ISSN 2395-3411 Available online at www.ijpacr.com 541

Research Article

Screening of Antioxidant Property of Methanolic Extract of *Euphorbia neriifolia* Latex

Chinmayi Upadhyaya*, Satish S and Karunakar Hedge

Srinivas college of Pharmacy, Valachill, Mangalore, Karnataka, India.

ABSTRACT

Antioxidants are the substances that removes potentially damaging oxidizing agents in a living organism and prevents the oxidative damage to the cells. The excess production of free radicals in cells plays a role in the pathophysiology of many disease conditions including cancer, atherosclerosis, cognitive impairment, immune dysfunction, Cataracts, muscular degeneration, alzheimer's, etc. The aim of the present study was to evaluate the antioxidant property of methanolic extract of *Euphorbia neriifolia* latex. The evaluation of the antioxidant property was done using four methods: DPPH radical reducing activity and inhibition of superoxide free radical by Riboflavin. The two antioxidant assays were carried out even with the standard antioxidant drug, Vitamin E. The results (IC_{50} values) obtained from the antioxidant assays of the standard were compared with the results obtained from the crude drug extract and the latex extract was found to have nearby values. The present study concluded that the methanolic extract of latex of *Euphorbia neriifolia* has significant antioxidant potential.

Keywords: *Euphorbia neriifolia*, antioxidant, free radical, Vitamin E.

INTRODUCTION

Human body, which is made up of approximately 100 trillion cells, form tissues, organs, organ system and the human body. Hence, damaged cells will lead to a damaged system and pay way for diseased conditions. Free radicals are something that attack and damage the cells. Damaged cells are made to oxidize and hence the antioxidants are required as many of the presently antioxidants have number of minor side effects when used for a long time.

Antioxidants are substances that inhibit the oxidation of other molecules and prevent or delay some types of cell damage[1]. Many advances have led to the development of several drugs, based primarily on antioxidant mechanism for the treatment of various diseases. Some with side effects[2]. The search and development of effective and multiple mechanism based therapeutics from traditional The latex of the plant *Euphorbia neriifolia* was collected from Panjya, Mangalore in February 2016. Immediately after the collection, toluene was added as it acts as a preservative and was stored in ambert color glass bottles. The extraction was carried out using methanol as a solvent in the magnetic stirrer. After extraction medicinal practices are the requirements of the day. One such plant which can be studied is *Euphorbia neriifolia.*

Euphorbia neriifolia is a spiny plant belonging to the family, Euphorbiaceae. It is proved to be potent analgesic, anti-inflammatory, mild CNS depressant, wound healing activity along with humoral and cell mediated immunostimulating activity. The leaves are used to treat asthma; wheezing in babies; colds; and stomach upset[3]. Antibacterial effect has been found in this plant[4]. *E. neriifolia* leaf extract was found to be mild depressant on central nervous system at higher doses and has anti-anxiety activity. It is also used against renal cancer[3].

EXPERIMENTAL MATERIALS AND METHODS

PLANT MATERIAL

the product was centrifuged, filtered and dried at room temperature.

THIN LAYER CHROMATOGRAPHY[5]

Solvent system used: - Toluene: Ethyl acetate: Formic acid (7:3:0.3).

Stock solution of extract: Stock solution of methanolic extract was prepared by dissolving 10mg of extract in 1ml of methanol. Solvent system was allowed to move through the stationary face which was spotted with the sample and the R_f values were calculated using the formula:

R_f value
= Distance moved by the solute front
Distance moved by the solvent front.

The bands were observed under short wave UV and the plate was tested with different spraying reagents to test for alkaloids, terpenoids, flavonoids, amines and phenols.

DPPH RADICAL REDUCING ACTIVITY[6]

DPPH radical reducing method is a rapid and simple method when compared to other invitro antioxidant assays. It involves the use of free radical, DPPH (2,2- Diphenyl-1-Picryl Hydroxyl). It involves the determination of the scavenging effect of DPPH, which is soluble in methanol. DPPH, being an artificial free radical compound, has been used widely to determine free radical scavenging ability of various samples invitro. The odd electron in the DPPH free radical gives a strong absorption maximum at 517nm and is purple in color. The color turns from purple to yellow when DPPH radical becomes paired with hydrogen from an antioxidant (which is a free radical scavenger) to form the reduced DPPH-H. The resulting decolorisation is stoichiometric with respect to the number of electron captured.
The stock solution of 20mg/ml was prepared. Different concentrations of the drug were taken in different test tubes. The Vehicle was added to one more test tube. The test tubes were added with Methanol as to makeup to a625µl. 375µl of DPPH was added in the dark. Incubated in dark for 20min. Drug blank was taken for all the concentrations as the solution appeared turbid. The absorbance was read at 515nm against Methanol as a blank. The concentration of extract to scavenge 50% of the DPPH radical (IC_{50} value) was calculated using the formula,
Percentage inhibition of the extract

$$= \frac{\text{OD of control} - \text{OD of sample}}{\text{OD of Control}} \times 100$$

The same procedure was carried out for 2mg/ml Vitamin E (standard = 2mg Vitamin E in 1ml water) without any drug blanks.

INHIBITION OF SUPEROXIDE RADICAL BY RIBOFLAVIN[7]

The superoxide radical scavenging activity was determined by Nitro Blue Tetrazolium (NBT) reduction method. The assay is based on the ability of the drug to inhibit the reduction of NBT by superoxide which is generated by the reaction of photoreduction of Riboflavin (Vitamin B_2) within the system. The superoxide thus generated reduces to NBT to a blue colored complex.
Stock solutions of the drug were prepared with 20mg/ml for Methanolic extract (20mg in 1ml of Methanol). All the reagents were prepared. Different concentrations of the drug were added to different test tubes and are made up to 2650µl with Phosphate buffer. 200 µl EDTA was also added. 100µl NBT and 50µl Riboflavin was added in dark, since both are light sensitive. The tubes were illuminated under incandescent light for 20-30min. The optical density at 560nm was measured before and after the illumination. Various concentrations of Vitamin E was taken from 20mg/ml stock and followed with same procedure. The ability to scavenge superoxide radical was calculated by comparing the absorbance of control with that of the extract. Concentration of the extract required to scavenge 50%superoxide anion was calculated using the formula,

Percentage inhibition of extract

$$= \frac{\text{OD of control} - \text{OD of sample}}{\text{OD of control}} \times 100$$

RESULTS AND DISCUSSION

THIN LAYER CHROMATOGRAPHY

The TLC showed the presence of 10 different bands when viewed under short wave UV.

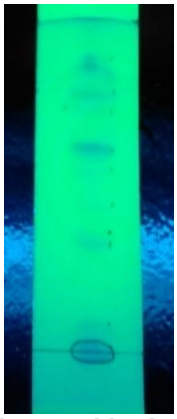


Fig. 1: Bands observed in the TLC of extract

This signifies the presence of 10 different Components which can give colored bands in short wave UV. Flavonoids were identified as the major component when performed TLC with spraying reagents.

DPPH RADICAL REDUCING ACTIVITY:

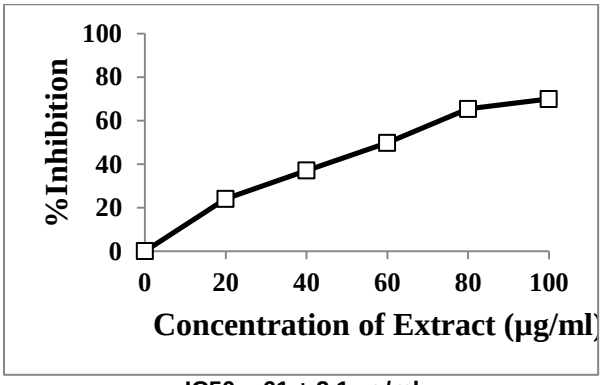
The results of In vitro antioxidant assays are as shown in the Table no1 and 2 for Methanolic extract and Standard drug (Cyclophosphamide), respectively. The percentage inhibition of the methanolic extract and Standard with increasing concentrations is depicted graphically in the graph no 1 and 2.

Table 1: DPPH radical reducing activity of Methanolic extract of *E. neriifolia*

Conc.Of drug (µg/ml)	50	100	150	200	250	Vehicle
% inhibition of Methanolic extract(%)	25± 0.11	380± 0.243	49.1±0. 632	66.9±0. 425	69.6±0. 121	1.34±0.243

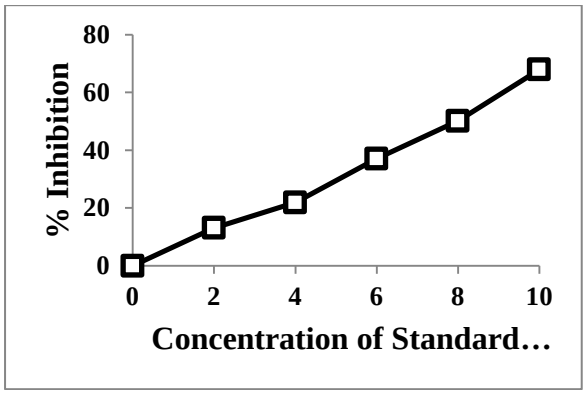
Table 2: DPPH radical reducing activity of Vitamin E

Conc.of the Standard drug (µg/ml)	2	4	6	8	10
%inhibition by standard drug (%)	13.1±0. 454	21.9±0. 333	37.1±0. 332	50.1±0. 534	67.9±0. 122



IC50 = 61 ± 2.1 µg/ml

Graph 1: DPPH radical reducing activity of Methanolic extract of *E. neriifolia.*



IC_{50} = 8.3 ±1.2 µg/ml

Graph 2: DPPH radical reducing activity of Standard drug

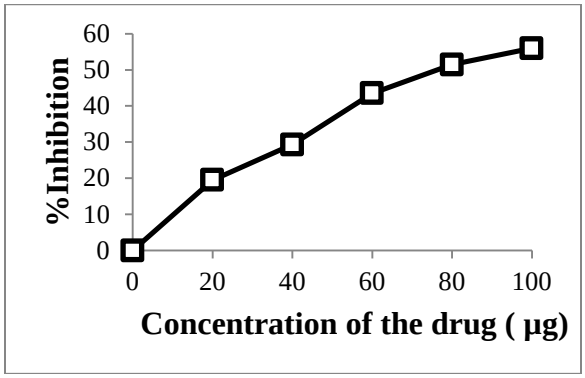
INHIBITION OF SUPEROXIDE RADICAL BY RIBOFLAVIN

Table 3: Superoxide radical scavenging activity of Methanolic extract of *E. neriifolia*:

Conc.of the drug (µg/ml)	20	40	60	80	100	Vehicle
%inhibition of Methanolic extract (%)	19.5±0.33	29.3±0.154	43.5±0.8	51.45±0.12	55.9±0.116	1.85±0.555

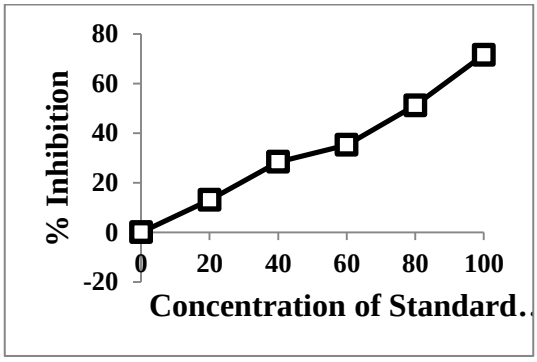
Table 4: Superoxide radical scavenging activity of Standard drug

Conc. the Standard drug (µg/ml)	20	40	60	80	100
%inhibition by standard drug (%)	13±0.215	28.35±0.332	35.2±0.165	51.1±0.232	71.5±0.323



IC_{50} = 78 ±1.06 µg/ml

Graph 3: superoxide radical scavenging activity of Methanolic extract of *E. neriifolia.*



IC_{50} = 79 ±0.46µg/ml

Graph 4: superoxide radical scavenging activity of Vitamin E.

Oxidative stress in large quantities of reactive oxygen species (ROS) are generated is one of the earliest responses to stress[8]. The antioxidant system protects the pathogens against the ROS induced oxidative damage. DPPH radical scavenging activity and Superoxide radical scavenging activity are most widely used methods for screening the antioxidant activity of plant extract. The methanolic extract showed good scavenging property with the highest dose showing maximum scavenging activity. There was a dose dependent free radical scavenging property. Thus the plant can be a potential source for antioxidant property. The presence of flavonoids may be the reason for antioxidant activity. Thus, the present investigation would be useful to treat and prevent the free radical damages occurring in human worldwide.

In future, it is worthy to identify, isolate the exact component that shows antioxidant activity and go for the treatment. Going for in vivo antioxidant assay is also recommended for the perfect investigation.

REFERENCES

1. I S Young, J V Woodside. Antioxidants in health and disease. J of clinical pathology. 2001;54 :176–186.
2. N W Booker, Z Diana. Antioxidants and cancer risk: the good, the bad, and the unknown. Cancer prevention and treatment fund. 2015.
3. P. Bigoniya, A.C. Rana. Subacute Effect of Euphorbia neriifolia Linn. On Hematological, Biochemical and Antioxidant Enzyme Parameters of Rat. Academic Journal of Plant Sciences. 2009; 2 (4): 252-259.
4. K swamy M, N Pokharen, S Dahal and Anuradha M. Phytochemical and antimicrobial studies of leaf extract of Euphorbia neriifolia. Journal of Medicinal Plants Research. 2011; 5(24): 5785-88.
5. Sibylle Z, Jiang H L, Hermann S, Rudolf B. Comparative study of roots of *Angelica sinensis* and related umbelliferous drugs by thin layer chromatography, high-performance liquid chromatography, and liquid chromatography – mass spectrometry. J of phyto chemical analysis.1998:1099-1155.
6. K S Rajani, K Manoranjan, R Rasmirani.DPPH Free Radical Scavenging Activity of Some Leafy Vegetables used by Tribals of Odisha, India. Journal of Medicinal Plants Studies. 2013; 1(4):21-27.
7. G C Sindhu. Free Radical Scavenging Activity Of *Aesculus Indica* Leaves. International J of PharmTech Research. 2009; 1(3):524-526.
8. S Pallavi, A J Bhushan, M Pesserkli, etal. Review Article on Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions. Journal of Botany. 2012: 1-27.