The sources of pharmaceuticals for human ailments include plants, either as compounds extracted as such or as their synthetic derivatives. With increasing trend in the resistant to antimicrobial drugs, there is an urgent need for the development of the new drugs to fight the microbial diseases. With the new methods in the drug discovery, identification of bioactive compounds from plants plays an important role in drug development. A vast number of medicinal plants containing the secondary metabolites exhibit antioxidant properties. Oxidative process is one of the most important routes for producing free radicals in foods, drugs and even in living systems (Halliwell, 1994). The most effective way to counter the action of free radicals which cause the oxidative stress is the antioxidative defense mechanisms. Recently there has been great interest in the screening of therapeutic potential of medicinal plants as antioxidants in reducing oxidative stress-induced tissue injury (Pourmorad et al., 2006). Euphorbia prostrata is used in fever, abdominal disorder and as blood purifier in many parts of the world. The extract of E. prostrata has been found to have significant anti-inflammatory, analgesic, haemostatic (stops bleeding) and wound healing properties (Singla and Pathak, 1989). Ethanolic extract of E.prostrata are tested for the antioxidant and antimicrobial activity.

MATERIALS AND METHODS

Plant Material

Euphorbi prostrata Ait., belonging to the family Euphorbiaceae were collected from the coastal areas of Kanyakumari district of Tamilnadu, India. These are branched, prostrate with many stems spreading from the roots, slender up to 20 cm long; leaves green but occasionally purplish red. The oval-shaped leaves are up to 1.0 cm long with finely toothed edges. The inflorescence is a cyathium less than 2 mm wide, with white petal-like appendages surrounding the actual flowers. There are four male flowers and a single female flower, the latter developing into a lobed, hairy fruit 1.0 – 2.0 mm wide.

Fresh plants were collected from sandy coasts of Kanyakumari district and washed properly with distilled water. The leaves were shade dried at room temperature. Dried leaves were uniformly pulverised using mechanical grinder. The ethanol extract was prepared with 25.0 g of powdered plant material using 150 ml of ethanol with soxhlet apparatus and it was used for the following tests and analysis.

ANTIOXIDANT ACTIVITY

Enzyme Extraction

For extracting antioxidant enzymes, fresh leaves (0.5 g) were ground using a tissue grinder in 5.0 mL of 50 mM cooled phosphate buffer (pH 7.8) placed in an ice bath. The homogenate was centrifuged at 15000 x g for 20 min at 4°C. The supernatant was used for determining the activities of enzymes. Protein concentration of the extract was measured by the method of (Lowry et al.,1951)

Antioxidant assay

Antioxidant activity was analysed in terms of Catalase (Sinha,1972), Peroxidase (Addy and Goodman, 1972) and Superoxide dismutase (Beauchamp and Fedovich, 1971)

ANTIBIOTIC ACTIVITY TEST (disc diffusion method)

In vitro antibacterial and antifungal activities were examined for ethanolic extracts. Antibacterial and antifungal activities of plant extracts against five bacteria (three Gram-positive and two Gram negative) and three pathogenic fungi were investigated by the agar disk diffusion method For the determination of zone of inhibition by the plant extract for Gram-positive, Gram-negative, and fungal strains, their zone of inhibition against standard antibiotics were taken as reference for comparison of the results. Netilmycin (100 µg/disc) was used as positive control for antibacterial activity and Flucanazole (100 µg/disc) was used as positive control for antifungal activity studies. The extracts were screened for their antibacterial and antifungal activities against the Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus cereus, Candida albicans, Candida parapsolis and Aspergillus niger. The plant extract with a dilution of 100 µg/ml and standard drugs prepared in double-distilled water in the same concentration were used as the positive control while discs soaked in ethanol were used as the negative control.

Mueller-Hinton sterile agar plates were seeded with each bacterial strain and incubated at 37°C for 24 hours. For analysing the antifungal activity cultures were taken, and swabbed on Sabouraud dextrose agar medium and incubated at 30°C for 48 hours. The zones of growth inhibition around the disks were measured after 24 hours of incubation at 37°C for bacteria and 48 hours for fungi at 30°C. The sensitivities of the microorganism species to the plant extracts were determined by measuring the sizes of inhibitory zones (including the diameter of disk) on the agar surface around the discs and values <8 mm were considered as not active against microorganisms.

Determination of relative percentage inhibition

The relative percentage inhibition of the test extract with respect to positive control was calculated by using the following formula (Gaurav Kumar,2010)

Relative percentage inhibition of the test extract = $\frac{100 \times (x-y)}{(z-y)}$

Where,

x: total area of inhibition of the sample ,

y: total area of inhibition of the solvent

z: total area of inhibition of the standard drug

The total area of the inhibition was calculated by using area $\pi r2$;

where, r = radius of zone of inhibition.

RESULTS AND DISCUSSION

A. Antimicrobial activity

The antimicrobial activity of the extracts of Euphorbia prostrata was studied against bacteria and fungi. Antibacterial and antifungal potential of extracts were assessed in terms of zone of inhibition of bacterial growth. The results of the antibacterial and antifungal activities are presented in tables (Table 1)

Table 1: Antimicrobial activity of ethanolic extracts of *E. prostrata*

Test organisms	*Sample*	*Positive control (Netilmycin)*	*Negative control*	*Relative inhibitory percentage (%)*
Escherichia coli	*18 mm*	*22 mm*	-	*81.8*
Klebsiella pneumonia	*20 mm*	*24 mm*	-	*83.3*
Pseudomonas aeruginosa	*18 mm*	*24 mm*	-	*75.0*
Staphylococcus aureus	*24 mm*	*29 mm*	*16 mm*	*61.5*
Bacillus cereus	*21 mm*	*24 mm*	-	*87.5*
Candida albicans	*12 mm*	*14mm (Flucanazole)*	-	*85.7*
Candida parapsolis	*10 mm*	*13mm*	-	*76.92*

Aspergillus	-	-	-	-

The results show that the extracts of *Euphorbia prostrata* were effective against all the bacteria and fungi tested except *Aspergillus niger* where the inhibitory effect was not much pronounced. When compared with standard drugs, the results revealed that in the extracts showed more activity against *Bacillus cereus* followed by *Klebsiella pneumonia*. The growth inhibition zone measured ranged from 18 to 24 mm for all the sensitive bacteria, and ranged from 10 to 12 mm for sensitive fungal strains.

The present work reveals that the extracts obtained from *Euphorbia prostrata* has strong activity against all the tested bacterial and fungal strains. When compared with standard antibiotic drugs used in this screening work, extracts of *Euphorbia prostrata* were found to be active against both the Gram-positive, Gram-negative, and fungal strains.

The antibacterial and antifungal activities are formed to be significant. This confirms presence of different phytoconstituents with biological activity that can be of valuable in therapeutic purpose in future.

Antioxidant activity

The present study shows the antioxidant potential and radical scavenging activity of ethanolic extract of *E. prostrata* by the determination of various antioxidants. The results are given in the table (Table 2). Highly reactive antioxidant capacity of *E. prostrate* strongly justifies the wide variety of use of this plant in various ailments like fever, blood purifier, anti-inflammatory, analgesic and even antibiotic.

Table 2: Enzymatic antioxidant activity of ethanolic extracts of *E. prostrata*

SOD (units per mg protein)	Catalase (jig of H_2O_2 decomposed/minute/mg protein)	Peroxidise (units/ mg protein)
6.725	63.933	0.579

The ethanolic extract showed a strong enzymatic antioxidant activity. Complex antioxidant systems are very important for protecting cellular membranes and organelles from the damaging effects of active oxygen species. These include both enzymatic and non enzymatic antioxidants. High superoxide dismutase (6.725 units/mg protein) and catalase (63.933 /jig protein), and peroxidase (0.579 units/mg protein) activities were detected. These enzymes are reported of participating mainly playing a major role in protection against oxidative stress.

The imbalance between antioxidant and reactive oxygen species, such as superoxide radical (O_2^-), hydroxide radical (-OH), peroxide radical (ROO^-), and nitric oxide radical produced due to excessive metabolism in the cell and its ability to detoxify these reactive intermediates is called oxidative stress. These reactive oxygen species damage the biological system and causes different chronic diseases like cancer and heart diseases (Prakash *et al*., 2007). The modern research claims that oxidative stress is the cause of various disorders and diseases, therefore, the researcher focus on the role of antioxidants in the maintenance of biological system (human health), its remedy and treatment (Etsuo, 2010). Natural antioxidants that are present in herbs and spices are responsible for inhibiting or preventing the deleterious consequences of oxidative stress.

Catalase (CAT) is another important antioxidant enzyme that converts H_2O_2 to water in the peroxysomes (Fridovich, 1989). In this organelle, H_2O_2 i~ [STJGXFeG[IToP [El-oxidation of fatty acids and photorespiration (Morita *et al*., 1994). Higher activity of CAT along with Ascorbate Peroxidase (APX) will decrease H_2O_2 level in cell and will increase the stability of membranes and CO_2 fixation because several enzymes of the Calvin cycle within chloroplasts are extremely sensitive to H_2O_2 and high level of H_2O_2 directly can inhibit CO_2 fixation (Yamazaki *et al.,* 2003). ROS can lead to oxidation of amino acid side chains, formation of protein-protein cross-linkages and oxidation of the protein backbone, resulting in protein fragmentation (Berlett and Stadtman, 1997). Harmful influence of ROS on cell macromolecules may also be alleviated by the activity of non-enzymatic antioxidant compounds such as ascorbic acid, phenolic compounds, glutathione, thioredoxin and carotenoids (Xiong and Zhu, 2002).

SUMMARY AND CONCLUSION

The selected plant *Euphorbi prostrata* Ait., belonging to the family Euphorbiaceae were collected from the coastal areas of Kanyakumari district of Tamilnadu. The leaves were shade dried at room temperature.

Dried leaves were uniformly pulverised using mechanical grinder. The ethanol extract was prepared with 25.0 g of powdered plant material using 150 ml of ethanol with soxhlet apparatus and it was used for the potential for its antimicrobial and antioxidant activity. Standard procedures were used for all the analysis. Five bacteria and three fungal species (*Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus cereus, Candida albicans, Candida parapsolis* and *Aspergillus niger* were taken as test organisms). SOD, Peroxidase and Catalase enzyme activity were analyzed with the ethanolic extract of the plant. The results confirm the plant has antioxidant and antimicrobial activity.

REFERENCE

[1] Addy S.K and Goodman, RN. 1972. Polyphenol oxidase and peroxidase in apple leaves inoculated with a virulent or an avirulent strain for Ervinia amylovora. Ind. phytopath. 25: 575- 579

[2] Bandow JE, Brotz H and Leichert LIO, 2003. Proteomic approach to understanding antibiotic action. Antimicrob Agents Chemother, 47: 948-955

[3] Beauchamp C and Fridovich I, 1971. Superoxide Dismutase: Improved Assays and an Assay Applicable to Acrylamide Gels. Anal. Biochem., 44: 276.

[4] Berlett BS and Stadtman ER, 1997. Protein oxidation in aging, disease and oxidative stress. J. Biol. Chem. 272: 20313 20316

[5] Etsuo N, 2010. Assessment of antioxidant capacity in vitro and in vivo.J. Free Radic. Biol. Med.8: 503-515

[6] Fridovich I, 1989. Superoxide dismutases: An adaptation to a paramagnetic gas, The J. of Bio. Chem. 264: 7761-7764.

[7] Gaurav Kumar, Karthik L and Bhaskara Rao KV, 2010. In vitro anti-Candida activity of Calotropis gigantea against clinical isolates of Candida, Journal of Pharmacy Research, 3: 539-542.

[8] Halliwell B, 1994. Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? Lancet. 344: 721-724

[9] Lowry OH, Rosenbrough NJ, Farr A and Randall RJ, 1951. J Biol Chem. 193:265-75

[10] Morita CT, Parker CM, Brenner MB and Band H, 1994. T cell receptor usage and functional capabilities of human yS T cells at birth. J. Immunol. 153: 3979-3988.

[11] Pourmorad. F, Hosseinimehr SJ and Shahabimajd N,2006. Antioxidant activity, phenols, flavanoid contents of selected Iranian medicinal plants. S. Afr. J. Biotechnol. 5: 1142- 1145.

[12] Prakash D, Upadhyay G, Sing BN and Sing HB, 2007. Antioxidant and free radical Scavenging activities of seeds and agri-wast of some varieties of soybean. Food Chem., 104: 783-790

[13] Rojas R, Bustamante B and Bauer J, 2003. Antimicrobial activity of selected peruvien medicinal plants. J Ethnopharmacol, 88: 199-204.

[14] Singla AK and Pathak K, 1989. Anti-inflammatory studies on Euphorbia prostrata. Journal of Ethnopharmacology. 27:1-2, 55-61

[15] Sinha A, 1972. Colorimetric Assay of Catalase. Analytical Biochemistry, 47:389-394

[16] Xiong L, and Zhu JK, 2002a. Salt-stress signal transduction. In: Scheel D and Wasternack C (eds) Plant Signal Transduction. Frontiers in Molecular Biology Series. Oxford University Press. pp 165-19

[17] Yamazaki, Y., Hyodo, F., Morita, T., 2003. Wide distribution of cysteine-rich secretory proteins in snake venoms: isolation and cloning of novel snake venom cysteine-rich secretory proteins. Arch. Biochem. Biophys. 412: 133 141