

Original Research Article

ANTI-OXIDANT EFFECTS OF CHROMATOGRAPHIC FRACTIONS OF *ABRUS PRECATORIUS* LEAVES (LINN) LEAF EXTRACT IN WISTAR RATS

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ABSTRACT

Aims: Plant antioxidants are considered safe, nutritional, and therapeutic, offering promising solutions to oxidative stress-related health issues. This study explores the antioxidant potential of column fractions derived from the methanol extract of *Abrus precatorius*, a plant with limited prior evaluation of its bioactive fractions.

Study design: The study used an experimental design

Place and Duration of Study: Orlu LGA, Imo State, Nigeria

Methodology: Plant materials were collected from Orlu LGA, Imo State, Nigeria. The dried, powdered leaves were screened for phytochemical constituents using standard methods. Methanol extract was obtained through cold maceration and further fractionated using column chromatography packed with silica gel (60–120 mesh), with elution performed using a gradient mixture of hexane, ethyl acetate, and methanol. The phytochemical analysis confirmed the presence of alkaloids, flavonoids, tannins, cardenolides, and saponins, compounds known for their antioxidative properties. Column chromatography yielded five fractions (F1–F5), which were assessed for antioxidant activity using both in vivo (rat models) and in vitro methods, including 2,2-diphenyl-1-picryl hydrazyl (DPPH) and nitric oxide assays.

Results: Notably, fraction F1 demonstrated a significant increase in glutathione and catalase activity, while F4 showed a marked elevation in superoxide dismutase activity in erythrocytes. In liver tissue, F1 and F5 showed exceptional catalase activity, with respective increases of 258% and 290%. Fraction F3 exhibited a 290% increase in superoxide dismutase activity. Furthermore, F3 effectively reduced DPPH radical activity with an IC50 of 0.2 mg/mL, while F4 significantly lowered nitric oxide levels with an IC50 of 0.3 mg/mL.

Conclusion: This study highlights the novel identification of potent antioxidant fractions from *Abrus precatorius* methanol extract, offering valuable insights for developing plant-based therapies for oxidative stress-related disorders such as cardiovascular diseases, diabetes, and neurodegenerative conditions. It underscores the plant's potential as a safe, natural antioxidant source for further pharmacological exploration.

Keywords: Antioxidant, *Abrus precatorius*, LINN, Orlu LGA

1. INTRODUCTION

Medicinal plants have been used for centuries as an alternative to synthetic medicine, with many people relying on their therapeutic properties for treating various diseases. Traditional medicine has played a significant role in the search for new cures, especially as diseases become increasingly resistant to modern treatments and concerns about cost and side effects grow. Approximately 400 million people worldwide have utilized herbal therapy, with 80% incorporating it into their routine for managing health issues (Balakrishnan et al., 2011; Olajide et al., 2013; Ogbuehi et al., 2015; Olajide et al., 2013). Oxidative stress, caused by reactive oxygen species, is a major factor in chronic and degenerative diseases. It can damage biological molecules and lead to infertility issues. Antioxidants play a key role in

averting oxidative damage and associated diseases. In relation to female reproduction, oxidative stress is linked to infertility and conditions like endometriosis (Veeru et al., 2009; Oladimeji et al., 2014; Chisolm, 2000; Wood et al., 2006; Hirst et al., 2008).

Studies have shown that antioxidants prevent oxidative damage by forming nonreactive compounds when they observe free radicals, thus preventing alteration of metabolic processes and rapid cellular damage, which are factors associated with diseases, aging and cancer (Chisolm, 2000; Wood et al., 2006; Hirst et al., 2008; Oladimeji et al., 2014). The role of oxidative stress in female reproduction has gained enormous interest, particularly because of the idiopathic linked infertility which is very common among women of reproductive age and married couples. Additionally, it has been implicated in some diseases of the reproductive system such as endometriosis (Oladimeji et al., 2014). Antioxidant therapy is a popular therapeutic approach aimed at fighting the harmful property of reactive oxygen species (ROS) and their associated diseases. Research has shown that phytochemicals found in plants and vegetables possess strong antioxidant properties, making them effective in protecting cells against ROS produced during oxidative stress (Veeru et al., 2009; Oladimeji et al., 2014).

According to the WHO, the percentage of chronic illnesses linked to oxidative stress was projected to reach 64% by 2030 in a 2002 report. However, a 2017 report indicated that this number had already reached 70%, underscoring the urgent need for research into antioxidant agents to combat oxidative stress. Some plants are known for their antioxidant properties, and *Abrus precatorius*, a plant with pharmacological significance, may also exhibit such potential. Traditionally, the leaves of *Abrus precatorius* have been used in treating various diseases, highlighting their potential therapeutic benefits.

Further investigation into the antioxidant potential of *Abrus precatorius* leaves is crucial for advancing oxidative stress management strategies. Conducting a study using chromatographic fractions of *Abrus precatorius* will help determine how further purification of the plant's crude extract affects its antioxidant activity, particularly in animal models such as rats.

The following objectives were formulated to guide this study:

1. To separate the crude extract of *Abrus precatorius* into fractions using column chromatography.
2. To carry out phytochemical screening of the fractions.
3. To determine the minimum dose at which these fractions exhibit antioxidant activity.
4. To investigate the antioxidant properties of the fractions in Wistar rats.

The findings from this study have significant implications for health and therapeutic settings. If the antioxidant activity of *Abrus precatorius* fractions is confirmed, these fractions could be developed into natural antioxidant therapies to manage oxidative stress-related diseases such as cardiovascular disorders, neurodegenerative diseases, diabetes, and infertility. Additionally, the plant's pharmacological properties may provide a safer alternative to synthetic antioxidants, reducing side effects and offering an affordable option for patients in resource-limited settings. Such advancements could also support the development of functional foods or supplements designed to improve oxidative stress resistance and overall health.

2. EXPERIMENTAL DETAILS

2.1 Materials used

The materials and experimental reagents utilized in this study included a range of laboratory equipment, chemicals, and specialized instruments. The primary reagent, DPPH (2,2-Diphenyl-1-picryl hydrazyl), was procured from Sigma Aldrich in St. Louis, USA. Other essential laboratory tools included 1ml syringes, 100ml beakers, 100ml volumetric flasks, sterilized latex gloves, universal containers, surgical blades, chromatography tanks, thin-layer chromatography plates, desiccators, and fume cupboards.

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Key instruments used in the experimental procedures were an ultraviolet spectrophotometer (Search Tech N4), cuvettes, a water bath, a rotary evaporator (DFS KW 1000DB, Techmel and Techmel, USA), and a weighing balance (Ohaus Adventurer™). Additional items included filter papers, funnels, white muslin cloth, crucibles, cotton wool, chromatography needles, and spatulas.

Reagents and solvents employed in the study comprised ethyl acetate, methanol, n-hexane, dimethyl sulfoxide (DMSO), vitamin C (100mg, Emzor), silica gel, distilled water, α -naphthyl-ethylenediamine, and ferric chloride. These were used alongside other materials, such as a chromatography tank, desiccators, and appropriate storage conditions in a laboratory fridge, to ensure optimal experimental outcomes.

2.2 Plant Material and its preparation

The plant, *Abrus precatorius* leaves were freshly harvested from Umuowa in Orlu LGA of Imo State in the month of November 2016 from a particular location and were identified at the Department of Plant Science & Biotechnology, in the Faculty of Science, University of PortHarcourt. The voucher specimens UPH/V/1258 were deposited at the Departmental Herbarium for further reference purpose.

Fresh leaves of *Abrus precatorius* were physically cleaned, separated, and dried in the Pharmacognosy Laboratory at the University of Port Harcourt. The samples were spread thinly on lab benches and dried with fans for 10 days. After drying, the samples were sieved and reduced into a powder form using an electric blender. The powdered sample was then stored in an airtight container. A total of 360g of the powdered sample was extracted with 70% ethanol for 72 hours. The extract was filtered and concentrated using a rotary evaporator at 45°C. The final extract was stored in a refrigerator at 4°C until further use.

2.3 Phytochemical analysis of crude extracts of *Abrus precatorius* leaves

The methanolic crude extracts of *Abrus precatorius* leaves were subjected to qualitative phytochemical analysis. The following tests were performed.

Test for Alkaloids

A 0.5g of *abrus precatorius* leaf extract was tested for the presence of alkaloids using various reagents such as Drangedorff's, Mayer's, and Hager's. Precipitates formed with the reagents indicated the presence of alkaloids in the extract. (Harborne, 199; Sofowora, 1993)

Test for Flavonoids

A 0.53g of the powdered sample was detained with acetone. Acetone was evaporated by placing the simple on a water bath. Boiling distilled water was added to the detained sample. The mixture was filtered while hot and 5mls of dilute sodium hydroxide was added to equal volume of the filtrate when cooled. A yellow solution was recorded as indication of the presence of flavonoids (Ogbuehi *et al*, 2015).

Test for Tannins

After agitating 5g of the sample extract with 10ml of distilled water, it was filtered, and ferric chloride reagent was added to the filtrate. It was believed that a blue-black staining constituted proof of tannins (Sofowora, 1993).

Test for Anthraquinones

Ten milliliters of benzene were mixed with five grams of extract from *Abrusprecatorius* leaves. After filtering, 5 milliliters of 10% ammonia solution (Born Trager's test) was added to the filtrate. Free hydroxyl-anthraquinones were present when the mixture was agitated and the ammoniacal (Lower) phase took on a pink or red hue.

Test for Triterpenoid/Steroids

A 0.1g of the sample extract was dissolved in 1ml of chloroform and it was filtered. The filtrate gotten was treated with few drops of concentrated sulphuric acid shaken and allowed to stand. A green yellow colour was indicated and appeared as an evidence for the presence of triterpenes.

Test for Saponins

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A 0.5g of the sample extract was shaken with 10mls of distilled water in a test tube frothing which persisted a warming was an evidence for the presence of saponins.

Test for Glycosides (Lieberman's Test)

About 0.5g of the sample extract was dissolved in 2ml of acetic anhydride and cooled well in ice. Sulphuric acid was added to the test and a violet to blue to green colour change was indicative of the presence of a steroidal nucleus (i.e. aglycone portion of the cardiac glycoside).

Test for Carbohydrate (Molisch's Test)

A 0.1g of the sample extract was weighed and two drops of alcoholic anaphthol solution was added violet colour formation at the junction indicates the presence of starch.

Test for Carotenoids

A 0.5g of sample extract was extracted with 10ml of chloroform in a test tube and was vigorously shake. The resulting mixture was filtered and 85% tetraoxosulphate(XI) acid was added. A blue colour at the interface was indicative of carotenoids.

2.4 Acute Toxicity study on the of crude extracts of *Abrus precatorius* leaves

The acute toxicity study was conducted following Lorke's method (Lorke, 1983) using 13 rats weighing 200-280kg. The study consisted of two phases: in the first phase, 9 rats were divided into 3 groups and administered different doses of the crude extract (10, 100, and 1000mg/kg) after an 8-hour fasting period. The rats were observed for toxicity and mortality. In the second phase, 4 rats were individually administered higher doses (1500, 2500, 3500, and 5000 mg/kg) and monitored for toxic effects.

2.5 Fractionation and screening of the methanolic crude extracts of *Abrus precatorius* leaves

2.5.1 Vacuum Liquid Chromatography (VLC)

The VLC with a 4.5 cm diameter was packed with silica gel (60-120 mesh) to a height of 15 cm. 20_g of pre-absorbed extracts was loaded onto the column and fractionated using gradient mixtures of n-hexane, ethyl acetate, and methanol. Five fractions (F1-F5) were obtained and tested on animal and in vitro models.

2.5.2 Qualitative Thin Layer Chromatography (TLC)

Qualitative screening was done on the n-hexane extract and fractions using thin layer chromatography (TLC). Silica gel GF254 plates were used with different solvent systems. The plates were marked and air dried before being placed in chromatographic tanks. After development, the plates were air dried and viewed under daylight and UV.

2.6 Determination of Appropriate Mobile Phase

The crude extract and fractions obtained from the column were spotted on analytical TLC plate (silica gel G (0.25 mm). The plates were developed in the following mobile phases to determine the most appropriate.

Hexane: Ethyl acetate: Methanol (10:5:5)

Hexane: Dichloromethane: methanol (10:25:5)

Pet ether: Chloroform: Methanol (2: 1:1)

Hexane: Dichloromethane (10: 30)

2.7 Determination of Appropriate Spray Reagent

The spray reagents prepared are;

a) Ferric chloride / Sulphuric acids: For detecting phenolics. Spray with a solution of 2_g FeCl₃ in 83_mL n-butanol and 15_mL conc. sulphuric acid. Heat at 110_°C View at 5 min intervals to see if spots appear at 254 and 360nm .Heat until spots are green or violet.

b) P -Anisaldehyde / Sulphuric acid:

For detection of phenols steroids, and terpenes. A solution of freshly prepared 0.5ml p-anisaldehyde in 50ml glacial acetic acid and 1ml conc. Sulphuric acid was sprayed. Heat to 105_°C until maximum visualization of spots. The Components give violet, blue, red, grey or green spots.

c) Dragendorff's spray:

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For detecting alkaloids and quaternary nitrogen compounds 0.11 gm of potassium iodide and 0.18 gm bismuth sub nitrate (OBiNO_3) was dissolved in 20 mL acetic acid and make up to 100 mL.

d) 70% H_2SO_4

The 70 % H_2SO_4 was sprayed and activated at 100 °C for 5-10 minutes in an oven. A brown or black colour indicates a positive result.

e) Vanillin /Sulphuric acid

For detection of steroids. Dissolve 1 gm of vanillin in 100 mL conc. sulphuric acid and spray plates. Dry at 120 °C until.

2.4 Treatment of experimental animal and protocol for sacrificing

This study involved 40 rats of both male and female sex weighing between 180-300 grams. The rats were obtained from the animal house of the department of Pharmacology at the University of Port Harcourt. They were acclimatized for 2 weeks with free access to food and water. The rats were randomly divided into eight groups of five each. Group 1 was the control, Group 2 received DMSO orally, Group 3 received 100 mg/kg of vitamin C, and Groups 4-8 received different fractions orally at a dose of 40 mg/kg for 28 days in a sub-acute study.

The rats were sacrificed 24 hours after the last administration on the 28th day. This was done under di-ethyl ether as anesthesia using the jugular puncture and the blood samples were collected into nonheparinized bottles and centrifuged. The serum was further collected for hematological studies. The liver was collected and rinsed with water to remove blood clot. It was then kept in a universal container with normal saline.

2.8 Determination of anti-oxidant effects

2.8.1 Determination of Reduced Glutathione (GSH) Level

The method outlined by Sedlack and Lindsay (1968) involved adding a small sample to distilled water followed by the addition of a precipitating solution (5% sulphosalic acid). After mixing and centrifugation, the filtrate was combined with phosphate buffer and DTNB. Readings were taken at 416 nm using a UV spectrophotometer to estimate GSH levels in $\mu\text{gGSH}/\text{min}/\text{mg}$ protein.

2.8.2 Determination of Catalase Activity (CAT)

Catalase activity was tested using a sample of 50-100 μg protein in 100 mM phosphate buffer with 100 mM H_2O_2 . Absorbance at 546 nm was measured after 5 minutes at 37 °C. Activity was calculated using H_2O_2 molar extinction coefficient. One unit of catalase activity was defined as the amount of protein converting 1 μmol $\text{H}_2\text{O}_2/\text{min}$. Samples were analyzed in quadruplicate.

2.8.3 Determination of Superoxide Dismutase (SOD)

The activity was determined using the Misra and Fridovich method. Tissue homogenates were diluted and mixed with carbonate buffer and epinephrine. Absorbance was measured at 516 nm every 25 seconds for 5 minutes.

2.8.4 Determination of Malondialdehyde (MDA)

A small sample of 0.5 ml of supernatant was mixed with 1.8 mL of Tris-KCL buffer and 0.6 mL of 30% TCA was added. 0.6 mL of 0.75% TBA were also added and was boiled for 1 hr in a boiling water bath. It was allowed to cool and centrifuged at 3000 g. The absorbance of the clear supernatant sample was measured against the reference blank of distilled water at 512 nm. The MDA level was determined using Todorova *et al* (2005) method and the result was expressed as $\mu\text{mol MDA}/\text{mg}$ protein.

2.8.5 Determination of Nitric Oxide Scavenging activity

The experiment involved using Griess reaction to determine the nitric oxide scavenging activity of *Abrus precatorius* fractions. Sodium nitroprusside (SNP) and *Abrus precatorius* fractions were mixed in phosphate buffer. After incubation, Griess reagent was added and the absorbance was measured at 546 nm. Nitric oxide scavenging activity was calculated using a formula.

2.8.6 Determination of DPPH Radical Scavenging activity

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A 2 mL of various concentration of *Abrus precatorius* fractions, 1.5 mL of DPPH 0.1Mm was added to the various concentrations. Methanol and DPPH in equal amount served as the control. After 20 mins incubation in the dark, absorbance was taken at 516 nm. The experiment was carried out in triplicates. The percentage scavenging of *Abrus precatorius* was calculated

Scavenging activity (%) = $100 \times (A_o - A_s) / A_o$

A_o = absorbance of the blank and A_s = absorbance of the sample.

2.9 Statistical Analysis

This was done using t-test and p value of p < 0.05 was taken as significant value

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3. Results and discussion

3.1 Results of Preliminary Phytochemical Screening of the Plant Extract:

Phytochemical screening of the methanol leaf extract of *Abrus precatorius* by qualitative study showed the presence of alkaloids, triterpenes, flavonoids, tannins, saponins and carbohydrates. This is presented in table 1.

Table 1: Phytochemical constituents of Methanol extract of *Abrus precatorius* leaf extract

A. <i>precatorius</i> leaves extract	Present/Absent
Alkaloids	+ve
Flavonoids	+ve
Tannins	+ve
Anthraquinones (Bontragers test)	-ve
Triterpenoid/ Steroids	+ve
Fixed oils	+ve
Carbohydrates	+ve
CardenolideKedde test	+ve
Keller Killuni test	-ve
Cyanogenic glycosides	-ve
Saponins	+ve

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Table 2: Blood Enzymatic and Non-enzymatic Parameters of Rats Treated with Fractions of *Abrus precatorius* for 28 days

GROUPS	TREATMENT	PARAMETERS	GSH	MDA	CAT	SOD
1	CONTROL		1.56±0.18	0.78±0.06	18.10±1.14	0.27±0.01
2	F1		2.73±0.11	2.07±0.52	34.20±0.84	0.26±0.04
3	F2		2.39±0.37	2.64±0.26	21.80±3.26	0.26±0.02
4	F3		1.18±0.03	2.12±0.18	10.55±2.64	0.31±0.04
5	F4		1.28±0.10	1.46±0.39	9.15±1.23	0.41±0.10
6	F5		1.67±0.06	2.70± 0.30	22.02±3.58	0.11±0.00
7	STD DRUG		2.56±0.04	1.73±0.16	13.13±2.39	0.23±0.05
8	DMSO		2.56±0.03	2.11±0.15	18.55±6.85	0.15±0.05

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Each value is represented as Mean ± Standard Error of Mean of five rats; represent values not significantly different from the control (p>0.05).

DTSLD = Distilled water; DMSO = Dimethyl sulfoxide; F1-F5 = Fractions 1 to 5; STD = Standard; GSH = Reduced glutathione; MDA = Malondialdehyde; CAT = Catalase; SOD = Superoxide dismutase.

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From the result of the blood Table 2, fraction F1, F2 and F5 increased the GSH, MDA and CAT except the SOD of the rats. Fraction F3 and F4 exhibited increased MDA, CAT and SOD except the GSH of the rats when they are all compared with the control.

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Table 3: Liver Enzymatic and NonEnzymatic Parameters of Rats Treated with Fractions of *Abrus Precatorius* for 28 days

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GROUPS	TREATMENT	PARAMETERS			
GSH	MDA	CAT	SOD		
1	CONTROL	1.33±0.01	0.27±0.01	3.30±0.01	0.30±0.01
2	F1	1.71±0.03	0.25±0.07	11.83±2.00	0.14±0.02
3	F2	1.63±0.02	0.63±0.07	7.39±0.86	0.16±0.05
4	F3	1.51±0.08	0.26±0.13	4.97±0.16	0.16±0.03
5	F4	1.53±0.13	1.11±0.01	4.97±0.88	0.27±0.05
6	F5	2.31±0.16	0.60±0.01	13.80±1.15	0.21±0.01
7	STD DRUG	2.10±0.28	0.25±0.05	13.90±1.36	0.19±0.06
8	DMSO	1.73±0.05	0.16±0.02	8.45±0.45	0.01±0.00

Each value is represented as Mean ± Standard Error of Mean of five rats; represent values not significantly different from the control ($p < 0.05$)
 DTSLD = Distilled water; DMSO = Dimethyl sulfoxide; F1-F5 = Fractions 1 to 5; STD = Standard; GSH = Reduced glutathione; MDA = Malondialdehyde; CAT = Catalase; SOD = Superoxide dismutase.

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Table.3 revealed that fraction F1 toF5 increased the GSH, MDA and CAT of the liver tissues of the rats significantly except the SOD.

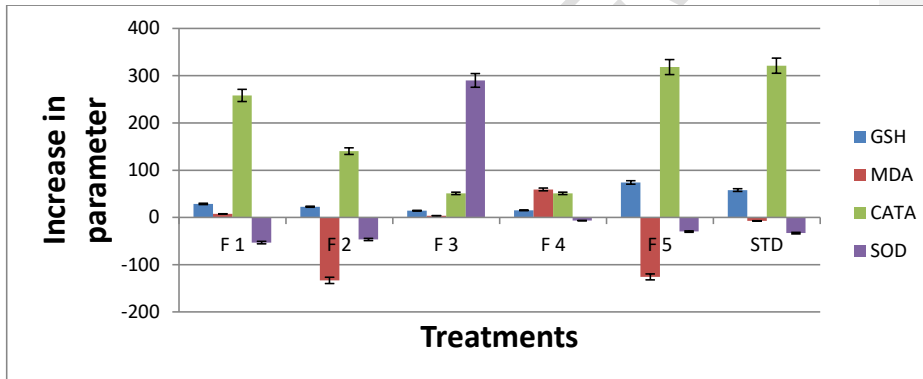


Fig 1: Percentage increase in serum antioxidant parameters

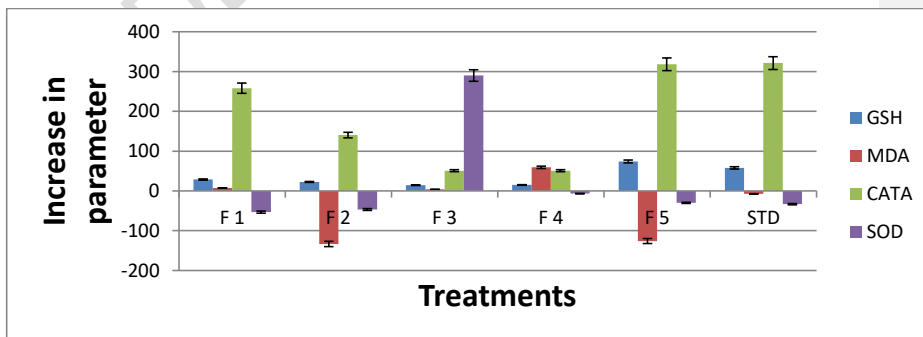


Fig 2: Percentage Increase in Liver antioxidant Parameters

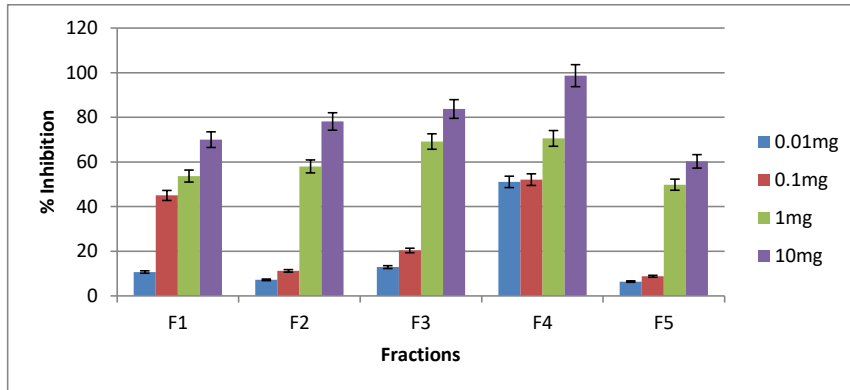


Fig 3: DPPH Radical scavenging activity

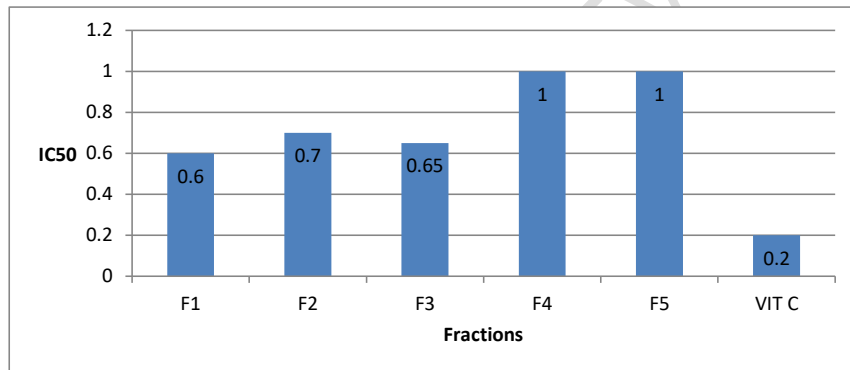


Fig 4: Bar Chart Representing the IC50 of Fractions of Scavenging DPPH

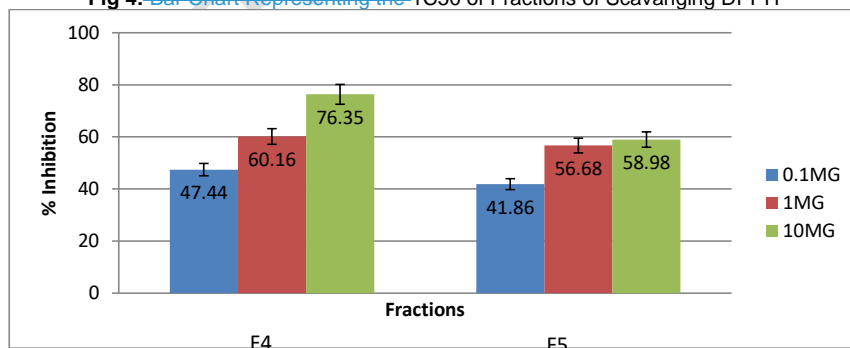


Fig 5: Nitric Oxide Scavenging activity

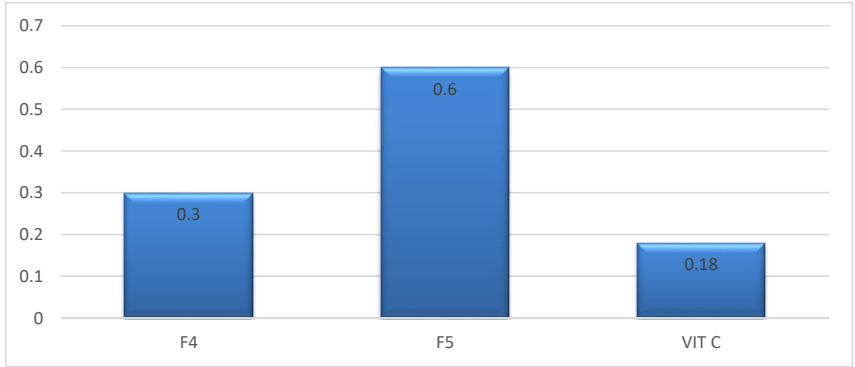


Fig 6: Bar Chart Representing the 1C50 Of Fractions Of Scavanging Nitric Oxide

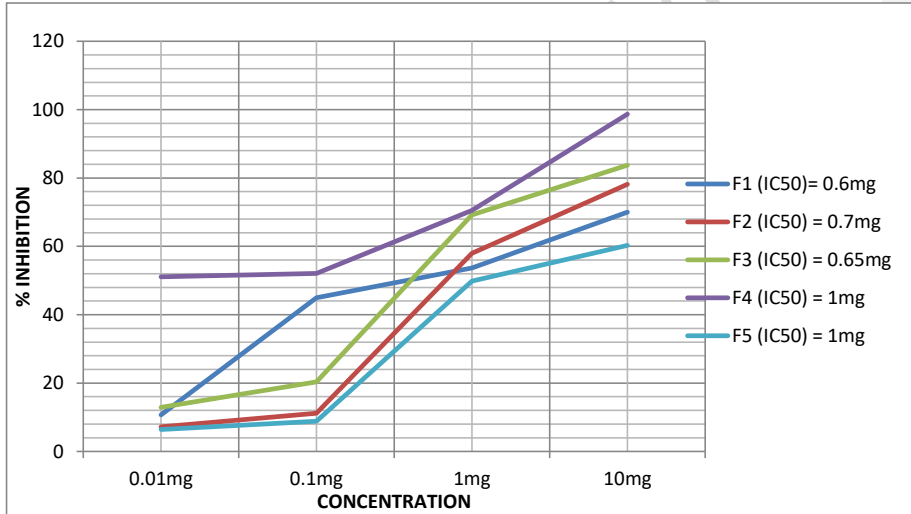


Fig 7: Inhibitory Concentration of DPPH

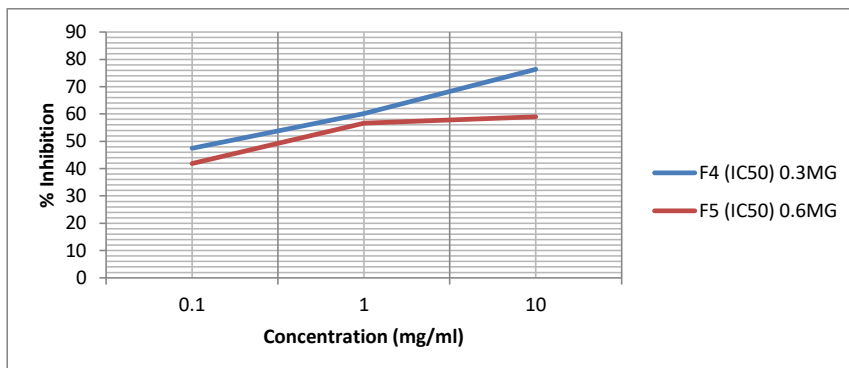


Fig.8: Inhibitory Concentration Of Nitric Oxide

DISCUSSIONS

Plants and plant products have demonstrated significant potential as sources of medicinal agents, but concerns about their pharmacological properties and safety have limited their integration into conventional medicine. This study explored the antioxidant effects of *Abrus precatorius* in rats, revealing the presence of flavonoids that suggest antioxidant activity. Acute toxicity tests indicated that the plant is safe, as no abnormal behavior was observed even at high doses.

Free radicals are known to damage tissues and biomolecules, contributing to the development of various diseases. Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione play vital roles in protecting cells from oxidative stress. The study demonstrated that certain fractions of *A. precatorius* increased the levels of these enzymes, indicating strong antioxidant activity in both blood and liver. Fraction F3, in particular, showed promise as an anti-inflammatory agent due to its protective effects on the liver. Overall, the findings suggest that the plant fractions possess antioxidant properties through their modulation of liver and blood enzyme activities.

The radical scavenging activity of the plant extracts was evaluated using the DPPH assay, with all fractions displaying dose-dependent activity. Fraction F3 exhibited the highest radical scavenging capacity, as evidenced by a 300% increase in SOD levels during in vivo assays. Additionally, nitric oxide scavenging activity was assessed for fractions F4 and F5. Fraction F4 showed significant inhibition of nitric oxide, with a 73.6% reduction observed at a dose of 10 mg/mL, further supporting its antioxidant potential.

Despite these promising findings, the study has certain limitations. The results are based solely on animal models, which may not fully replicate human biological responses. Moreover, only acute toxicity was evaluated, leaving questions about chronic toxicity and long-term safety unanswered. The focus on a limited number of fractions and antioxidant markers means other potential bioactive compounds and mechanisms may have been overlooked. Furthermore, the specific pathways underlying the observed antioxidant effects were not explored in detail.

To address these limitations, future research should include clinical studies to validate the antioxidant and therapeutic properties of *A. precatorius* fractions in humans. Investigating the chronic toxicity and long-term safety of the plant fractions will be essential. Additionally, studies should focus on identifying other bioactive compounds in *A. precatorius* and elucidating their pharmacodynamic mechanisms. The effectiveness of these fractions in managing oxidative stress-related diseases in humans, such as cardiovascular disorders, neurodegenerative diseases, and infertility, should also be explored. Finally, developing

formulations of these fractions, such as dietary supplements or functional foods, could pave the way for practical pharmacological applications.

While the study highlights the promising antioxidant properties of *A. precatorius* fractions, their implications for human health and pharmacological applications remain minimal at this stage due to the lack of clinical trials. Further research is needed to establish their safety, efficacy, and appropriate dosage for human use. However, the findings provide a valuable foundation for exploring *A. precatorius* as a natural source of antioxidant agents with potential therapeutic applications.

CONCLUSION

The various fractions obtained from column chromatography exhibited varied antioxidant activities in both the blood and liver of the rats, as well as in the DPPH and nitric oxide assays. Importantly, all fractions demonstrated antioxidant activity, with significant effects observed on different indices of antioxidant status, except for fraction F2. The major class of compounds likely responsible for this activity were flavonoids, as indicated by the phytochemical screening.

These findings have practical significance in the context of developing natural antioxidant therapies. The identification of flavonoid-rich fractions with proven antioxidant activity provides a promising avenue for creating plant-based treatments aimed at managing oxidative stress-related diseases, such as cardiovascular diseases, neurodegenerative conditions, and metabolic disorders. By isolating and utilizing the active antioxidant fractions, it may be possible to formulate targeted supplements or functional foods that could help mitigate oxidative damage, improve cellular health, and potentially reduce the risk of diseases associated with chronic oxidative stress.

Furthermore, the study highlights *Abrus precatorius* as a potential source of natural compounds that can be harnessed for therapeutic purposes. The clear evidence of its antioxidant potential opens the door to further exploration of this plant in the context of functional medicine, offering a safer, more accessible alternative to synthetic antioxidants, which often come with side effects. Thus, the findings provide a strong foundation for future pharmacological applications, with potential for broad health benefits.

CONSENT

All authors declare that 'written informed consent was obtained from approved parties for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee

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Abraham, Y., Mensah, A. S., Bonsu, A. S., & Fleischer, T. C. (2011). Investigation of the bronchodilator activity of *Abrus precatorius*. *International Journal of Pharmaceutical Sciences Review and Research*, 6(2), 003. Available online at www.globalresearchonline.net.

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Commented [H15]: The entire discussion should be re-written. There are no references in support or against your findings. This is alien to good scientific findings. Discuss your parameters one after the other including the phytochemical results and provide references where necessary.

Commented [H16]: This conclusion did not support your findings. You can not over generalized your findings when you only worked on oxidative stress. All the disorders mentioned were not part of your investigations and should be expunged and strictly state your findings conclusion in simple sentence of one paragraph.

Commented [H17]: Most of your references are too old, kindly update them with latest references form 2019 and above.

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