CHAPTER 5

EVALUATION OF ANTIOXIDANT ACTIVITY AND PHENOLIC CONTENT OF ROOT BARK OF *CROTON CAUDATUS* GEISELER AND STEM BARK OF *PHYLLANTUS ACIDUS*(L.) SKEELS

5.1: Introduction

An antioxidant is a substance capable of slowing or preventing the oxidation of molecules. In living systems, free radicals are constantly generated and they can cause extensive damage to tissues and biomolecules leading to various disease conditions, especially degenerative diseases ***(Halliwell and Gutteridge, 1998).***

Many synthetic drugs protect against oxidative damage but they have adverse side effects. An alternative solution to the problem is to consume natural antioxidants from food supplements and traditional medicines ***(Yazdanparast et al., 2008).***

Plants are good sources of natural antioxidants and some of these have significant antioxidative properties ***(Exarchou et al., 2002***). Recently, many natural antioxidants have been isolated from different plant materials (***Packer and Ong, 1997, Jovanovic and Simic, 2000***).The antioxidant activity is mainly due to phenolic contents such as flavonoids, phenolic acids, tannins and phenolic diterpenes ***(Awaad and Al-Jaber, 2010, Shahidi et al., 1992)***.

The evaluation of the antioxidant activities of polyphenols from ethnomedicinal plants may also be necessary because they are among desired medicinal properties of plants due to their nutraceutical effects ***(Zhuet et al., 2004)***. Antioxidant activities of polyphenols have been suggested to exert beneficial pharmacological effects on neurological

disorders on the basis of in vitro observations ***(Moosmann and Behl, 1999, Parr and Boolwell, 2000)***. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing, free radicals, quenching singlet and triplet oxygen, or decomposing peroxides ***(Osawa, 1994).***

Phenolic compounds are also thought to be capable of regenerating endogenous α-tocopherol, in the phosphor lipid bilayer of lipoprotein particles, back to its active antioxidant form. They are also known to inhibit various types of oxidizing enzymes. These potential mechanisms of antioxidant action make the diverse group of phenolic compounds an interesting target in the search for health beneficial phytochemicals ***(Halliwell and Gutteridge, 1989, Hall and Cuppett, 1997).***

So, the present study is to investigate the total phenolic content and the antioxidant activities of ethanol extracts of the root bark of *Croton caudatus* Geiseler and stem bark of *Phyllantus acidus* (L.)Skeels.

The antioxidant activities were determined by DPPH radical scavenging assay by Spectropotometric measurement at 517nm. The demonstrated modified Spectropotometric method makes use of the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical and its specific **absorbance properties. The absorbance decreases when the radical is** reduced by antioxidant.

5.2: Materials and Methods

5.2.1 Standards and reagents:

The chemicals were purchased from SIGMA-ALDIRICH (DPPH), REAGENTS, INC (gallic acid), Fine Chemicals (Ascorbic

acid), LOBA CHEMIE PVT.LTD (Folin–Ciocalteu phenolic reagent) and QUALIGENS (methanol, analytical grade).

5.2.2 Scavenging activity on DPPH radical:

The radical scavenging activities of the plant extracts against 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical were determined by UV spectrophotometry(electric GEAESYD-20 thermo spectronic) at 517nm.Radical scavenging activity was measured by a slightly modified method previously described by ***Ayoola et al., 2006*** and ***Brand et al., 1995***. Briefly, 1ml of the crude extract solution at variable concentrations (0.05, 0.1, 0.2, 0.5, 0.75,1,2 and 5mg/ml in methanol) was placed in a test tube and 3ml of methanol was added followed by 0.5ml of 1mM DPPH in methanol, kept for 35mins at room temperature till it produced a stable colour and absorbance was measured at 517nm.L-ascorbic acid was used as the positive control. A blank solution was prepared containing the same amount of methanol and DPPH. Ascorbic acid was used as the antioxidant standard at concentration of 0.05, 0.1, 0.2, 0.5, 0.75,1,2 and 5mg/ml in methanol (Analar grade).

The radical scavenging activity was calculated using the following formula.

% of inhibition = {[Ab-Aa]/Ab} X100

Where Ab is the absorption of the blank sample and Aa is the absorption of the extract.

5.2.3 Determination of the effective concentration (EC_{50}):

The EC_{50} value expresses the amount of extracts necessary to decreases the absorbance of DPPH by 50 % ***(Antolovich et al., 2002***).

The value can be determined graphically by plotting the absorbance against the used extract concentration.

5.2.4 Determination of total phenolic content (TPC):

The total phenolic content was determined using Folin-Ciocalteu reagents ***(Waterman and Mole, 1994)*** with analytical grade gallic acid as the standard.1ml of extract or standard solution (25mg/ml)was added to deionized water(60ml) and Folin-Ciocalteu phenol reagents (5.0mL). After 5minutes, 20% sodium carbonate (15.0mL) was added to the mixture. After being kept in darkness for 2h, the absorbance was measured at 760nm using a spectrophotometer (GEAESYS-20, Thermospectronic).The same solution was used without the extract as blank solution.Amount of TPC were calculated based on gallic acid standard

$$\text{Total Phenol\%} = \frac{\text{Asample X Wstandard X 50}}{\text{Astandard X Wsample X 50}} \text{ X 100\%}$$

The results were expressed as gallic acid equivalent (GAE) mg/mg of extract.

5.3: Results and Discussion

5.3.1. Radical scavenging avtivity by DPPH method:

Reactive oxygen species (ROS) produced in vivo includes superoxide radical, hydrogen peroxide and hypochlorous acid. Hydrogen peroxide and superoxide can interact in the presence of certain transition metal ions to yield a highly-reactive oxidizing species, the hydroxyl radical. The antioxidants react with the stable free radical DPPH and convert it to 2, 2-diphenyl-1-picrylhydrazyl with decoloration.

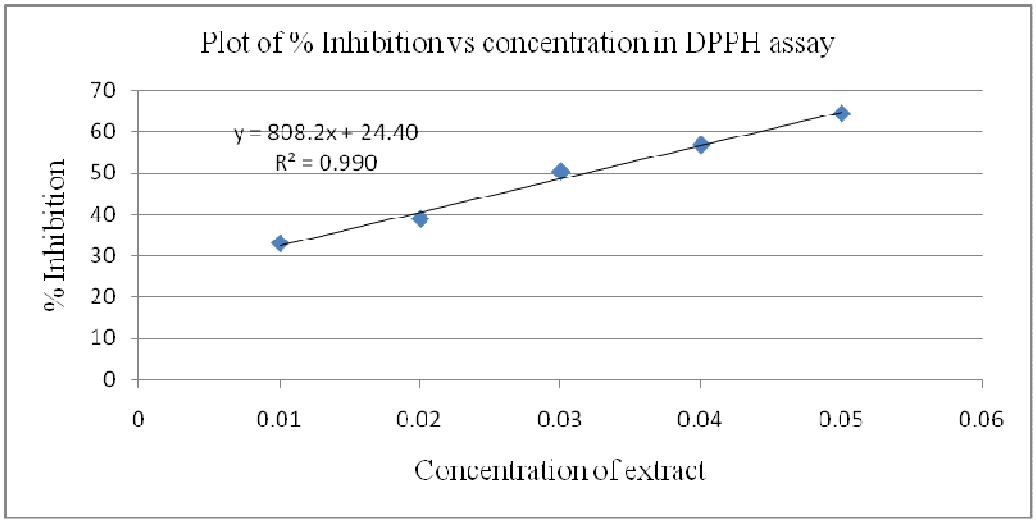
The results are express in EC_{50} (Effective concentration to reduce the initial concentration of DPPH to 50%). Lesser the EC_{50} value for an extract is associated with higher ability to donate hydrogen radical i.e antioxidant activity. In the present study EC_{50} value were found to be 0.318, 0.031 and 0.0024 for the *Phyllantus acidus* (L.) Skeels, *Croton caudatus* Geiseler and Ascorbic acid.These data clearly indicates that the antioxidant activity of the *Croton caudatus* Geiseler is higher than that of the *Phyllantus acidus* (L.)Skeels**.**

Table 5.3.1: Absorbance of extracts and ascorbic acid at different concentrations at 517nm.

Absorbance of blank = 1.835(Spectrophotometric absorbance at 517nm)

Table 5.3.1(a): Absorbance for *Croton caudatus* Geiseler

Concentration	Absorbance	Radical scavenging activity(%)	Mean (S.D)
0.01	1.230 1.231 1.230	32.97 32.91 32.97	32.95± 0.03
0.02	1.120 1.122 1.124	38.96 38.85 38.74	38.85± 0.11
0.03	0.912 0.910 0.914	50.29 50.40 50.19	50.29±0.10
0.04	0.795 0.790 0.793	56.67 56.94 56.78	56.79±0.13
0.05	0.650 0.654 0.656	64.57 64.35 64.25	64.39±0.16

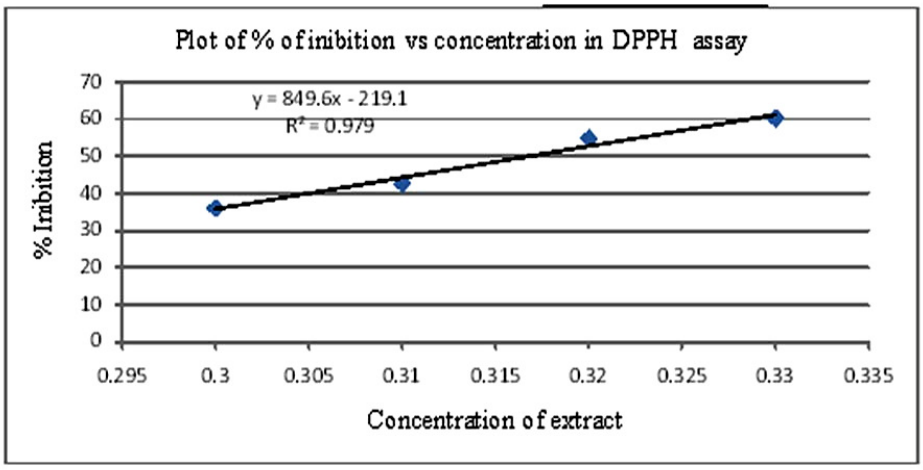


EC_{50} = 0.0317

Fig 5.3.1(a): Plot of % inibition vs *Croton caudatus* Geiseler **extracts at different concentration**

Table 5.3.1(b): Absorbance for *Phyllanthus acidus* (L) Skeel

Concentration	Absorbance	Radical scavenging activity (%)	Mean (S.D)
0.30	1.153 1.190 1.172	37.16 35.14 36.13	36±1.01
0.31	1.001 1.096 1.058	45.44 40.27 42.34	42.68±2.60
0.32	0.851 0.835 0.802	53.62 54.49 56.29	54.80±1.36
0.33	0.732 0.725 0.729	60.10 60.49 60.27	60.28±0.19
0.34	0.615 0.632 0.627	66.48 65.55 65.83	65.95±0.47

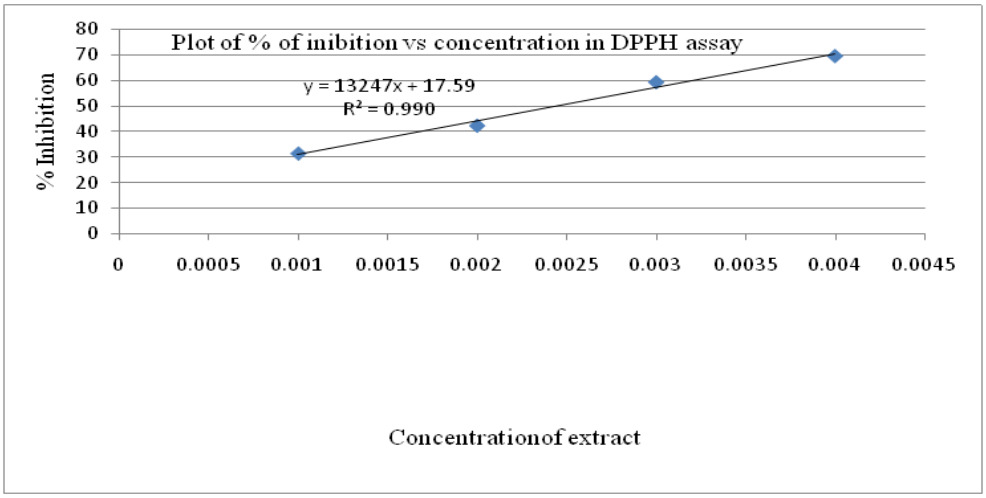


EC_{50} = 0.317

Fig 5.3.1(b): Plot of % inibition vs *Phyllanthus acidus* (L) Skeel extracts at different concentration

Table 5.3.1(c): Absorbance for Ascorbic acid

Concentration	Absorbance	Radical scavenging activity (%)	Mean (S.D)
0.001	1.261	31.28	31.33±0.90
	1.243	32.26	
	1.276	30.46	
0.002	1.059	42.28	42.30±0.73
	1.072	41.58	
	1.045	43.05	
0.003	0.734	60.00	59.45±0.52
	0.745	59.40	
	0.753	58.96	
0.004	0.541	70.51	69.77±0.70
	0.567	69.10	
	0.556	69.70	



EC_{50} = 0.0051

Fig 5.3.1(c): Plot of % inibition vs ascorbic acid **at different concentration**

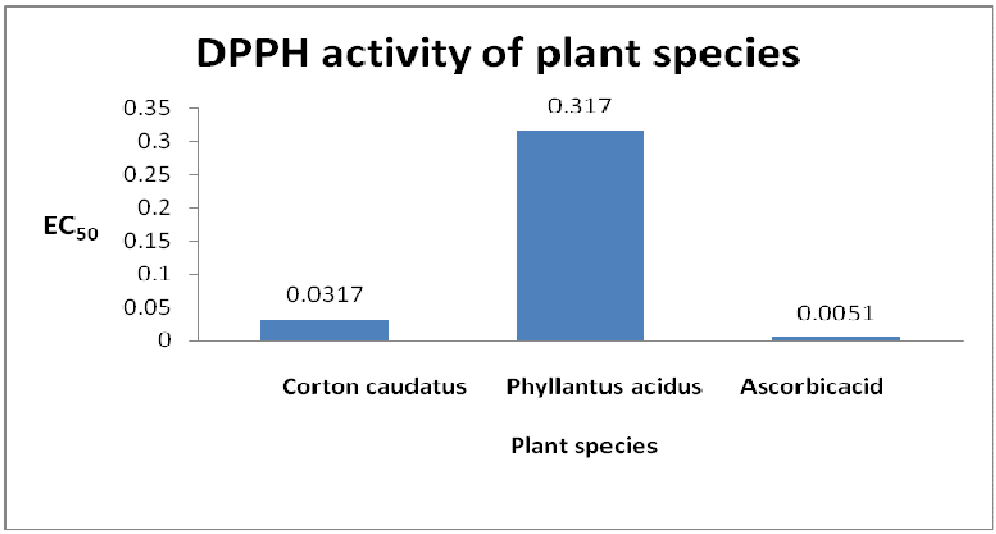


Fig 5.3.1(d): EC_{50} (mg/ml) values of plant species for free radical scavenging activity by DPPH radical. Lower EC_{50}value indicates higher antioxidant activity.

5.3.2 Estimation of total phenolic content:

Phenolic compounds are having wide bioactivity including antioxidant properties. The antioxidant activity of phenolic compound

is due to hydroxyl functional group, however other factors presence of electron withdrawing or releasing group in the aromatic ring having hydroxyl moiety will increase or decrease the activity. In the current study total phenolic content were found to be 26.66±1.15mgGAE/mg dw and 57.35±0.75 mg GAE / mg dw for *Phyllanthus acidus* (L.)Skeels and *Croton caudatus* Geiseler (using MS Excel v 2003). This shows that *Croton caudatus* Geiseler has higher content of phenolic compounds.

Table 5.3.2(a): Total phenolic content for *Phyllanthus acidus* (L.)Skeels

No of observation	Weight of sample (mg)	Weight of Standard (mg)	Absorbance of sample	Absorbance of standard	Total phenol (%)	Mean (S.D)
1	20	20	0.35	1.325	26.41	26.66 ±1.15
2	20	20	0.34	1.325	25.66	
3	20	20	0.37	1.325	27.92	

Table 5.3.2(b): Total phenolic content for *Croton caudatus* Geiseler

No of observation	Weight of sample (mg)	Weight of Standard (mg)	Absorbance of sample	Absorbance of standard	Total phenol (%)	Mean (S.D)
1	20	20	0.745	1.325	56.22	56.27 ±2.09
2	20	20	0.698	1.325	52.67	
3	20	20	0.794	1.325	59.92	

5.4: Conclusion

On the basis of the results obtained in the present study, it is concluded that methanol extract of root bark of *Croton caudatus* Geiseler and stem bark of *Phyllanthus acidus* (L.)Skeels posses *in vitro* antioxidant potential as comparable to those of the standard compound such as ascorbic acid. The methanol extract of root bark of *Croton caudatus* Geiseler showed higher potency in scavenging of DPPH free radical as compared to that of the extract of stem bark of *Phyllantus acidus* (L.)Skeels.This may be due to the presence of higher amount of phenols, flavonoids and other phytochemical components in this extract. The results obtained support the facts that more work needs to be done on the identification and quantification of the active components with the view of their use for *in vivo* studies.

5.5: Bibliography

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