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IN VITRO ANTI OXIDANT ACTIVITY OF ETHANOLIC EXTRACT OF DIOSPYROS PANICULATA Dalz, BARK

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ABSTRACT

Diospyros Paniculata is a tree which belongs to the family Ebanacea which has been used as a folkforic medicine in ayurveda and siddha. It has been commonly used in the treatment of ulcer, burns, poisoning etc. Here the study was conducted to evaluate the In vitro Anti oxidant activity of bark extract of diospyros paniculata by DPPH, Hydroxyl radical scavenging methods. The effect of the extract is assessed by the ability to scavenge DPPH radicals and Hydroxyl radicals. The results obtained indicate that the Ethanolic extract of Diospyros Paniculata (EEDP) has significant In vitro Anti oxidant activity ($P<0.001$).The IC 50 value of EEDP by DPPH method was found to be 100µg/ml as compared to standard which is 70µg/ml and Percentage Inhibition of Hydroxyl radical at dose 1000µg/µl of EEDP is 38.07% as that of standard which is 75.44%.

KEYWORDS: Diospyros Paniculata, In vitro, Anti oxidant, DPPH, Hydroxyl radical scavenging.

INTRODUCTION

Oxygen is essential for the survival of all on this earth. During the process of oxygen utilization in normal physiological and metabolic processes approximately 5% of oxygen gets univalentely reduced to oxygen derived free radicals[1,2] like superoxide, hydrogen peroxide, hydroxyl and nitric oxide radicals. These substances are called as Reactive Oxygen Species [ROS]. These ROS play an important role in degenerative or pathological processes, such as aging, cancers, coronary heart diseases, Alzheimer's disease, neurodegenerative disorders, atherosclerosis, cataracts and inflammations.[3] Living organisms have antioxidant defense systems that protects against oxidative damage by removal or repair of damaged molecules.[4] The 'antioxidant' refers to the activity of numerous vitamins, minerals and phytochemicals which provide protection against the damage caused by ROS.[5] Antioxidants interfere with the oxidative processes by scavenging free radicals, chelating free catalytic metals and by acting as electron donors.[6] The natural antioxidant mechanisms maybe insufficient in variety of conditions and hence dietary intake of antioxidant compounds are important.[7]

Antioxidant agents of natural origin have attracted special interest because they can protect the human body from free radicals.[8] Plant based antioxidants are preferred to the synthetic ones because of their multiple mechanisms of actions and non-toxic nature. These facts have inspired widespread screening of plants for possible medicinal and antioxidant properties; the isolation and characterization of diverse phytochemicals and the utilization to antioxidants of natural origin to prevent the diseases.[9] Current study is performed to evaluate the in vitro anti oxidant activity of Diospyros Paniculata.

Diospyros paniculata is a moderate sized handsome tree attaining a height of 50 ft and a diameter of 1.25 m. The fruits are green and ovoid, about 1 in long. The wood is whitish grey, occasionally with narrow stripes of black. This plant does not yield black heartwood. Bark is soft and moderately heavy (wt. 46 lb /cu ft). Leaves of the tree are used as fish poison; dried and powdered fruits are applied to heal burns; Decoction of the fruit is used in gonorrhoea, biliousness and blood poisoning; powdered stem bark is used for rheumatism and ulcer.[10] The preliminary photochemical screening of diospyros paniculata indicate the presence of essential oil, saponins, terpenoids, flavanoids and alkaloids.[11,12]

MATERIALS AND METHODS

PLANT COLLECTION AND AUTHENTICATION

Diospyros Paniculata was collected from Gootrical Forest range, sabarimala, Pathanamthitta district, kerala in October 20, 2015 and it was authenticated by Mr. M V Krishna raj M.Sc., B.Ed., Ph.D Assistant Professor, Department of Botany, Baselius College Kottayam.

PREPERATION OF THE ETHANOLIC EXTRACT OF BARK OF DIOSPYROS PANICULATA

Fresh bark of Diospyros Paniculata was collected and washed thoroughly with distilled water and dried in open

air at shade. Later the dried bark were chopped into small pieces and the material were properly packed and kept in soxhlet extractor and is made to undergo successive soxhlet extraction using ethanol as solvent. After 48hrs the extract was collected and it is air dried to remove the solvent. The extract collected is properly packed and kept for further studies.

IN VITRO MODELS

DPPP FREE RADICAL SCAVENGING ACTIVITY[13]

The radical scavenging activity of different extracts was determined by using DPPH assay according to Chang et al 2001.The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517 nm

Ascorbic acid (10mg/ml DMSO) was used as reference.

Principle

1,1-diphenyl-2-picryl hydrazyl is a stable free radical with purple colour which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as,

DPPH + [H-A] →DPPH-H + (A)

Antioxidants react with DPPH and reduce it to DPPH-H and as consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

Reagent preperation

0.1mM DPPH solution was prepared by dissolving 4mg of DPPH in 100ml of ethanol.

Procedure

Different volumes of samples were made up to 40µl with DMSO and 2.96ml DPPH (0.1mM) solution was added. The reaction mixture incubated in dark condition at room temperature for 20 minutes. After 20 minutes, the absorbance of the mixture was read at 517nm.3ml of DPPH was taken as control.

CALCULATION

% inhibition = $\frac{control-test}{control} X100$

HYDROXYL RADICAL SCAVENGING ACTIVITY[14]

This assay is based on the qualification of the degradation product of 2 deoxy ribose by condensation with TBA (Elizabeth and Rao,1990). Hydroxyl radical was generated by the Fe^{3+} - ascorbate- EDTA $–H_2O_2$ system (The Fenton reaction). The reaction mixture contained in the final volume of 1 mL. 2 deoxy 2 ribose (2.8mM), FeCl3 (100µm), EDTA (100µm), H_2O_2 (1.0mM), ascorbic acid (100µm) in KH_2PO_4 - KOH buffer (20 mM pH 7.4) and various concentrations (125-2000µg/µl) of the test sample. After incubation for 1hour at 37°C, add 1ml of 2.8% TCA, then 1ml 1% aqueous TBA was added and the mixture was incubated at 90°C for 15 minutes to develop the colour. After cooling the absorbance was measured at 532nm against an appropriate blank solution.

CALCULATION

% inhibition = $\frac{control-test}{control} X100$

STATICAL ANALYSIS

The data where analysed statistically using one way ANOVA followed by Tukey-Kramer Multiple Comparison test. Values are expressed as mean ± SD, n=3 and P<0.001*** is considered as significant.

RESULTS

1. EXTRACTION

The ethanolic extract of diospyros paniculata (EEDP) is prepared from bark.

2. IN VITRO ANTI OXIDANT ACTIVITY

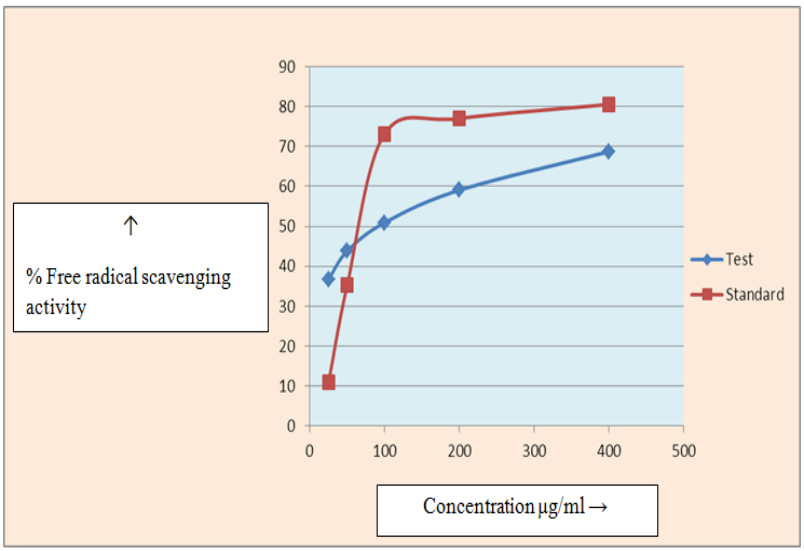
2.1. DPPP FREE RADICAL SCAVENGING ACTIVITY

In vitro free radical scavenging activity of Ethanolic Extract Diospyros Paniculata (EEDP) is shown in the Table no 1.The percentage radical scavenging activity of the Diospyros Paniculata is compared with the standard Anti oxidant Ascorbic acid. . The activity of the extract is assessed by the ability to scavenge DPPH radicals. It is found that the extract is having significant free radical scavenging activity with increase in the Percentage inhibition in a concentration dependent manner. The IC 50 value of standard is 70µg/ml and that of EEDP is 100µg/ml.

Table no:1 Comparison of the DPPH free radical scavenging activity of EEDP and Ascorbic acid

SL NO	CONCENTRATION µg/ml	% RADICAL SCAVENGING ACTIVITY	
		EEDP	ASCORBIC ACID
1	25	36.8 ± 0.065***	10.96 ± 0.184***
2	50	43.83 ± 0.052***	35.35 ± 0.184***
3	100	50.92 ± 0.025***	73.17 ± 0.184***
4	200	59.06 ± 0.050***	77.02 ± 0.184***
5	400	68.73 ± 0.028***	80.54 ± 0.0184***

Values are mean ± SD, n=3, p<0.001=***, indicates extremely significant increase in scavenging of free radical by EEDP as compared with Ascorbic acid. Statistical analyses were carried out by using one-way ANOVA followed by Tukey-Kramer Multiple Comparisons Test.



GRAPH 1 DPPH free radical scavenging activity of EEDP and Ascorbic acid

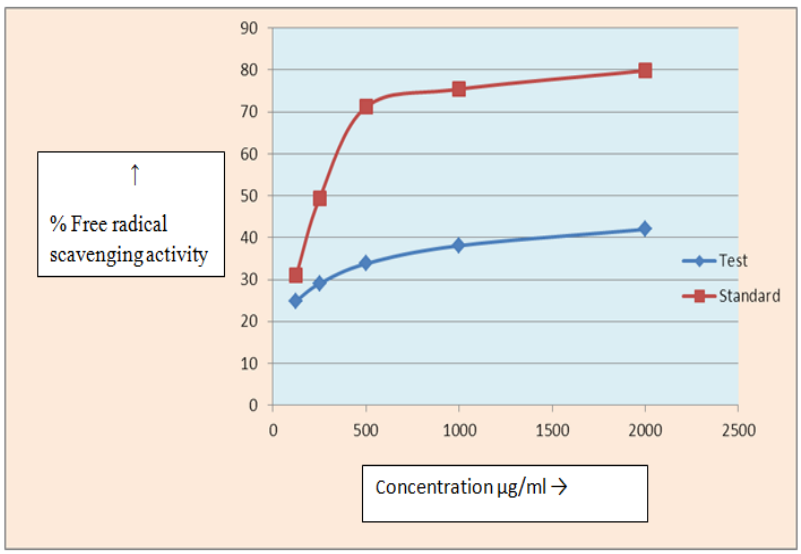
2.2 HYDROXYL RADICAL SCAVENGING ACTIVITY

In vitro Hydroxyl radical scavenging activity of Ethanolic Extract Diospyros Paniculata (EEDP) is shown in the Table no 2. The percentage radical scavenging activity of the Diospyros Paniculata is compared with the standard Anti oxidant Gallic acid. The activity of the extract is assessed by the ability to scavenge OH radicals. It is found that the extract is having significant free radical scavenging activity with increase in the Percentage inhibition in a concentration dependent manner. The Percentage inhibition of EEDP at dose 1000µg/µl is 38.07% as that of standard dose which is 75.44%.

Table no 2: Hydroxyl radical scavenging activity

SL NO	CONCENTRATION µg/µl	% HYDROXYL RADICAL SCAVENGING ACTIVITY	
		EEDP	GALLIC ACID
1	125	24.92 ± 0.010***	31.06 ± 0.144***
2	250	29.00 ±0.010***	49.34 ± 0.132***
3	500	33.78 ± 0.030***	71.17 ± 0.138***
4	1000	38.07 ± 0.010***	75.44 ± 0.138***
5	2000	41.99 ± 0.010***	79.86 ± 1.391***

Values are mean ± SD, n=3, p<0.001=***, indicates extremely significant increase in scavenging of free radical by EEDP as compared with Ascorbic acid. Statistical analyses were carried out by using one-way ANOVA followed by Tukey-Kramer Multiple Comparisons Test.



GRAPH 2 Free Radical Scavenging Activity of EEDP and GALLIC ACID

DISCUSSION

The antioxidant ability of the bark extracts was analyzed based on their ability to scavenge free radicals. DPPH radical scavenging is considered a good in vitro model widely used to assess antioxidant efficacy within a very short time. In its radical form, DPPH has disappears on reduction by an antioxidant compound or a radical species to become a stable diamagnetic molecule resulting the colour changes from purple to yellow, which could be taken as an indication of the hydrogen donating ability of the tested sample.[15,16] The extract is having significant free radical scavenging activity at various concentrations when compared with standard in a dose dependent manner in DPPH method.

Hydroxyl radical is particularly reactive and dangerous. Hydroxyl radical damages proteins in various ways and damages membrane by initiating the oxidation of fatty acid in membrane lipids, a process termed lipid peroxidation. It also damages nucleic acid, both by causing polynucleotide strand breakage and by changing the structure of DNA bases.[17] The extract is showing good free radical scavenging activity when compared with the standard compound.

The preliminary phyto-chemical analysis indicate that the chemical constituents like flavanoids, saponins, alkaloids where present in the extract which are having Anti oxidant activities which are scientifically proved. So that the Anti oxidant activity of the extract may be due to the presence of these chemical constituents.

CONCLUSION

From the results obtained from the In vitro methods like DPPH, Hydroxyl Radical scavenging activities it is confirmed that the extract is showing significant Anti oxidant activity in a concentration dependent manner as shown in Table 1&2.The activity may be due to the presence of chemical constituents like flavanoids, alkaloids etc. Even though further isolation and study of the chemical constituents is needed to confirm the Anti oxidant effect of the bark extract.

REFERENCES

1. Yu B P, Cellular defenses against damage from reactive oxygen species, *Physiol Rev.*, 1994; 74: 139.
2. Halliwell B & Gutteridge J M C, in free readicals in biology and medicine, 2nd ed (Clarendon Press, Oxford), 1988; 1.
3. Huang DH, Chen C, Lin C, Lin Y, Antioxidant and antiproliferative activities of water spinach (Ipomoea aquatica Forsk.) constituents. Botanical Bulletin of Academic Science, 2005; 46: 99-106.
4. Sun J, Chen Y, Li M, Ge Z. Role of antioxidant enzymes on ionizing radiation resistance. Free Radical Biology and Medicine, 1998; 42: 586-592.
5. Khilfi S, Hachimi E, Khalil A, Es-Safi A, Belahyam A, Tellal A, El Abbouyi A. In-vitro antioxidant properties of Salvia verbenaca. L. hydromethanolic extract. Indian Journal of Pharmacology, 2006; 38: 276-280.
6. Gulcin I, Alici HA, Cesur M. Determination of in-vitro antioxidant and radical scavenging activities of propofol. Pharmacology Bulletin, 2005; 53: 281-285.
7. Terao J, Piscula M, Yao MC. Protective effect of epicatechin, epicatechin gallate and quercetin on lipid peroxidation in phoshpolipid bilayers. Archives of Biochemistry and Biophysics, 1994; 308: 278-284.
8. Osawa T, Kavakishi S, Namiki M. In: Kuroda Y, Shankai DM, Waters MD. Antimutagenesis and anticarcinogenesis mechanisms II. New York: Plenum, 1990; 139.
9. Akinmoladun AC, Ibukun EO, Afor E, Akinrinlola BL, Onibon TR, Akinboboye AO, Obutor EM, Farombi EO. Chemical constituents and antioxidant activity of Alstonia boonei. African Journal of Biotechnology, 2007; 6: 1197-1201.
10. Shastri BN. The wealth of India; A dictionary of Raw Material and Industrial Research. New Delhi, 1952; 76-87.
11. Odebiyi, A, Sofowora, A.E. Phytochemical Screening of Nigerian Medicinal Plants. Part III. *Iloydia*, 1978; 41: 243-246.
12. Maridass, M. Essential oils of ethnomedicine of *Diospyros malabarica* fruits (Kostal). M.Sc., *Dissertation*, SriParamakalyani Centre for Environmental Sciences, Manonmaniam Sundaranar University, Alwarkurichi, Tamil Nadu, 1999.
13. Chang et al Antioxidant Activity of Extracts from *Acacia confusa* Bark and Heartwood, 2011; *49:* 3420-3424.
14. Elizabeth K, Rao MWA, Oxygen radical scavenging activity of Curcumin, Int. J. Pharmaceu., 1990; 58: 237-240.
15. Marxen K, Vanselow KH, Lippemeier S, Hintze R, Ruser A, Hansen UP, Determination of DPPH radical oxidation caused by methanolic extracts of some micro algal species by linear regression analysis of spectrophotometric measurements, Sensors, 2007; 7: 2080-2095.
16. Lee YR, Woo KS, Kim KJ, Son JR, Jeong HS, Antioxidant activities of ethanol extracts from germinated specially rough rice, Food Sci Biotechnol., 2007; 16: 765-770.
17. Harsha et al, Asian journal of pharmaceutical and clinical research, 2012; 5(1).