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IN VITRO ANTI OXIDANT POTENTIAL SCREENING OF BERRY EXTRACTS OF *DIOSPYROS VIRGINIANA* LINN USING DPPH FREE RADICAL MODEL

S. N. V. L. Sirisha[1]*, K. Prabhu[2] and A. Sambasiva Rao[3]

[1]JNTU Institute of Science and Technology, Centre for Pharmaceutical Sciences, Hyderabad.
[2]Vivekananda School of Pharmacy. Batasingaram, Hayath nagar R.R District -501505.
[3]Sri Indu Institute of Pharmacy, Sheriguda, Ibrahimpatnam, R.R District -500030.

***Corresponding Author: S. N. V. L. Sirisha**
JNTU Institute of Science and Technology, Centre for Pharmaceutical Sciences, Hyderabad.

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ABSTRACT
Free radicals are toxic by products of natural cell metabolism and are responsible for causing a wide number of health problems. In this study petroleum ether extract, chloroform, Ethanolic berry extracts of Diospyros Virginiana Linn In vitro by experimental parameters such as DPPH free radical scavenging action, Diospyros Virginiana Linn is used as an antioxidant, antidiabetics, hepatoprotective, cytotoxic activity, genetoxicity, antitumour activity, antilice agent. It is related to contain flavonoids, carbohydrates, fixed oils, tannins & phenolics.

KEYWORDS: *Diospyros Virginiana, Antioxidant activity, DPPH free radical model.*

1. INTRODUCTION
According to the WHO survey 80% populations living in the developing countries rely almost exclusively on traditional medicine for their primary health care needs.[1] The use and search of drugs and dietary supplements from plants have been intensified in recent years.[2] Medicinal plants are a source of great economic value in the Indian sub continent. For most of the disease, plant materials are used as drugs because of its active compounds. In many disorders the free radical mediated damage may play an important role. Free radicals are responsible for causing a wide number of health problems which include cancer, aging, heart diseases and gastric problems etc. Antioxidants cause protective effect by neutralizing free radicals, which are toxic by products of natural cell metabolism. The human body naturally produces antioxidants but the process is not 100 percent effective in case of overwhelming production of free radicals and that effectiveness also declines with age.[3] Antioxidant compounds can decrease oxidative stress and minimize the incidence of these diseases. The mechanism of the action of these antioxidant compounds include suppression of reactive oxygen species formation either by inhibition of the enzymes or by chelating of trace elements involved in free radical production, scavenging of reactive species and up- regulating or protecting antioxidant defense.[4]

Diospyros Virginiana L. is referred as Divine fruit belongs to Ebenaceae family having a smooth skin fruit that becomes dull red when ripe. Less volatile substances such as alkaloids, diterpenoids, and acetogenins[5] have been identified so far from oil of various parts of the plant. However, the antioxidant potential of *Diospyros Virginiana* leaf is not yet been explored properly. With this in mind, the present work was an attempt to perform anti oxidant studies on the berry extracts of *Diospyros Virginiana* Linn.

2. MATERIALS AND METHODS
2.1 Collection of plant material
D.Virginana Linn berries were collected in the months of October 2016 from the market of Missouri and were authenticated by Prof D. Ramakanth Raju retired botanist Acharya Nagarjuna University, a voucher specimen (Snvl/jntu/2017-05) has been deposited in the Viswabharati college of pharmacy, Guntur, A.P.

2.2 Preparation of plant extracts: Obtained plant material has been dried under shade and made into coarse powder passed though sieve# 20 and has been successively soxhelated using solvents like petroleum ether, chloroform, and ethanol for 72 hours. Obtained extracts were made solvent free using rota evaporator and stored in vacuum desiccators. Yield was found to be 0.4%, 2.5% and 3.72% respectively. Obtained extracts were tested for preliminary phytochemical screening.[6]

2.3 DPPH Radical Scavenging Activity
The free radical scavenging activity of the extracts, based on the scavenging activity of the stable 1,1 diphenyl-2-picrylhydrazyl (DPPH) free radical, was determined by

the method.[6] To a methanolic solution of DPPH(200µ M), 0.05ml of test compounds dissolved in ethanol were added at different concentration (100-500µg/ml). an equal amount of ethanol was added to the control after 20 min the decrease in the absorbance of test mixture (due to quenching of DPPH free radicals) was read at 517 nm and the percentage inhibition calculated by using the formula.[7]

% Inhibition = [(A0–A1)/A0] X 100,

Whereas A_0 is the absorbance of the control, and A_1 is the absorbance of the test/ standard. ascorbic acid was used as standard.

2.4 Statistical analysis

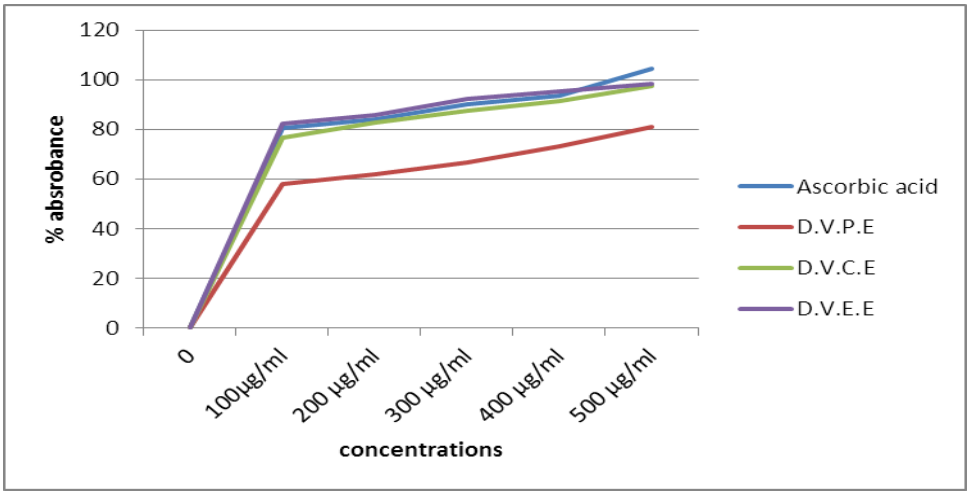
All the experiments were performed in triplicate and results were averaged. Linear regression was used to calculate the IC_{50} values.[8]

In vitro Anti oxidant potential screening of berry extracts of *Diospyros virginiana* Linn using DPPH free radical model.

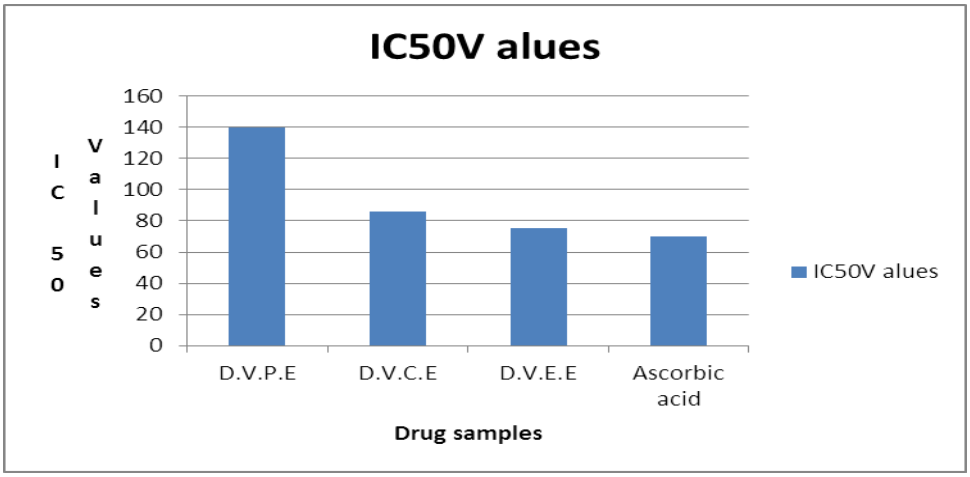
Table 1: Results of *Diospyros Virginiana* Linn Berry extracts in DPPH free radical scavenging.

S.no	Name of the extract	Concentrations					IC_{50}Values
		100 µg/ml	200 µg/ml	300 µg/ml	400 µg/ml	500 µg/ml	
1.	D.V.P.E	80.42	84.23	89.99	93.54	104.36	140
2.	D.V.C.E	57.86	61.88	66.46	72.99	80.91	86
3.	D.V.E.E	76.78	82.87	87.65	91.34	97.34	75
4.	Ascorbic acid	82.21	85.82	92.13	95.47	98.32	70

Values are mean of triplicate



Graph 1: Graphical representation of *Diospyros virginiana* Linn berry extracts in DPPH free radical Scavenging model.



Graph 2: Graphical representation of IC_{50} Values of *D.Virginiana* berry extracts and standard drug ascorbic acid.

3. RESULTS

Several concentrations ranging from 100-500µg/ml of the *petroleum ether, chloroform and ethanolic extracts of Diospyros Virginiana berries were* tested for their antioxidant activity in DPPH free radical scavenging *in vitro* models. It was observed that free radicals were scavenged by the test compound in a concentration dependent manner.

4. DISCUSSION

Oxidative stress has been implicated in the pathology of many diseases and condition including diabetes, cardiovascular diseases, inflammatory conditions, cancer and aging. DPPH is a stable free radical. The *in vitro* study carried out on this radical is based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH. This radical reacts with suitable reducing agents, the electrons become paired off and the solution loses color stochiometrically depending on the number of electrons taken up.[9] From the present results, it may be concluded that the extracts reduce the radical to the corresponding hydrazine when they react with the hydrogen donors in the antioxidant principles.

5. CONCLUSION

From the present research work we conclude that *Diospyros Virginiana* Linn ethanolic berry extract has got maximum antioxidant activity than compare to other extracts in a dose dependent manner. This may be attributed to the presence of acetogenins, phenols which probably play a role as an effective free radical scavenger.[10]

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