ANTIOXIDANT ACTIVITY OF TRADITIONAL HERBAL DRUGS USED IN THE TREATMENT OF JAUNDICE

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Abstract: Jaundice is not a disease rather a symptom that can show up in several disease conditions. The manifestation of jaundice is due to the severe hepatic damage and excessive release of a yellow pigment called bilirubin to blood. The process of RBC destruction is called haemolysis and the jaundice caused this way is called haemolytic jaundice. The other two types of jaundice are called hepatocellular jaundice and obstructive jaundice. In our body, a balance between oxidative free radicals and antioxidants is usually maintained and this is a prerequisite for a normal health. Overloading with the free radicals and significant depletion in the supply of antioxidants lead to an act of imbalance termed as oxidative stress in health science. Oxidative stress causes haemolysis. Severe oxidative stress is considered one of the principal factors causing jaundice. The hepatoprotective property of certain herbal drugs may be attributed to their antioxidant potential. All plants produce chemical compounds as part of their normal metabolic activities. The curative properties of drugs are due to the presence of complex chemical substances of varied composition (present as secondary plants metabolites) in one or more parts of these plants. A large number of plant based materials are recognized as hepatoprotective herbal drugs, and these are traditionally being used for the reversal of jaundice in ethno-medicine.

Keywords: Jaundice, medicinal plant, traditional herbal drug, oxidative stress, free radical, antioxidant, haemolysis, bilirubin, hepatic damage, DPPH.

1. Introduction:

Jaundice is not a disease rather manifestation of severe hepatic damage and excessive release of a pigment, bilirubin, to blood. Yellow staining of the skin and eye caused by high level of bilirubin in blood is often termed as the incidence of jaundice [1]. Bilirubin is a liphophilic linear tetrapyrrole (Figure 1) which is toxic and insoluble in blood [2]. The colours of skin and eye of the affected person may vary depending upon the level of bilirubin [1].

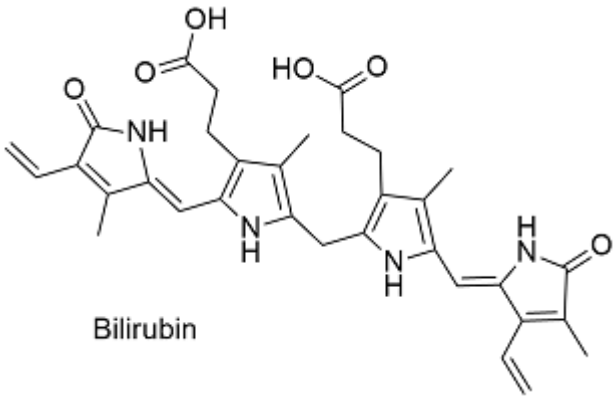

Figure 1: Molecular Structure of bilirubin (C33H36N4O6) [1]

Accumulation of the pigment bilirubin in the blood stream may result from either over production of bilirubin or impaired hepatic metabolism of the pigment [3]. If the production of RBC in blood falls below normal as well as for any reason RBCs die at a faster rate than normal, then bilirubin can accumulate in the blood and cause jaundice

[3]. The process of RBC destruction is called haemolysis and the jaundice caused this way is called haemolytic jaundice. The other two types of jaundice are called hepatocellular jaundice and obstructive jaundice [4,5]. Hepatocellular jaundice occurs when bilirubin is unable to leave the liver cells and cannot be removed from the body by the kidneys. It is usually caused by liver failure, liver disease (cirrhosis), hepatitis (inflammation of the liver), or by taking certain types of medication. Obstructive jaundice is a condition in which there is blockage of the flow of bile out of the liver. This results in redirection of excess bile and its by-products into the blood, and bile excretion from the body is incomplete.

Metabolic activity in the liver generates reactive free radicals such as oxidative free radicals [6] which are usually countered by the presence of another group of chemicals called antioxidants. A balance between oxidative free radicals and antioxidants is usually maintained in our body, and this is very important for normal health. Overloading with the free radicals and significant depletion in the supply of antioxidants lead to an act of imbalance termed as oxidative stress in health science. Oxidative stress causes haemolysis [7]. Experimental and clinical studies have demonstrated the pivotal role of oxidative stress in the promotion of hepatic and intestinal injury [8]. Severe oxidative stress is considered one of the principal factors causing jaundice [9,10].

The hepatoprotective property of certain herbal drugs may be attributed to their antioxidant potentials [6]. Antioxidants detoxify the oxidative free radicals in our body. Antioxidant rich plant based materials are therefore potential hepatoprotective drugs [11].

A large number of plant based materials are recognized as hepatoprotective herbal drugs, and these are traditionally being used for the reversal of jaundice in ethno-medicine. Scientific study on antioxidant potentials of such drugs is scanty. In this report, an attempt has been made to correlate the antioxidant potentials of a couple of herbal drugs to their efficacy in reversing jaundice.

2. Materials and Methods:

We focused on medicinal plants used by the rural people of the district of Goalpara under the guidance of folk medicine practitioners. Both patients and practitioners were interviewed and discussed. They were specifically asked about the plant materials, modes of use, duration of treatment and success of recovery. Plant specimens were identified with the help of available literature and also verified with the taxonomists in the Department of Botany, Gauhati University. Antioxidant activity of the plant materials was assessed using DPPH Assays [12,13].

2.1 Procedure for the measurement of antioxidant activity

Shade dried plant materials were powdered using a mechanical device and stored at ambient temperature in polybags. Methanolic extracts of the plant materials were prepared and the antioxidant activity was assessed following the standard procedure as described below.

2.2 Preparation of extract

A mixture of powdered sample (5g) and methanol (HPLC grade, 50mL) was stirred for 3 hours (Magnetic Stirrer) and filtered through sintered glass crucible. Solvent was removed completely from the filtrate under vacuum using a rotary vacuum evaporator followed by drying using a high vacuum pump. The soluble part recovered as a residue was dissolved in methanol (1g/100mL) and preserved at 0 ^{o}C to 10 ^{o}C for further use.

2.3 Measurement of antioxidant activity

A freshly prepared solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH,1mg) in methanol (HPLC grade, 10 mL) was mixed with methanolic extract (10 mg/mL, 10 mL) in equal part and kept stirring in dark. An aliquot of the mixture was pipetted out and absorbance measured at 515 nm at 0 time and thereafter at intervals of 30 minutes using Spectrophotometer (Hitachi U-3210) until two consecutive constant absorbance were observed. Absorbance of a blank prepared by mixing DPPH solution with methanol in 1:1 ratio was also measured. Antioxidant activity was then calculated using the following relation:

$$\% \, Activity = \left[\frac{A_0 - A_t}{A_0}\right] x100$$

where, A_0 = Absorbance of the blank and A_t = Absorbance of reaction mixture at time t. The stock solution of extract was diluted so as to make solution of different concentrations such as 1000mg/L, 200mg/L, 100mg/L, 50mg/L and 10mg/L and their antioxidant activities were recorded following the same procedure.

3. Results and Discussion:

A total of 10 folk medicine practitioners and 68 patients were consulted. Parts of the plant used, mode of preparation, frequency of applications and duration of treatment are recorded in Table 1. Number of users (patients) and information on success rate provided by them are shown in Table 2. From the data recorded in Table 2 it is observed that number of users of the plant Cajanus cajan is highest (14) as compared to other plants, Centella asiatica (05), Ananus comosus (04) and Scoparia dulcis (02). Also the success rate is highest with Cajanus cajan (85.71%) followed by Centella asiatica (80%) and Ananus comosus (75%). Number of users (02) and success rate (50%) is found to the lowest with Scoparia dulcis.

Table 1: Parts of the plants used, modes of preparation, frequency of applications and duration of treatment etc. of medicinal plants used as folk medicine against jaundice.

Sl No.	Name of the plants	Parts of the plants used	Modes of preparation	Frequency of application	Duration of treatment
1	*Ananus comosus*	Leaf	2 tea spoons fresh young leaf juice mixed with 1tea spoon of honey.	Orally, twice a day	2 weeks
2	*Cajanus cajan*	Leaf	2 tea spoons fresh leaf juice mixed with 1 cup of curd and 1 tea spoon of table sugar.	Orally, thrice a day	2 weeks
3	*Centella asiatica*	Leaf	1 tea spoon fresh leaf juice mixed with water.	Orally, thrice a day	2 weeks
4	*Scoparia dulcis*	Leaf	1 tea spoon fresh leaf juice mixed with water.	Orally, thrice a day	2 weeks

Table 2: Success rate of medicinal plants used as folk medicine against jaundice.

Sl No.	Name of the plants	No. of patients used the plant (N_P)	No. of patients fully or partially cured (N_C)	No. of patients recording no effect	Effectiveness (%) (N_C/N_P) x100
1	*Ananus comosus*	04	03	01	75
2	*Cajanus cajan*	14	12	02	85.71
3	*Centella asiatica*	05	04	01	80
4	*Scoparia dulcis*	02	01	01	50

Antioxidant property of the plant extracts at different concentrations as assessed through DPPH assay and their respective antioxidant activity along with IC_{50} values are shown in Table 3. Antioxidant activity is evaluated at five different concentrations (10, 50, 100, 200 and 1000 mg/L), and the leave extract of the plant *Cajanus cajan* shows higher activity at all concentrations as compared to other plant extracts. This is as expected and in agreement with the higher success rate shown by *Cajanus cajan*. *Centella asiatica* shows the second highest antioxidant activity followed by *Ananus comosus* and *Scoparia dulcies* (Table 3).

Graphical plots of antioxidant activity against concentrations for each plant extract are shown in Figure 2 to 5. IC_{50} values evaluated from plots are recorded in Table 3. Lower IC_{50} value is expected for a plant extract displaying higher antioxidant activity, and this is in agreement with the observed results. *Cajanus cajan* plant extract has the lowest IC_{50} (66.57) as expected as the extract shows highest antioxidant activity. This is followed by *Centella asiatica* (IC_{50} 82.2), *Ananus comosus* (IC_{50} 103.12) and *Scoparia dulcies* (IC_{50} 191.89). A comparative bar diagram of IC_{50} values are shown in Figure 6.

It is evident from the collected information that Cajanus cajan is the widely used plant that records success rate of 85.71%. The leave extract of this plant shows highest antioxidant activity and lowest IC_{50} value among the four different plants studied. These results indicate that antioxidant activity of herbal drugs used in the treatment of Jaundice has a direct correlation with their bioactivity.

Table 3: Antioxidant activity at different concentrations of medicinal plant extracts used as folk medicine against jaundice.

Sl No.	Name of the plants	Antioxidant activity (%)					IC_{50} values
		1000 mg/L	200 mg/L	100 mg/L	50 mg/L	10 mg/L	
1	*Ananus comosus*	48	48	26	13	03	103.12
2	*Cajanus cajan*	76	74	39	20	03	66.57
3	*Centella asiatica*	62	60	32	16	03	82.21
4	*Scoparia dulcies*	28	26	13	07	01	191.89

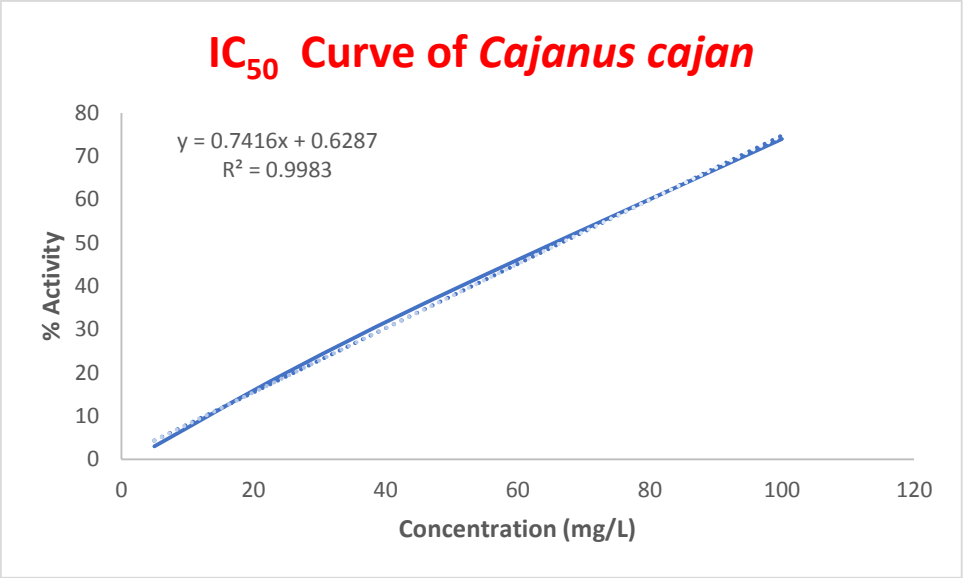

Figure 2. Antioxidant activity(%) vs Concentration(mg/L) plot of *Cajanus cajan* leave extract.

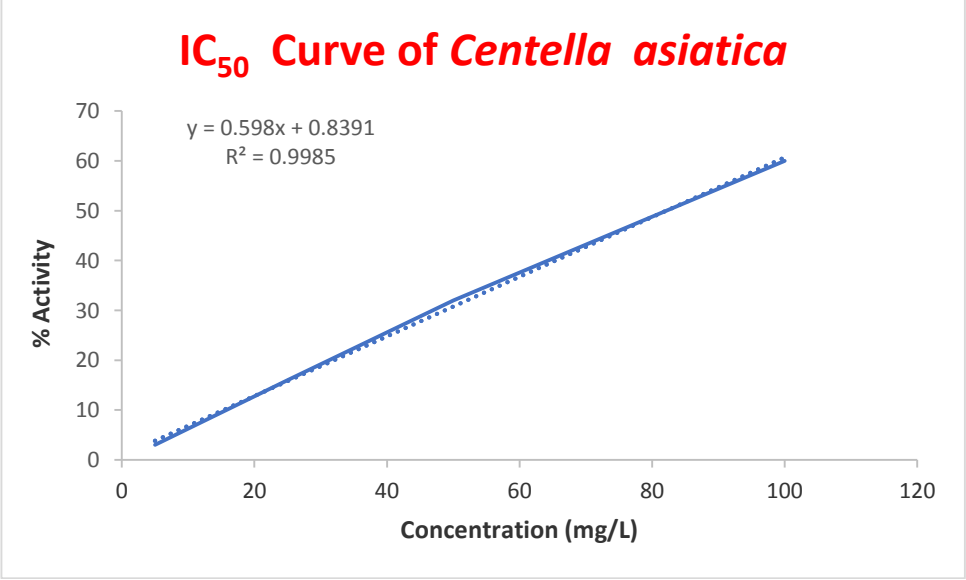

Figure 3. Antioxidant activity(%) vs Concentration(mg/L) plot of *Centella asiatica* leave extract.

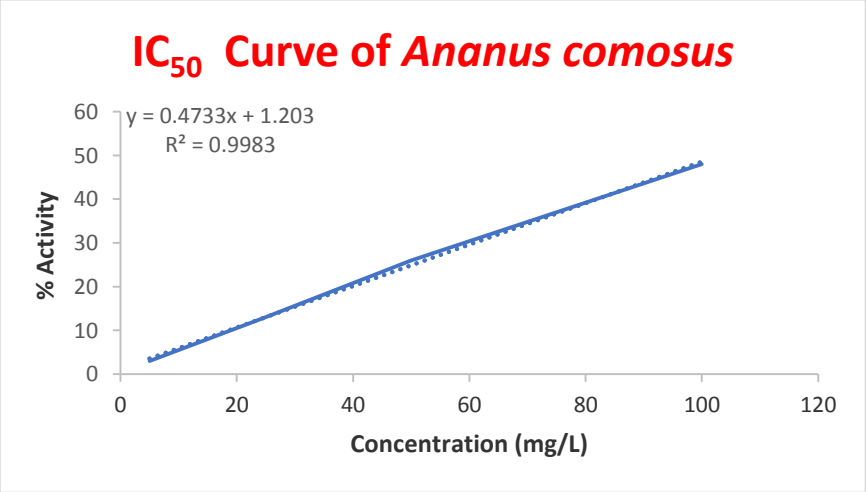

Figure 4. Antioxidant activity(%) vs Concentration(mg/L) plot of *Ananus comosus* leave extract.

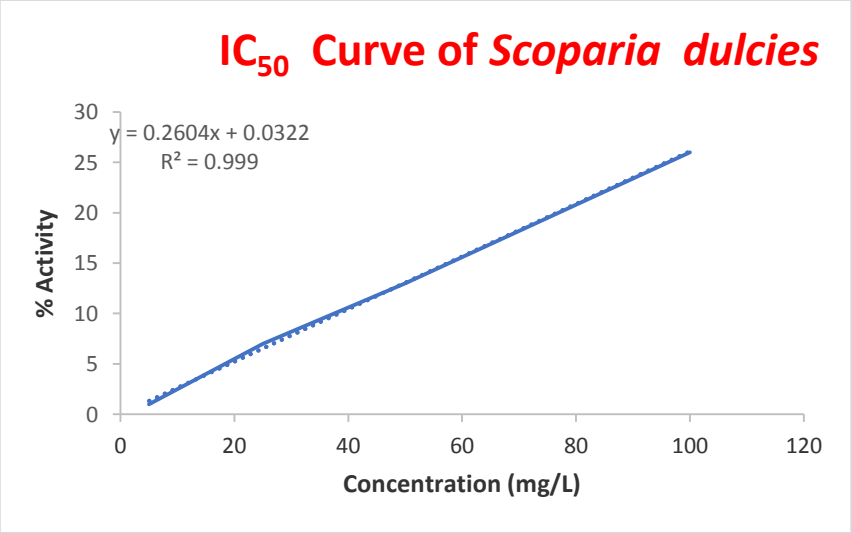

Figure 5. Antioxidant activity(%) vs Concentration(mg/L) plot of *Scoparia dulcies* leave extract.

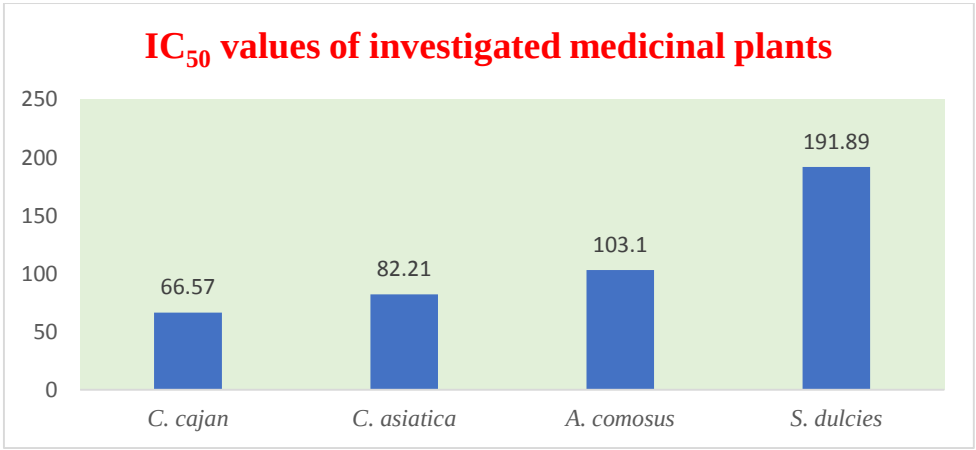

Figure 6: The bar diagrams of IC_{50} values of investigated medicinal plants used as folk medicine against jaundice.

4. Conclusion:

The present study indicates that there is a relationship between the curative power and the antioxidant property of herbal drugs used against jaundice. Herbal drugs with high antioxidant property are expected to work better against jaundice. The findings can be a useful information while screening potential herbal drugs used against jaundice for further study.

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