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EVALUATION OF ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF ETHANOLIC EXTRACT OF *EUPHORBIA HIRTA* LEAVES

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ABSTRACT

This study was undertaken to evaluate the antioxidant and antimicrobial effects of the ethanolic extracts of the leaves of *Euphorbia hirta*. The antioxidant and antimicrobial effects of the ethanolic extracts of the leaves of Euphorbia hirta were determined using the in-vitro methods. Its antioxidant effect was determined by evaluating its ferric reducing ability and its scavenging effects on some free radicals such as; 2,2 diphenyl-2-picrylhydrazyl (DPPH), hydrogen peroxide (H_2O_2), hydroxyl radical, superoxide radical, and nitric oxide (NO) radicals. Its total phenol content was also determined at 1, 2, 3mg/ml. The antimicrobial effects was determined by evaluating its inhibitory effects against some microorganisms like *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella spp, Enterococcus feacalis, Klebsilla pneumonia, Proteus maribalis and Shigella dysenteri*. Its inhibitory effect was also compared with some standard antibiotics such as ciprofloxacin, streptomycin, gentamicin and amoxicillin. The result shows a concentration dependent increase in the activity of the extract against the radicals. The ethanolic extracts of the leaves of *Euphorbia hirta* exhibited a reasonable inhibitory effects against all the microorganisms tested with its minimum inhibitory concentration (MIC) at 0.8mg/ml. Comparing with the standard antibiotics, It shows a wider zone of inhibition against some microorganisms than the standard antibiotics which was more pronounced against *Proteus maribalis, Escherichia coli* and *Bacillus subtilis*. This study demonstrated the significant free radical scavenging and antimicrobial potential of the leaves of *Euphorbia hirta* and could be explored for treatment of various infections caused by these microorganisms and also in the treatment of free radicals mediated aliments.

KEYWORDS: *Euphorbia hirta*, antioxidants, free radicals, microorganisms, antimicrobials.

INTRODUCTION

Antioxidants are compounds that can prevent or delay the oxidation of oxidizable products by scavenging free radicals and reducing oxidative stress. Oxidative stress is an imbalance between oxidants and antioxidants in favour of the oxidants, potentially leading to damage. Oxidative stress has been implicated in the pathogenesis of human degenerative diseases such as: atherosclerosis, sickle cell anaemia, myocardial infarction, heart failure and Parkinson disease.[1] This is due to their ability to react with electron donors thereby damaging proteins, lipids and DNA.[2] Antioxidants are increasingly being recommended because they act directly on oxidative processes and may be a method to prevent diseases and health problems related to aging. Thus, there is a constant search for antioxidant natural resources. Infectious diseases are disorders caused by pathogenic microorganisms like bacteria, viruses, fungi, protozoa and multi-cellular parasites. Bacterial infections are one of the prominent causes of health problems, physical disabilities and mortalities around the world.[3]

Symptoms and complications associated with bacterial infections such as fever, chills, headache, nausea, vomiting and organ failures affect patient's life severely. Many of infectious microorganisms are resistant to synthetic drugs; hence an alternative therapy is very much needed.[4] Antibiotic resistance has become a global concern in recent years. This problem is of great significance especially in developing countries because infectious diseases are one of the major causes of mortality in these countries.[5]

Euphorbia hirta, an important medicinal herb, belongs to genus *Euphorbia*, family *Euphorbiaceae*. It is a very common herb in the pan-tropic and partly sub-tropic areas worldwide, including China, India, Philippines, Australia, Africa, Malaysia, and so on. The leaves have been found to possess some phytochemicals which confers a lot of pharmacological importance on the plant. This study is therefore aimed at evaluating the antioxidant and antimicrobial effects of the ethanolic extracts of the leaves of *Euphorbia hirta*.

MATERIALS AND METHODS

Plant Materials

The *Euphorbia hirta* leaves were collected from Ekiti State University campus, Ekiti state, Nigeria. The samples were identified at the Herbarium section of Plant Science Department,

Ekiti State University, Ado-Ekiti, Nigeria. A voucher specimen was deposited in the Departmental Herbarium. The leaves were air-dried at room temperature; pulverized into fine powder using an electric blender.

Chemicals

Chemicals and reagents used are 1,10-phenanthroline, Gallic acid, Folin- Ciocalteu reagent, 2,2 diphenyl-2-picrylhydrazyl (DPPH),Trichloroacetic acid (TCA), Hydrogen peroxide, Methanol, $FeCl_3$, Sodium carbonate, $AlCl_3$, Tris-HCl buffer, $FeSO_4$,NaOH. $NaNO_3$, Sodium phosphate buffer, KFC, Sodium nitroprusside, Phosphate saline, Greiss reagent, Deoxyribose, Phosphate buffer, TBA, Sodium azide, H_2HPO_4, DNTB, Carbonate buffer, Adrenaline, $NaPO_4$, EDTA were products of Randox Laboratories Ltd., United Kingdom. All other solvents used were of analytical grade.

Preparation of the extract

Fifty grams (50g) of each powdered form of the plants was macerated in 250ml of ethanol (70%) for 48hours. It was filtered using a Buchner funnel with Whatman's No 1 filter paper and the filtrate concentrated to dryness under vacuum with rotary evaporator to concentrate at 90^{o}c. After concentration, the extract was dispensed into an air tight bottle.

Determination of Ferric Reducing Property of the Extracts

The reducing property of the extracts was determined by.[6] 0.25ml of the extracts was missed with 0.25ml of 200mml of sodium phosphate buffer pH 6.6 and 0.25ml of 1 % potassium ferro cyanide (KFC). The mixture was incubated at 50^{0}c for 20mins, there after 0.25ml of 10% trichloro acetate (TCA) was also added and centrifuge at 2000 rpm for 10mins, 1ml of the supernatants were mixed with 1ml of distilled water and 0.1% iron (iii) chloride ($FeCl_3$) and the absorbance was measured at 700nm.

DPPH Scavenging Assay of the Extracts

The free radical scavenging ability of the extracts against 1,1-diphenyl-2-picryhydrazyl (DPPH) using.[7] method. 1ml of the extracts were mixed with 1ml of 0.4mm methanolic solution of the DPPH, the mixture was left in the dark for 30mins before measuring the absorbance at 570nm. and the scavenging ability of the extract was calculated as.

DPPH radical scavenging activity (%) = [(Abs control – Abs sample)]/ (Abs control) ×100

Where Abs control is the absorbance of DPPH radicals + methanol; Abs sample is the absorbance of DPPH radical + extract or standard

Hydrogen peroxide scavenging assay

The modified method of [8] was employed for the hydrogen peroxide scavenging assays of the extracts. The stock solution contained 4mM hydrogen peroxide prepared in 0.1M phosphate buffer (pH 7.4). A volume of 0.6 ml of the solution was added to 2 ml of the extract and standard (200 – 1000 µg/ml) and incubated for 15 min at room temperature. The absorbance was read at 230 nm and the percentage inhibition of hydrogen peroxide was calculated as: H2O2 scavenging activity (%) = [(Abs control – Abs sample)] / (Abs control) ×100 Where Abs control is the absorbance of H2O2 radicals; Abs sample is the absorbance of H2O2 radical + extract or standard.

Determination of Nitric oxide (NO) Radical Scavenging Ability of the Extracts

Sodium Nitropruside in an aqueous solution at physiological pH spontaneously generates No, which interacts with oxygen to produce nitrite ions that can be estimated by the use of Greiss reagent. Scavenging of NO compete with oxygen, reading to reduce production of NO. Briefly 5mm sodium nitropruside in phosphate saline were mixed with the extracts before incubation at 25^0c for 15mins. Thereafter, the reaction mixture was added to Greiss reagent. Before measuring the absorbance at 546nm, relative to the absorbance of standard solution of potassium nitrate treated in the same way with Greiss reagent.[9] The percentage nitric oxide inhibition by the extracts was calculated using the following equation.

NO scavenging activity (%) = [(Abs control – Abs sample)]/ (Abs control) ×100 Where Abs control is the absorbance of NO radicals; Abs sample is the absorbance of NO radical + extract or standard.

Superoxide Anion Scavenging Activity Assay of the Extracts

The superoxide anion radicals were produced in 2ml of phosphate buffer (100mm), pH 7.4 with78µm β-nicotinamide adenosine dinucleotide (NADH) 50µm nitro blue tetrazolium chloride (NBT) and test samples at different concentrations. The reaction mixture was kept for incubation at room temperature for 15mins. It was then added with 5-methyl phenazinium methosulphate (PMS) (10µm) to initiate the reaction and incubated for 5mins at room temperature. The colour reaction between superoxide anion radical and NBT was read at 560nm. Gallic acid was used as a positive control agent for comparative analysis. The reaction mixture without sample is used as control and without PMS is used as blank.[10] The percentage inhibition of superoxide anion radical was calculated as.

Superoxide anion radical scavenging activity (%) = [(Abs control – Abs sample)] / (Abs control) ×100

Where Abs control is the absorbance of superoxide anion radicals; Abs sample is the absorbance of superoxide anion radical + extract or standard.

Hydroxyl (OH) Radical Scavenging Ability of the Extracts

The ability of the extracts to prevent fe^{2+}/ H_2O_2 induced decomposition of deoxyribose will be carried out using the method of.[11] Freshly prepared extracts (0-100µl) added to a reaction mixture containing 120µl, 20mml deoxribose, 400µl, 500µm, $FeSO_4$ and volume were been made to 800µl with distilled water. The reaction mixture was incubated at 37°c for 30min and the reaction was stop by the addition of 0.5ml of 2.8% trichloro acetate (TCA) this was followed by the addition of 0.4ml of 0.6% thiobabithoric acid solution (TBA). The tubes were subsequently incubated in boiling water for 20mins. The absorbance was measured at 523nm in spectrophotometer.

Determination of Total Phenol in the Extracts

The method of Wolfe *et al*,[12] was used to determine the total phenolic content. The reaction mixture contained 2.5 ml of 10 % (v/v) Folin- Ciocalteu reagent, 2 ml of 7.5 % (w/v) of sodium carbonate and 0.5 ml (1 mg/ml) of the extract. The mixture was mixed together and incubated at 40oC for 30 min after which the absorbance was measured at 765nm. Gallic acid was used as standard. The total phenolic content was calculated from the equation obtained from the calibration curve of gallic acid and expressed as mg/g of dry extract.

Determination of antimicrobial activities

The extract was subjected to antimicrobial assay using the agar well diffusion method of.[13] 20ml of nutrient agar (NA) was dispensed into sterile universal bottles, and then inoculated with 0.2ml of 24hrs old culture of the test organisms, mixed gently and poured into sterile petri dishes. After setting, a number of 9cups borers (6mm diameter) was properly sterilized by autoclaving and used to make 3 or 5 uniform cups/wells in each petri-dish. A drop of molten agar was used to seal the base of each cup. The cups/wells were filled with the extract solution and allow diffusing for 45minute. The plates were incubated at 37°c for 24hrs. The zones of inhibition were measured with digital venial caliper in mm and the experiment was carried out in duplicates.

2.4.2 Determination of Minimum Inhibitory Concentration (MIC)

Different concentration of the extract was prepared (0.2mg, 0.4mg, 0.6mg 0.8mg and 1mg/ml) and subjected to antimicrobial assay as describe above using the agar well diffusion method.[13] The MIC was determined as the least concentration of the extract that exhibit bactericidal action by the zone of inhibition around the well. The result was recorded appropriate. MIC of Euphobia hirta is 0.8mg/ml.

Statistical analysis

All results were expressed as mean ± standard deviation (SD) of three replicates and were subjected to analysis of variance (ANOVA). Significant levels were tested at $P < 0.05$.

RESULTS

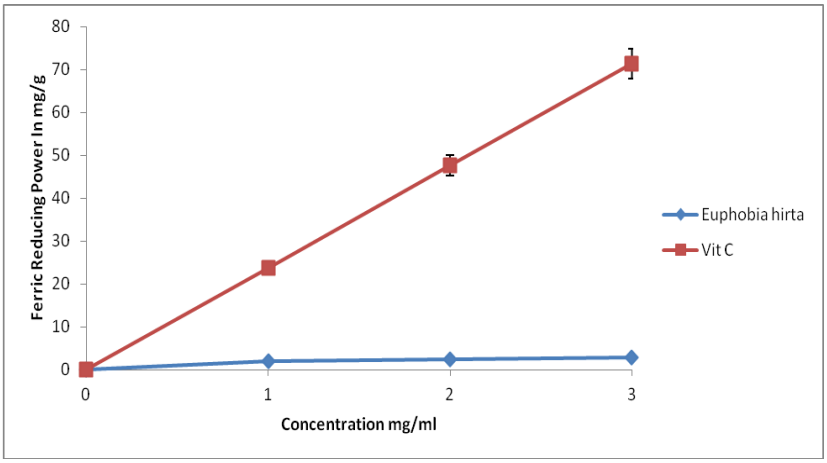

Figure 1: **Ferric reducing ability of *Euphorbia hirta* leaves. The results are means ± SD (n=3).**

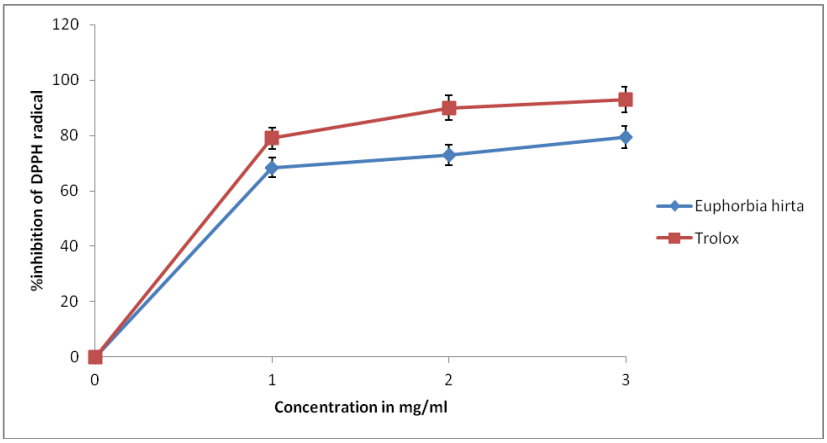

Figure 2: **Scavenging effects of *Euphorbia hirta* leaves on DPPH radical. The results are means ± SD (n=3).**

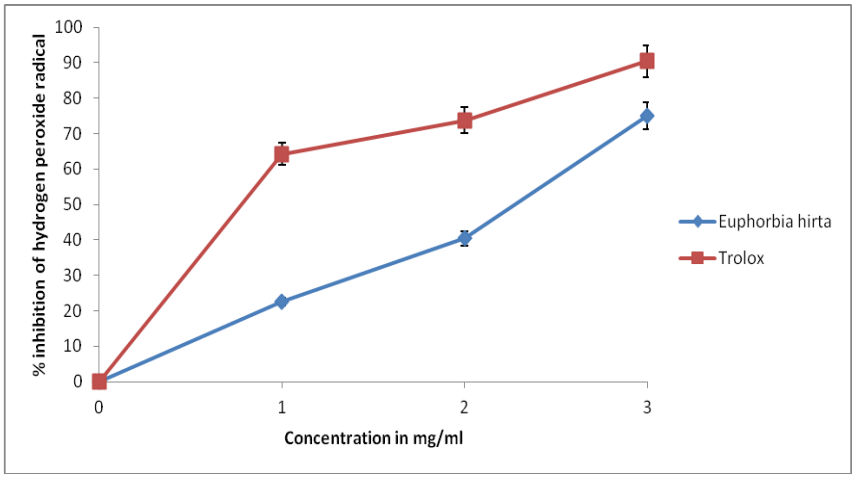

Figure 3: Scavenging effects of *Euphorbia hirta* leaves on Hyrodgen peroxide radical. The results are means ± SD (n=3).

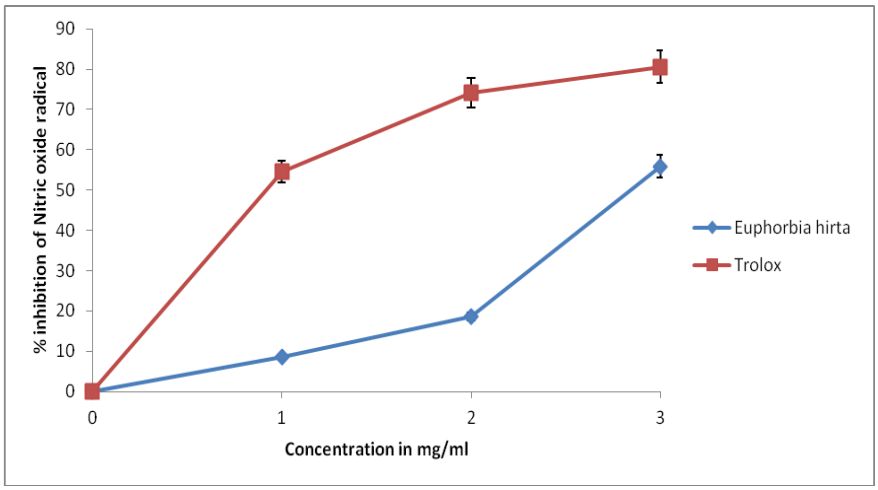

Figure 4: Scavenging effects of *Euphorbia hirta* leaves on nitric oxide radical. The results are means ± SD (n=3).

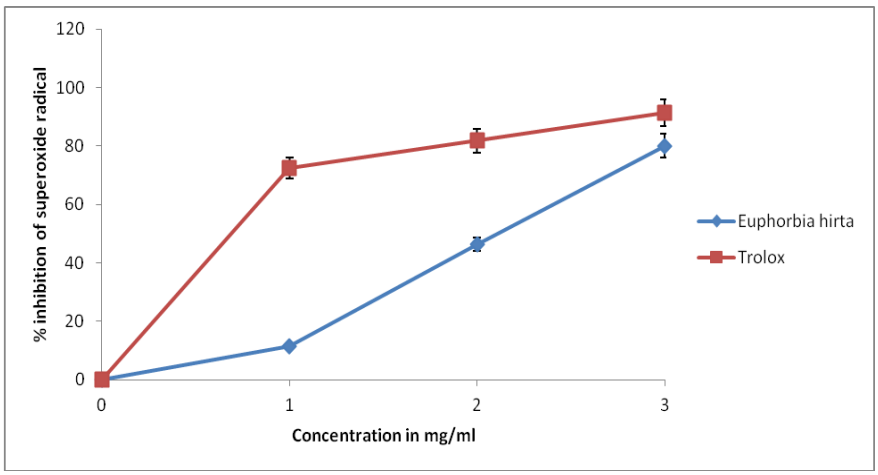

Figure 5: Scavenging effects of *Euphorbia hirta* leaves on superoxide radical. The results are means ± SD (n=3).

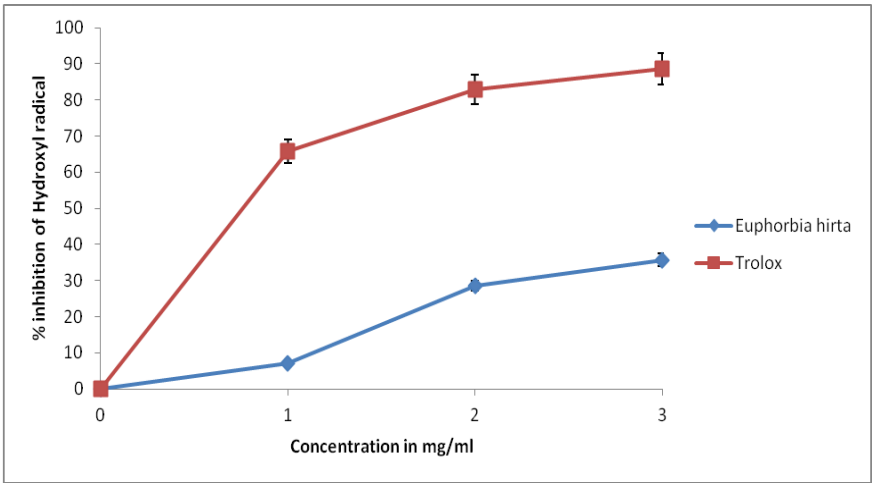

Figure 6: **Scavenging effects of *Euphorbia hirta* leaves on hydroxyl radical. The results are means ± SD (n=3).**

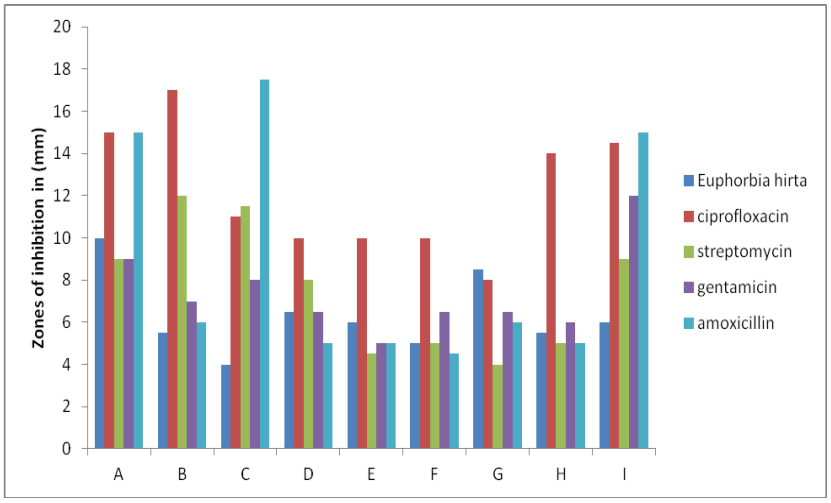

Figure 7: **Comparative effect of *Euphorbia hirta* leaves and some standard antibiotics against nine selected microorganisms.**

A-*Bacillus subtilis*, B-*Pseudomonas aeruginosa*, C-*Staphylococcus aureus*, D-*Enterococcus feacalis*, E-*Escherichia coli*, F-*Klebsilla pneumonia*, G-*Proteus maribalis*, H-*Salmonella spp, and I-Shigella dysenteri*.

DISCUSSION

Free radicals are highly reactive oxygen species produced through oxidative process within the mammalian body. They are chemically unstable atoms or molecules that cause extensive damage to cells as a result of imbalance between the generation of reactive oxygen species (ROS) and the antioxidant enzymes.[14] In normal conditions, the human body possesses many defence mechanisms against oxidative stress, including antioxidant enzymes and non-enzymatic compounds but under some circumstances including exposure to some

environmental pollutants, e.g., cigarette smoke, pesticides, smog, UV radiation, etc. the natural antioxidant mammalian mechanism become insufficient and then the excess of free radicals can damage both the structure and function of a cell membrane in a chain reaction leading to many degenerative diseases.[15] Many antioxidant based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer.[16] The role of free radical reaction in human disease, biology, toxicology, and the deterioration of food has become an area of intense research interest. Recently, interest has increased considerably in finding naturally occurring antioxidants for use in foods, cosmetics or medicinal materials to replace synthetic antioxidants, which are being restricted due to their carcinogenicity.[17] The antioxidant effect of *Euphorbia hirta* as evaluated by its ferric reducing ability and its scavenging effects on some free radicals such as; 2,2 diphenyl-2-picrylhydrazyl (DPPH), hydrogen peroxide (H_2O_2), hydroxyl radical, superoxide radical, and nitric oxide (NO) radicals shows that the leaves of *Euphorbia hirta* has a reasonable scavenging effects on all the radicals tested. It can be clearly seen that the scavenging activity of *Euphorbia hirta* extracts were concentration-dependent. The scavenging activity of the extracts increased with increase in the concentration of the extract. The extract of *Euphorbia hirta* possess efficient scavenging character when compared with the standards and the study reveals that the extract of *Euphorbia hirta* exhibits the proton- donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants.[18]

In present time, multiple drug resistance in microbial pathogens become a serious health problem to humankind world-wide.[19] It is aroused due to indiscriminate and repetitive use of antimicrobial drugs.[20] Synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often associated with adulterations and side effects. Therefore, there is need to search new infection fighting strategies to control microbial infections. Due to the same reason, during the past decade, traditional systems of medicines have become increasingly important in view of their safety.[21] The ethanol extracts of the leaves of *Euphorbia hirta* was subjected to a preliminary screening for antimicrobial activity against nine selected microorganisms (Bacillus *subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and *Salmonella spp, Enterococcus feacalis, Klebsilla pneumonia, Proteus maribalis, Shigella dysenteri).* This study clearly shows that the ethanolic extracts of the leaves of *Euphorbia hirta* exhibited a pronounced antimicrobial activity against the tested microorganisms. Comparing with some standard antibiotics shows that the extract was more

potent than some standard antibiotics against some organisms. The effects were more pronounced against *Proteus maribalis, Escherichia coli* and *Bacillus subtilis*.

CONCLUSION

The results in this investigation have shown that leaves of *Euphorbia hirta* may be a good source of natural antioxidants and antimicrobial agent. This preliminary work encourages continuous investigation of more potential antioxidant and antimicrobials and elucidate their mechanism of action.

REFERENCES

1. Flora, S. J. Role of free radicals and antioxidants in health and disease. Cell Mol. Biol, 2007; 53: 1-2.
2. Ahmed, M., Khan, M.I., Khan, M.R., Muhammad, N., Khan, A.U. Role of Medicinal Plants in Oxidative Stress and Cancer. Open Access Scientific Reports, 2013; 2(2): 641-643
3. Khosravi, A and Behzadi, A. Evaluation of the antibacterial activity of the seed hull of *Quercus barantii* on some gram-negative bacteria. Pak. J. Med. Sci, 2006; 22: 429-432.
4. Al-Bari, M.A.A., Sayeed, M.A., Rahman, M.S. Characterization and antimicrobial activities of extracts in a phenolic acid derivative produced by *Streptomyces bangladeshiensis*, a novel species collected in Bangladesh. J. Res. Med. Sci, 2006; 1: 77-81.
5. Solanki, R. Some medicinal plants with antibacterial activity. International journal of comprehensive pharmacy, 2010; 4(10): 1-4.
6. Warokar AS, Ghante MH, Duragkar NJ, Bhusari KP. Anti-inflammatory and antioxidant activities of methanolic extract of Buchanania lanzan Kernel. Indian J Pharm Educ Res 2010; 44(4): 363-368
7. Gyamfi, M.A., Yonamine, M. and Aaniya, Y. Free radical scavenging action of medicinal herbs from Ghana: thonningia sanguine on experimentally induced liver injuries. General Pharmacology., 1999; 32: 661 – 667.
8. Hazra B, Biswas S, Mandal N. Antioxidant and free radical scavenging activity of Spondias pinnata. BMC Complement & Alt Med, 2008; 8: 63-75.
9. Bao J. Y., Cai M., Sun G., Wang and H. Corke. Anthocyanins, Flavonoid and Free Radical Scavenging Activity of thines Baybery (Myrial rubia) extracts and their colour properties and stability. Journal of Agric Food Chem, 2005; 53: 2327-2332.

10. Selvakumar K. Madhan R., Srinivasan G. and Baskar V. Antioxidant Assays in Pharmacological Research. Asian J. Pharm. Tech., 2011; 1(4): 99-103
11. Halliwell B, Gutteridge J.M.C. Free radicals in biology and medicine. New York: Oxford University Press, 1999: 936.
12. Wolfe K, Wu X, Liu RH. Antioxidant activity of apple peels. J. Agric. Food Chem, 2003; 51: 609-614.
13. Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA . 9th edition. ASM Press, Washington DC. Man. Clin. Microbiol, 2007; 2260.
14. Lobo, V., Patil, A., Phatak, A., and Chandra, N. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogn Rev*, 2010; 4(8): 118–126.
15. Atta-ur-Rahman, Choudhary, M.I. Bioactive natural products as potential source of new pharmacophores. A theory of memory. Pure Appl. Chem, 2001; 73: 555–560.
16. Mosquera, O.M., Correa, Y.M., Buitrago, D.C., Niö, J. Antioxidant activity of twenty five plants from Colombian biodiversity. Mem. Inst. Oswaldo Cruz, 2007; 102: 631–634.
17. Sasaki, Y.F., Kawaguchi, S., Kamaya, A., Ohshita, M., Kabasawa, K., Iwama, K. The comet assay with 8 mouse organs: Results with 39 currently used food additives. Mutat. Res. Genet. Toxicol. Environ. Mutagen, 2002; 519: 103–109.
18. Pracheta, Sharma V, Paliwal R, Sharma S. Preliminary phytochemical screening and *in vitro* antioxidant potential of Hydro-Ethanolic extract of *Euphorbia neriifolia* Linn. International Journal of PharmTech Research, 2011; 3(1): 124-132.
19. Peng Y, Rakowskim S.A, Filutowiez M. Small deletion variants of the replication protein Pi and their potential for over- replication-based antimicrobial activity. FEBS Microbiol Lett, 2006; 261(2): 245-252.
20. Shariff, ZU. Modern Herbal Therapy for Common Ailments. Nature Pharmacy, 2001; 1: 9684.
21. Krishnaraju AV, Rao TVN, Sundararaju D, Vanisree M, Tsay HS, Subbaraju G. Biological screening of medicinal plants collected from Eastern Ghats of India using *Artemia salina* (brine shrimp test). Int. J. Appl. Sci. Eng, 2006; 4: 115-125.