To Study The Phytochemical Screening And Antioxidant Activity Of Cassia Fistula And Boerhaavia Diffusa Leaf Extracts On Hepatotoxicity

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Abstract
Hepatotoxicity is the medical term used to describe liver damage caused by toxins, dietary or herbal supplements, or pharmaceuticals. The most common reason for a pharmaceutical being withdrawn from the market after it has been approved is due to an adverse drug reaction. Hepatotoxicity can manifest as either acute or chronic, and its symptoms, which resemble those of various other hepatobiliary diseases, can pose a challenge in terms of identification. This study aimed to investigate the antioxidant activity and phytochemical properties of the leaves of the Boerhaavia diffusa and Cassia fistula plants. Phytochemical and antioxidant tests were conducted on leaves. Ascorbic acid was utilized as a reference antioxidant to assess the ability of leaf extracts from regularly used medicinal herbs to scavenge radicals. The experiment utilized the DPPH free radical to evaluate the scavenging activity. The antioxidant actions of phenolic compounds, including their bioactive characteristics, are widely recognized and accepted. Notably, the leaves of Boerhaavia diffusa and Cassia fistula had the highest overall antioxidant activity. The primary objectives of this work were to assess the antioxidant capacities of various leaf extracts using the DPPH and ABTS assays, and to determine their phenolic content and reduction power.

Keywords: Hepatotoxicity, *Cassia fistula* and *Boerhaavia diffusa* , antioxidant and phytochemical screening

1. INTRODUCTION

Living things are defined by their capacity for growth, reproduction, and response to external stimuli. Its survival is reliant on a wide range of physiological and metabolic processes.

The most important organ is the liver. It has a crucial role in regulating many physiological and biochemical functions in the body.[1,2] The most common causes of liver difficulties are infections, autoimmune diseases, excessive alcohol consumption, and the absorption of toxic substances from the digestive tract.[3,4] Liver damage is the umbrella term encompassing a number of disorders that can affect the liver, including cirrhosis (widespread inflammation and scarring leading to permanent cell damage and liver failure), hepatitis (inflammation), fibrosis (development of scar tissue), and liver cancer.[5,6] Adverse drug reactions play a major role in liver damage, frequently requiring hospitalization, liver transplantation in extreme situations, or stopping the toxic medicine. In the US, the most common cause of abrupt liver failure is drug-induced hepatotoxicity. The liver is particularly vulnerable to the harm that medications can cause because of its function in the concentration and metabolism of the majority of medicines.[7,8]

A plant that is commonly encountered and well-known for its medicinal properties is Cassia fistula L. It is an Indian Labernum that has been somewhat domesticated. Known as the fistula tree Cassia. There are important therapeutic ramifications for the Indian medical system. This plant has antibacterial, antipyretic, analgesic, anti-inflammatory, and hypoglycemic properties in the extracts made from its various parts.[9,10] They are used to treat a variety of illnesses, such as rheumatism, liver problems, skin concerns, eye problems, and haematemesis. It is also a component of the remedies called pilex, detoxifier, and Purian for hemorrhoids, in that order.

One species of blooming plant in the four o'clock family is Boerhavia diffusa. It goes by several names, including red spiderling, punarnava, spreading hogweed, and tarvine. It is used in herbal medicine for a variety of purposes, including as an analgesic. In many parts of India, boerhavia diffusa leaves are a popular leafy vegetable. One important botanical ingredient present in many ayurvedic remedies is boswellic acid (BD). Many formulations for the treatment of inflammatory conditions, jaundice, asthma, rheumatism, nephrological disorders, ascites, anemia, and gynecological issues have included it.[11,12]

2. METHODS

2.1 Preparation Of Plant Extracts

Using distilled water and a mortar and pestle, the fresh leaves were crushed. There were 300 milliliters of water and 100 grams of crushed leaves. After that, the ground leaves were cooked for an hour in a water bath. The leaf extract was then

strained through a muslin towel. A rotary evaporator was used to evaporate the liquid extract, producing a dry powder. By weight, the yield was 3.9%. Phytochemical analysis was used to further investigate the powder in order to determine the chemical components of the extract. For oral administration, a powdered extract of *Cassia fistula* and *Boerhaavia diffusa* leaves was dissolved in water without the need for any suspending agent.

2.2 PHYTOCHEMICAL SCREENING

Screening for extract phytochemicals was done according to protocol. Plant compounds have been classified through preliminary phytochemical testing of extracts.

2.2.1 Carbohydrate tests

A. Molisch test: emergence of purple ring in response to 1 ml of test + 2-3 drops of α-naphthol + Conc. sulphuric acid.
B. Fehling's test: Add heat, Fehling's solutions A and B, and one milliliter of the test sample to get a brick-red precipitate.
C. Benedict's test: An identical amount of Benedict's reagent was added to 1 milliliter of test sample, and it was then cooked. Precipitate with a red color indicated the presence of carbohydrates.

2.2.2 Alkaloids test

Prior to filtering, each extract was individually treated with dil. hydrochloric acid. The filtrate from each extract was put through the following examinations:
A. Mayer's test: 1 ml of the filtrate plus 1 ml of the cream precipitate Mayer's reagent
B. Hager's test: 1 mL of the filtrate plus 1 mL of the yellow precipitate-containing Hager's reagent
C. Wagner's test: 1 milliliter of the filtrate plus 1 milliliter of Wagner's reagent yields a reddish-brown precipitate.

2.2.3 Terpenoids tests

A. Salkowski test: An equivalent volume of concentrated sulfuric acid was added to the test sample's chloroform solution. The test sample's red coloration in the chloroform layer and green fluorescence in the acid layer indicated the presence of steroid components.

B. Libermann-Burchard test: Two milliliters of test sample were mixed with chloroform, and then two to three drops of acetic anhydride and concentrated sulfuric acid were added. The test solution's color was watched for changes, first from red to blue and subsequently to bluish green, which indicated that steroids were present in the test extracts.

2.2.4 Flavonoid tests

A. Lead acetate test: The extract was mixed with a lead acetate solution. The presence of flavonoids was verified by the production of a yellow precipitate.

B. Alkaline reagent test: A yellow precipitate formed when 1 milliliter of the test material was dissolved in a diluted sodium hydroxide solution.

2.2.5 Phenolic compound and tannin tests

A. 5% FeCl3 solution: By adding a few drops of the 5% FeCl3 solution to the small amount of extract, a rich blue-black color complex was formed.
B. 10% lead acetate solution: A white precipitate appeared when a few drops of 10% lead acetate solution were added to 2 milliliters of extract.
C. Gel test: A little amount of extract was dissolved in distilled water, and then 2 milliliters of 1% gelatin solution with 10% NaCl was added. This resulted in the production of a white precipitate, which was indicative of the presence of phenolic chemicals.

2.2.6 Saponin testing

A. Foam test: 1 milliliter of test material with 20 milliliters of distilled water + shaking for 15 minutes; foam should form persistently.

2.2.7 Check for amino acids and proteins

A. Ninhydrin test: Heat for 10 minutes, add 3 drops of 5% ninhydrin to 3 milliliters of the test solution, and watch for a color shift.
B. Biuret test: The test sample showed signs of violet or pink coloration after being treated with an identical volume of 1% copper sulfate and 4% sodium hydroxide solution.

C. Million's test: Three milliliters of the extract and five milliliters of the reagent were combined. When heated, a white precipitate turned brick red, signifying the presence of proteins.

2.2.8 Glycoside tests:

A. The Borntrager Exam: Five minutes were spent boiling three milliliters of extract solution with dil. H2SO4. After filtering, the mixture was chilled. Chloroform was added in the same amount while the mixture was being shaken. To the layer of organic solvent, ammonia was added. The ammonical layer's color changed to a pinkish red hue, indicating the presence of glycosides of the anthraquinone type.

B. Legal test: A color shift was noted after adding 1 ml of sodium nitroprusside to 1 ml of pyridine solution that included the test material.

C. Keller killani test: Three milliliters of glacial acetic acid, one drop of 5% ferric chloride, and two milliliters of extract were added. The observation was recorded after this solution was carefully added to the surface of two milliliters of concentrated
H2SO4.

2.2.9 Fat and oil test results

A spot test was conducted by placing a single droplet of extract onto filter paper and letting the solvent drain. The presence of fixed oil was detected by an oily stain on filter paper.

2.3 Antioxidant activity in vitro

2.3.1 ABTS radical cation decolorization assay: Using ABTS radical cation, ABTS solution was made. It was left out at ambient temperature for 12 to 16 hours and contained 2.45 milligrams of ammonium persulfate. Before the experiment, it was in use. A range of extract concentrations (from 2 to 1000 µg/ml) were added to 0.4 ml. At 745 nm, the absorbance was computed.

2.3.2 DPPH radical scavenging activity- Combine 1ml of the extract with 1ml of the DPPH solution and thoroughly mix the two. Methanol and DPPH are used as control substances. Subsequently, it was placed in a dark location for a duration of 20 minutes. Subsequently, the absorbance is measured at a wavelength of 517nm.

2.3.3 Superoxide Radical Scavenging – The superoxide radical is employed to assess the scavenging activity that inhibits the production of O2. The DMSO technique is utilized for the determination of this experiment. Potassium superoxide and DMSO are added to the solution and then filtered. The filtrate was mixed with an aqueous solution containing NBT, EDTA, and potassium phosphate buffer.

2.3.4 Nitric oxide radical scavenging

Nitric oxide was assessed from sodium nitroprusside and quantified using the Griess reaction. Various concentrations of the extract were incubated in sodium nitroprusside and phosphate buffer. The tubes are placed in an incubator at a temperature of 250°C for a duration of 5 hours. The test control was executed in the same manner as before. After incubation, 5 hours of the solution is withdrawn, and the Griess solution is used to dilute it. During the process of diazotization of nitrite, the chromophore exhibits absorption when combined with sulphanilamide and coupled with napthyl ethylene diamine at a wavelength of 546nm.

2.3.5 Iron chelating activity: The reaction mixture consists of O-phenathroline (0.05%), ferric chloride (2ml), and varying concentrations of the test substance. The mixture is incubated for 10 to 15 minutes, and then the absorbance is measured at 510nm.

2.3.6 Total antioxidant capacity:

The spectrophotometric approach is employed to determine the antioxidant property. A solution containing 0.1ml of extract was prepared by dissolving it in water, and then 1ml of reagent solution was added to the mixture. The tubes are incubated and sealed in a thermal block for a duration of 95 minutes at a temperature of 95 degrees Celsius. After cooling to room temperature, the sample's absorbance is measured at a wavelength of 695 nanometers.

3. RESULTS

3.1 Phytochemical screening

S.no	Test	Methods	Results
1.	Flavonoids	sodium hydroxide test	+
		ferric chloride test	+
		lead acetate test	+
2.	Glycosides	Molisch test	+
		Benedict's test	+
		Fehling's test	+
3.	Alkaloids	Dragendorff's test	+
		Wagner's test	+
		Mayer's test	+
4.	Tannins	Lead acetate test	+
		Ferric chloride test	+

5.	Saponins	Foam test	+
		Liberman's test	+

Table 1. Phytochemical screening of *Cassia fistula*

S.no	Test	Methods	Results
1.	Flavonoids	sodium hydroxide test	+
		ferric chloride test	+
		lead acetate test	+
2.	Glycosides	Molisch test	+
		Benedict's test	+
		Fehling's test	+
3.	Alkaloids	Dragendorff's test	+
		Wagner's test	+
		Mayer's test	+
4.	Tannins	Lead acetate test	+
		Ferric chloride test	+
5.	Saponins	Foam test	+
		Liberman's test	+

Table 2. Phytochemical screening of *Boerhaavia diffusa*

3.2 Antioxidant activity

3.2.1 DPPH scavenging activity

The IC_{50} values of the aqueous extracts of *Boerhavia diffusa* and *Cassia fistula* were 78.20 µg/ml and 80.75 µg/ml, respectively. The proton radical scavenging mechanism is the most crucial factor in measuring antioxidant activity. This test is utilized to determine the presence of DPPH in the antioxidant. In this investigation, the extract demonstrates a higher proportion of inhibition. The phytochemical analysis also indicates that the chosen plant extract has the ability to provide hydrogen to free radicals, which could potentially cause harm.

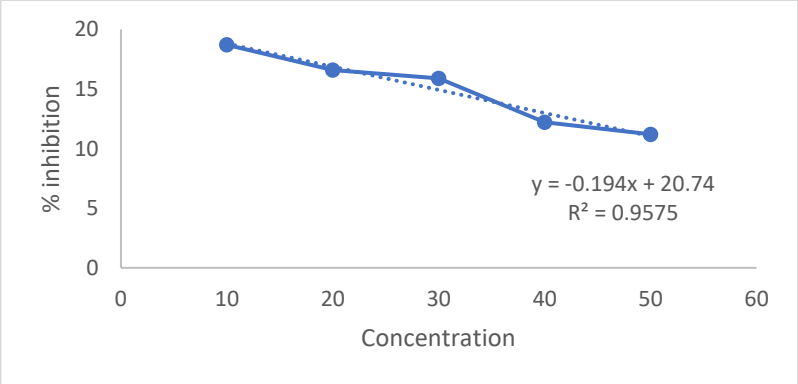

Figure 1. DPPH assay activity of ethanol extracts of *Boerhavia diffusa*

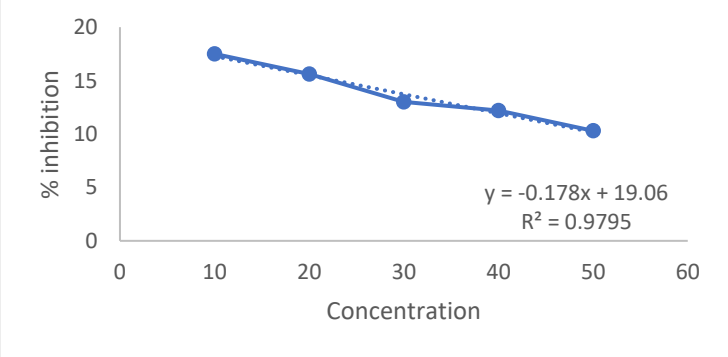

Figure 2. DPPH assay activity of ethanol extracts of *Cassia fistula* on DPPH assay

3.2.2 Hydrogen peroxide radical scavenging activity

The IC_{50} value was determined using aqueous extracts of Boerhavia diffusa and Cassia fistula. The IC_{50} was determined for each extract and the standard ingredient, ascorbic acid. The IC50 values for Boerhavia diffusa and Cassia fistula were 55.39 and 61.24 µg/ml, respectively.

It possesses potent oxidizing properties. These drugs initiate the signaling pathways that promote cellular growth and specialization. The enzyme superoxide dismutase functions in the biological system by catalyzing oxidation reactions. The hydrogen peroxide underwent breakdown, resulting in the generation of the hydroxyl radical. It triggers the process of peroxidation and causes harm to cells. Plant antioxidants in biological studies are capable of generating hydrogen peroxide. The chosen plants contain hydrogen peroxide due to the presence of phenolic groups, which contribute electrons to hydrogen peroxide. The components were neutralized.

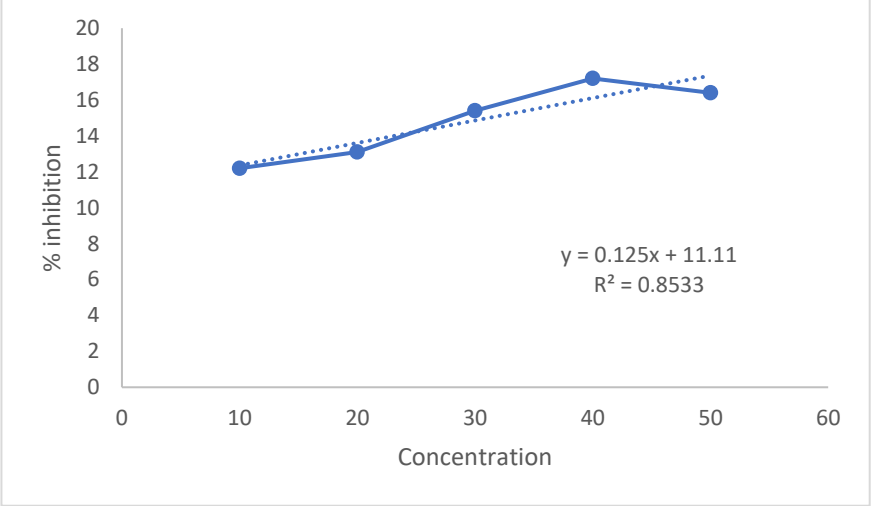

Figure 3. Different concentration of H_2O_2 radical of ethanol extracts of *Boerhavia diffusa*

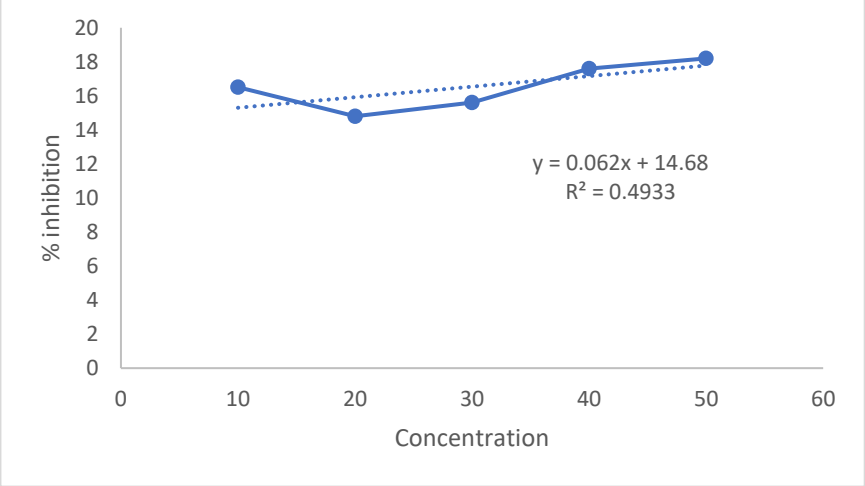

Figure 4. Different concentration of H_2O_2 radical of ethanol extracts of *Cassia fistula*

3.2.3 Nitric Oxide Scavenging

Boerhavia diffusa and Cassia fistula aqueous extracts were used in the experiment, and the outcomes were reported as IC_{50} values. For Cassia fistula and Boerhavia diffusa, the nitric oxide IC_{50} values were 79.28 µg/ml and 46.66 µg/ml, respectively. This is the important bioactive substance. It is employed in the preservation of homeostasis. It's also used to fight harmful microorganisms. Furthermore, it functions as a vasodilator to influence blood vessels. It modifies endothelial permeability and influences neurotransmitters.

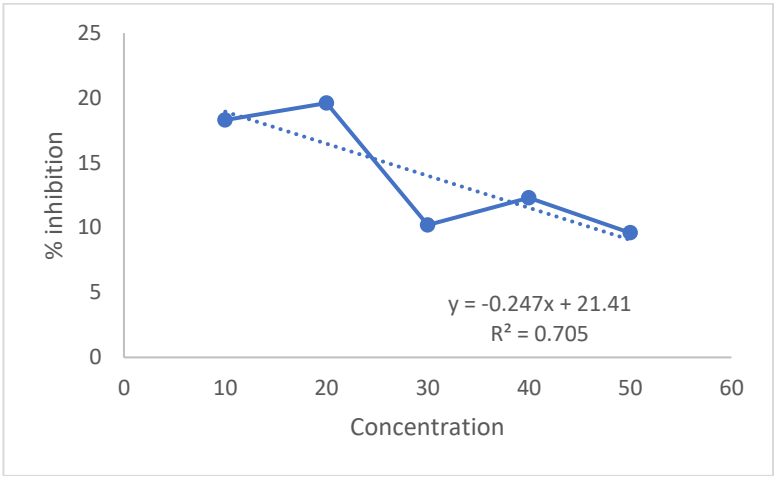

Figure 5. Scavenging effect of ethanol extracts of *Boerhavia diffusa* on nitric oxide assay

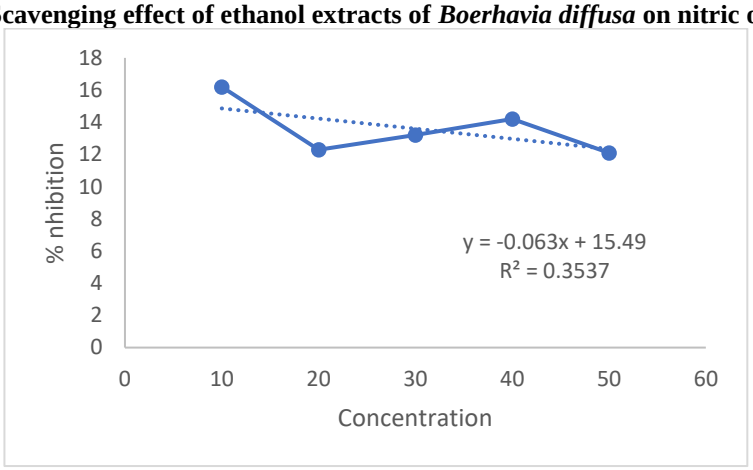

Figure 7. Scavenging effect of ethanol extracts of *Cassia fistula* on nitric oxide assay

3.2.4 Reducing power assay

Based on the power assay, the aqueous extracts of Cassia fistula and Boerhavia diffusa obtained IC_{50} values of 65.57 and 53.68 µg/ml, respectively. Free radicals were significantly reduced by the Boerhavia diffusa extracts, with an IC_{50} value of 53.68 µg/ml. Conversely, the Cassia fistula extract showed a lower reduction in free radicals, with an IC_{50} value of 81.41 µg/ml.

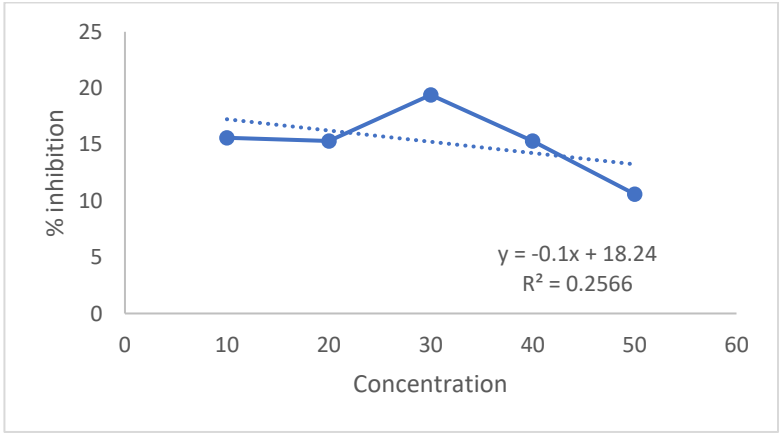

Figure 8. Scavenging effect of ethanol extract of *Boerhavia diffusa* on reducing power assay

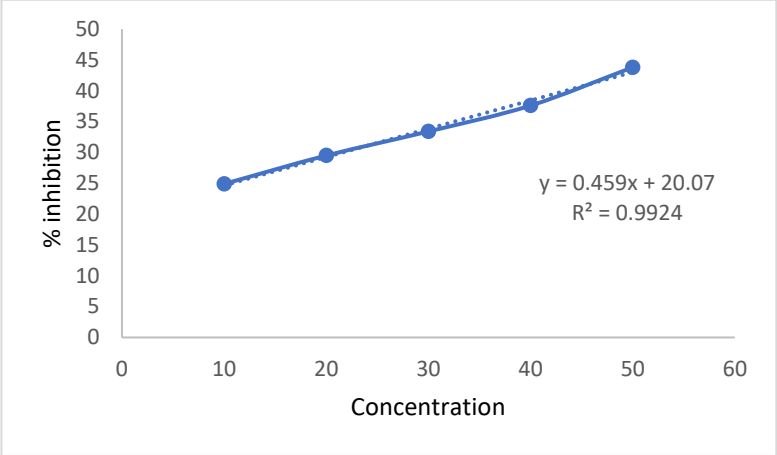

Figure 9. Scavenging effect of *Cassia fistula* on reducing power assay

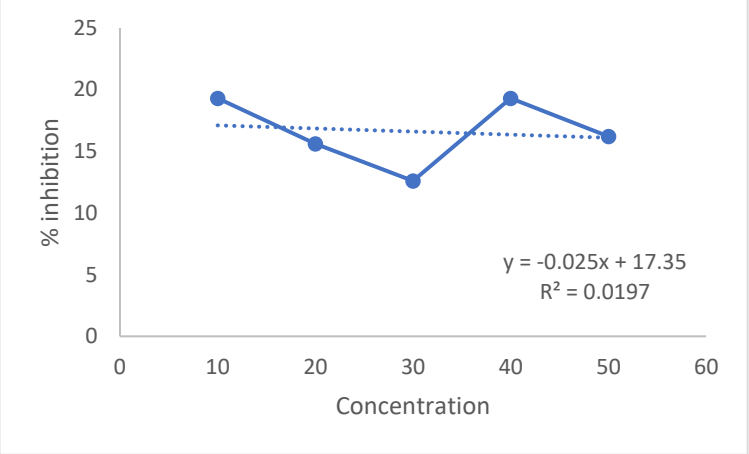

Figure 10. Reducing power assay of ethanol extract of *Cassia fistula*

4. CONCLUSION

This study shows Cassia fistula and Boerhavia diffusa's antioxidant and antidiarrheal capabilities. Multiple antioxidant tests indicated free radical scavenging activity in all fractions, including crude extract (ethanol extract). The ethyl acetate fractions scavenged free radicals better than the crude extract and all other fractions in every configuration. Polyphenols—including phenolic and flavonoid compounds—concentrated during fractionation make the ethyl acetate fraction effective at neutralizing free radicals. It is well known that free radicals affect several diseases. Cassia fistula and Boerhavia diffusa also fight diarrhea. This new information supports the idea that the plant's historic use as a cure originates from its free radical-fighting properties.

5. REFERENCES

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