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Investigation on Antioxidant Activity of Bioactive Molecules of *Bauhinia Purpurea (L*) Bark

Shamala.T[1], Krupanidhi.A.M [2], Kalleshappa C.M[3]
[1]*Dept. of Chemistry, Jain Institute of Technology, Davanagere, Karnataka, India.*
[2]*Dept. of Pharmacology, Bapuji College of pharmacy, Davanagere, Karnataka, India*
[3]*Dept. of Chemical engineering, BIET, Davanagere, Karnataka, India*

Abstract:- Oxygen free radicals usually induces tissue damage due to peroxidation to biomembranes and also damage the DNA,thus cause occurance of number of diseases.The naturally occurring antioxidants are more beneficial and therapeutical value and neutralize the effect of free radicals through different ways and may prevent the body from various diseases.Thus the present investigations was undertaken to appraise properties of antioxidant activity by invitro method.The total antioxidant activity of ethanolic extract,chloroform extract,ethylacetate extract and isolated fractions of Bauhinia purpurea(BP) was estimated by 2,2-diphenyl-1-picryl hydrazyl(DPPH) radical scavenging assay. The DPPH scavenging potential of ethanolic extract of stem bark of BP ranged from 60.80% to 63.43%.The isolated fractions of stem bark of bp fraction showned percentage of scavenging ranged from 60.80% to 63.78%.Those antioxidant activities were compared to standard's like ascorbic acid, when Bauhinia purpurea crude extracts and fraction showed potent invitro antioxidant activity and result revealed that Bauhinia purpurea is a potential source of natural antioxidant. Further isolation and characterization of a bioactive molecule has to be carried out and find out its clinical significance.

Keywords:- Bauhinia purpurea, Flavonoids, Soxhlet extraction, Column chromatography, DPPH, Antioxidant activity.

I. INTRODUCTION

There is an increasing interest in antioxidants, particularly for preventing the presumed deleterious effects of free radicals in the human body,and to prevent the deterioration of fats nand other constitutents of food stuffs in the both the cases,[1]there is a preference for antioxidants from natural rather than from synthetic sources.Therefore a parallel increase in the use of methods for estimating the efficacy of such substances as antioxidants.In connection to that the Bauhinia purpurea plants have a rich source of secondary metabolites likje Flavonoids, alkaloids etc.[2] Hence in present days an upward trend has been witnessed in the demand of herbal medicines.However the local ayurvedic as well as traditional vaidhyas who rely preliminarily on prescribing of Bauhinia purpurea for various cardiovascular disorders and antidiabetic activity.[3]Hence our major study has focused on investigation of antioxidant activity of Bauhionia purpurea.

The invitro methods are based on inhibition. Samples are added to a free radical scavenging system, inhibition of the free radical action is measured and this inhibition is related to antioxidant activity of the sample [4]. Methods vary greatly as to the generated radical, the reproducibility of the generated process and end point that is used for the determination.The effect of antioxidants on DPPH radicals scavenging is thought to be due to their hydrogen or electron donating abilities [5][6].

II. MATERIALS AND METHODOLOGY

A. Collection of plant materials and extraction:

The plant was collected (SP No:105/BPC)from local areas of around Davanagere District and the plant Bauhinia purpurea aunthentication was done according different flora references by taxonomist Dr.K.M Shivakumar Davangere university, Davanagere.

The air dried coarse powdered material was subjected to Soxhlet extraction successfully by using solvents of increasing polarity namely Petroleumether(60-80^{0}C) ,chloroform,ethylacetate,ethanol and distilled water.All the extracts were evaporated to dryness by using rotary evaporator under reduced pressure and controlled temperature. 1Kg of dried powder of stem bark of *Bauhinia purpurea* were subjected to thimble. Some of the desired compound will then dissolve in the warm solvent.When the Soxhlet chamber is automatically emptied by a arm of siphon,with the solvent running back down to distillation flask and this is repeated for 30-40 cycles/day for five days. The concentrated extracts were then subjected to various phytochemical analysis by standard procedures. Phytochemical investigations of all extracts were carried out in order to detect the presence of secondary metabolites.[7][8].

Preliminory qualitative phytochemical screening was carried out with the corresponding methods revealed the presence of Flavonoids.

B. Isolation of fractions by Column Chromatography:

As ethanolic extract was found to be biologically due to the presence of flavonoids,alkaloids etc.Hence ethanolic extract of stem bark of BP was subjected to column chromatography.In present work, crude ethanolic extracts of stem, bark and leaf of Bauhinia purpurea(L) was subjected to column chromatography..The ethanolic extract(20g) was chromatographed over silica gel (100-200mesh) on column. Elution was carried out with solvent mixtures of increasing polarities.Dried extracts were stored separately. A part of the fraction (50:50) was dissolved in ethanol and subjected for Shinoda test, confirms the presence of Flavonoids.

C. Pharmacological screening:

Study on *Invitro* antioxidant activity by using DPPH (2,2-diphenyl-1-picryl-hydrazyl).

1) *Preparation of extract solutions*: Extracts (130mg) dissolved in 50 ml of ethanol separately to obtain solution of 2mg/ml concentration. Solutions were serially diluted separately to obtain to lower concentrations like 100,200,300,400,500µg/ml.

2) *Procedure*: Samples were prepared and 1ml of each concentration was added to 3ml of 0.004% ethanol solution of DPPH.Further kept for incubation period of 30min.Absorbance was measured by using UV-Spectrophotometer at λmax 517nm against blank.

Ascorbic acid used as standard free radical scavenger, its absorbance was measured as same as ethanolic extract as in table 1

Antioxidant activity of samples of different concentrations was calculated by using the formula., % scavenging ={(A1-A2)/A1}*100

Where A1 is the absorbance of the blank,A2 is the absorbance of the standard/sample extract.

III. RESULTS AND DISCUSSION

Free radicals are constantly generated resulting is extensive damage to tissue and bio molecules leading to various diseases conditions. The different extracts and fractions of *Bauhinia purpurea* are employed as an alternative source of medicine to mitigate the disease with oxidative stress. An attempt has been made in the present study to evaluate the antioxidant activity of aerial parts of *Bauhinia purpurea* extracts and fractions was performed using DPPH.

The ethanolic extracts of aerial parts of at the concentration of 500 µg/ml showed excellent inhibition (63.43%) of DPPH radicals. Fractions of *Bauhinia purpurea* showed significant inhibition of (63.78%) at 500 µg/ml concentrations of DPPH radicals. Table I shows great differences in total antioxidant capacity among the different extracts of aerial parts of *Bauhinia purpurea.*

In general, antioxidant activity of flavonoids depends as the structure and substitution patterns of –OH groups. The essential requirements for effective radical scavenging is the 3, 4, - orthodihydroxy configuration in ring 3 and 4 carbonyl groups, giving a catechlo- like structure in ring C , is also beneficial for antioxidant activity of flavonoids. Probably, aerial parts of *Bauhinia purpurea* extracts and fractions may contain the above similar type of flavonoids. So that *Bauhinia purpurea* exhibits the excellent antioxidant activity.

Table: I Antioxidant activities of stem-bark extract and fraction of *Bauhinia purpurea* by DPPH method

Sl. No.	Drug Treatment	Absorbance at 517nm	% of Scavenging
1.	**Blank**	0.7552	0.00
Standard			
2.	100 µg/ml	0.0886	63.78
3.	200 µg/ml	0.0849	64.27
4.	300 µg/ml	0.0823	64.62
5.	400 µg/ml	0.0797	64.96
6.	500 µg/ml	0.0786	65.11
Ethanolic Extract			
7.	100 µg/ml	0.1111	60.80
8.	200 µg/ml	0.1041	61.73
9.	300 µg/ml	0.0991	62.39
10.	400 µg/ml	0.0935	63.13
11.	500 µg/ml	0.0886	63.43

Fraction(Ethylacetate:alcohol)			
12.	100 µg/ml	0.1050	60.80
13.	200 µg/ml	0.1025	61.94
14.	300 µg/ml	0.0996	62.33
15.	400 µg/ml	0.0953	62.90
16.	500 µg/ml	0.0913	63.78

IV. CONCLUSION

Extensive studies have been carried out in antioxidant activity of Bauhinia *purpurea* of different extracts and fractions. The antioxidant activity was measured by 2, 2- diphenyl- 1 –picryl hydrazyl solution (DPPH) radical scavenging assay and the in vitro studies showed considerable antioxidant activity, mainly based a scavenging of oxygen radicals. These Flavonoids mainly inhibit of low- density lipoproteins oxidation, likely due to their reductive capacity and protein- binding properties. Hence *Bauhinia purpurea* are to be claimed as good antioxidant properties. Further isolation and Characterization will be required for establishing a new bioactive antioxidant compound used as a natural source of antioxidant.

Flavonoids present in plant origin are also potential antioxidants [9]. At present, the research interest is focused on the active role of antioxidants in the treatment and cure of several diseases Therefore, the utilization of more effective antioxidants of plant origin are desired [10].

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REFERENCES

[1]. T.Kumar and K.S.Chandrashekar, Bauhinia purpurea linn:A review of ethnobotany, phytochemical and Pharmacological profile. *Research journal of medicinal plant*, 5: 2011,420-431.
[2]. Asolker L.V, K.K Kakkar and O.J.Chakre, second supplement to glossary of Indian medicinal plants, part I (A- K); National Institute of Science communication, New Delhi, 2000.
[3]. Kirtikar.K.R and D.B Basu, Indian medicinal plants; Oriental enterprises, Dehradun, 2001.
[4]. Yu Cy, Ghimire BK, Chung IM(2011). *A comparative evaluation of the antioxidant activity of some medical plants popularly used in Nepal*. J.Med.Plants Res.,5(10):1884-1891.
[5]. Yu-Ling,HO., Shyh-Shyun,HUANG.,Jeng-shyan,Deng,Yaw-huei,LIN.,Yuan-shiun,Chang and Guan-, Huang (2012). *Invitro antioxidant properties and total phenolic contents of Wetland medicinal plants,* Taiwan Botanical Studies, 53:55-66.
[6]. WHO,2000. General guidelines for methodologies on research and evaluation of traditional medicine. World Health Organization, Genera.
[7]. Harbone,JB,"Phytochemical methodfs",IIIrd edition,Chapman and Hall,London,P04(1988).
[8]. Evans,W C,"Trease and Evans Pharmacognosy",XIVth Ed,Hart Court Brace and Company Asia Ltd,Singapore,P 117 (1988).
[9]. Shajiselvin.G, Somasundaram.A., Kottai,Muthu, *Antioxidant capacity of various extracts from whole plant of Bauhinia purpurea (linn)*.Evaluated by Three Invitro Methods Pharmacologyonline 1:221-227, 2011.
[10]. Anagnostopoulou M.A,Kefalas P,Papageorgion VP and Boskon D. *Radical scavenging activity of various extracts and fractions of sweet orange peel(citrus sinensis).*Food Chem.2006,94,19-25.