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Evaluation of the total antioxidant capacity, total polyphenol content, and flavonoids content in ethanolic fruit extract of *Raphia hookeri* and determination of the correlation between the antioxidant effects and the phytochemical contents

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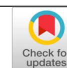
ABSTRACT



In order to assess the total antioxidant capacity (TAC), total polyphenol content (TPC), and total flavonoid content (TFC) in ethanol fruit extract, as well as to ascertain the relationship between the antioxidant effects and phytochemical levels, this study looked at all three variables. 11 groups of 10 rats each were formed out of the experimental animals. All experimental subjects received a single intraperitoneal injection of aluminium chloride, 4.2 mg/kg body weight. Groups 4 and 5 received n-hexane fraction at 10 and 20 mg/kg b. w., respectively, whereas the other groups received various fractions and crude extracts of R. hookeri. Ethyl acetate fraction was given to groups 6 and 7 at doses of 10 and 20 mg/kg b. w. Aqueous fractions at 10 and 20 mg/kg b. w. were given to groups 8 and 9. While groups 10 and 11 got ethanolic crude extract daily for 21 days at doses of 200 mg/kg b. w. and 400 mg/kg b. w., respectively. All groups that were given $AlCl_3$ - induced TBARS showed a significant (P 0.05) increase in vivo antioxidant activity. However, in the liver, kidney, and testes of all treatment groups, superoxide dismutase (SOD) and catalase (CAT) activity considerably increased (P 0.05). This suggests that a significant portion of the antioxidant activity displayed by the ethanolic fruit extract of R. hookeri is due to flavonoids.

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INTRODUCTION

Due to the widespread use of natural extracts as medicines since antiquity, researchers have recently become more interested in learning more about their phytochemical compositions, characteristics, and potential uses in a variety of industries, including cosmetics, food, and agriculture [1]. The majority of plants or herbal remedies used to cure various illnesses contain a variety of chemical compounds; some of these chemical constituents may have positive effects on the body system while others may be

poisonous [2].

In every molecule, a free radical is defined as an unpaired electron that sits alone in an atomic or molecular orbital. This reactive molecule pairs with another electron, starting an uncontrollable chain reaction that can harm a living cell's normal functions and lead to various diseases. The lone electron from free-radical molecules can be abstracted by many fruits and vegetables, herbs, grains, and seeds that contain natural antioxidants, assisting humans in maintaining control over these dangerous species. Anthocyanines, proanthocyanidins, flavans, flavonoids, and their glycosides, carotenoids like -carotene and lycopene, make up the majority of these antioxidants in plants [3]. [3] has also shown that the polarity of these molecules affects the ability to isolate antioxidants from plants.

Raffia palm, also called Raphia hookeri (Rh), is a member of the Palmaceae family. The Hausa call it Tukurwa, the Igbo call it Nkwu, and the Tiv call it Ichor. The plant, believed to have sprung from West African wetlands, is only found in tropical rainforests, which is its perfect biological setting [4, 5].

In recent years, especially in Nigeria, scientific research into the cultivation, management, and commercial products of R. hookeri has drawn more attention. In the southern, eastern, and northern parts of Nigeria, particularly in the states of Taraba and Benue, it was also reported to be a significant source of forested food species. As it is used in herbal therapy to treat a variety of ailments, R. hookeri has also demonstrated helpful therapeutic properties [6]. The root extract is typically administered to infants who are experiencing stomach ache.

Practitioners of traditional medicine assert that R. Hookeri is a liniment and laxative used to treat children's stomachaches. The fruits are deadly if taken raw, according to past reports [7–9], however this perception has led to serious abuse such as prolonged administration without proper dose monitoring, compromising the larger potential for adverse effects. According to a recent investigation, both male and female brood-stocks' reproductive capacities were lowered by the fruit extract [10]. Unfortunately, not all of the therapeutic herbs that are used have had their effects—whether good or harmful—validated by science. One such plant, Raphia hookeri (Rh), has been used for centuries to treat and prevent a number of illnesses [6]. There is a pressing need for its scientific examination because fruits are consumed by people as forest food and are thought to harm reproduction.

MATERIALS AND METHODS

Sample collection and preparation

In Benue State, the fruits of R. hookeri were harvested near the River Buruku. Dr. Ojobo, O. of the Botany Department, Joseph Saawuan Tarka University of Agriculture Makurdi, carried out the identification and validation of the plant sample with voucher number RH 206. Only the healthy fruits were chosen for use after the plant material underwent a thorough inspection for any signs of disease. The fruit's thin, golden mesocarp was extracted after it had been dehulled. The substance was completely cleaned, allowed to air dry at room temperature, and then put through a laboratory manual blender to turn it into a fine powder [11, 12].

Sample extractions

With only minor adjustments, sample extraction was carried out using the technique used by [13] and [14]. 400 grams (g) of powered plant material were macerated in 1600 mL of ethanol for 48 hours with sporadic stirring. Cheese cloth was used to filter the mixture first, and then Whatman No. 1 filter paper. The filtrates were conserved in moisture-free, airtight laboratory containers and concentrated using a rotary evaporator at decreased pressure before being kept in the refrigerator at 4 oC for later use.

Fractionation of Ethanolic fruit extract of R. hookeri

To separate the ethanol extract into its component fractions, it was run through a column chromatograph. The column was packed with silica gel (G60-120 mesh size), and several solvent mixtures with increasing polarity served as the mobile phase.

Packing of column

The packing of the column was completed using [12's approach]. With the help of glass rod, the lowest portion of the glass column was filled with glass wool. The slurry was created by dissolving 75 g of silica gel in 180 mL of pure chloroform. The silica gel-packed chromatographic column, which had a diameter of 30 mm and a height of 400 mm, allowed solvent to flow freely into the conical flask below. When the solvent drained easily without dragging either the silica gel or the glass wool into the tap, the setup was deemed to be in order.

Elution of the ethanolic fruit extract

Little alteration was made to the procedure of [13]. 15 mL of absolute n-hexane were used to dissolve the 2 g of ethanolic fruit extract before applying the mixture to the chromatographic column.

The extract was eluted using an escalating polarity solvent system, starting with n-Hexane, Chloroform, Ethyl acetate, Methanol, Ethanol, and Distilled water.

Solvent combination Ratios

n-Hexane: Chloroform 100:0, 80: 20, 60:40, 40:60, 20:80

Chloroform: Ethyl acetate 100:0, 80: 20, 60:40, 40:60, 20:80

Ethyl acetate: Methanol 100:0, 80: 20, 60:40, 40:60, 20:80

Methanol: Ethanol 100:0, 80: 20, 60:40, 40:60, 20:80

Ethanol: Distilled water 100:0, 80: 20, 60:40, 40:60, 20:80 Distilled water 100

Each time, a determined amount (400 mL) of each solvent mixture was added to the column using a separatory funnel. Beakers were used to collect the eluted fractions in 100 mL aliquots.

Determination of Total Antioxidant Capacity

According to the procedure outlined in [15], the scavenging activity of the plant extract and the fractions produced by the ethanol extract on 1,1-diphenyl-2-picrylhydrazyl (DPPH) was evaluated colorimetrically at 517 nm using Trolox as the standard. For each fraction, the absorbance was calculated in triplicate. Using the regression equation from the calibration curve, total antioxidant capacity (TAC) was determined as mg/mL of trolox equivalent (TE). To create a 0.1 mM, exactly 39.4 mg of DPPH were dissolved in 1L of 80% methanol. 100 L of the sample was pipetted into a cuvette after 2 mL of the DPPH solution.

Determination of Total Phenolic Content

Using the Folin-Ciocalteu reagent and colorimetry at 765 nm as described in [16], the total phenolic content was calculated and represented as gallic acid equivalent (GAE). By combining 20 g of sodium carbonate with 100 mL of distilled water, a precise 20% solution was created. A test tube was filled with 2.5 mL of the Folin-Ciocalteu reagent and 2 mL of a 20% sodium carbonate solution. The sample was introduced to the test tube in an exact quantity of 100 l, and it was let to remain at room temperature for 15–20 minutes. At 765 nm, the absorbance was measured against a blank for the reagent.

Determination of Total Flavonoids

Utilizing the aluminum chloride colorimetric method of [17], flavonoid content was calculated. The calibration curve was derived using quercetin. The amount of total flavonoids was given as mg/mL of quercetin equivalent (QE). By combining 10 g of aluminum chloride with 100 mL of distilled water, approximately 10% aluminum chloride was created. In order to make 1 M potassium acetate, 98.15 g were dissolved in 1L of methanol. A test tube was filled with 1.5 mL of methanol and then 0.1 mL of a 10% aluminum chloride (AlCl3) solution. Exactly 0.5 mL (500 L) of the diluted sample was added to the test tube after 0.1 mL of 1M potassium acetate (CH3COOK) was added. After 30 minutes of room temperature incubation, the reaction mixture's absorbance was measured at 415 nm.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The analysis was performed using a Perkin Elmer Turbo Mass Spectrophotometer (Norwalk, CTO6859 and USA), which is equipped with a Perkin Elmer Autosampler XLGC, in accordance with the instructions provided by [18]. Total ion count (TIC) was used to analyze the data after analysis utilizing electron impact ionization at 70 eV in order to identify and quantify the compounds. The component spectrums were compared to a database of known component spectrums kept in the GC-MS library. Turbo-Mass-OCPTVS-Demo SPL Software was used to measure peak areas and analyse data.

Experimental animals

Organ toxicity induction with Aluminum Chloride and administration of active fractions and crude ethanolic fruit extract of R. hookeri

For the study, male Wistar rats that were 8 weeks old, weighed 80–120 grams, and were in good health were utilized. They were obtained from the breeding colony of the animal house at Benue State University Makurdi. According to the OEC&D's guidelines, 110 Wistar rats were housed in cages for two (2) weeks prior to the experiment's start, where they were given free access to grower mash, Vital Feeds Company Nigeria, and clean tap water whenever they desired. The cages were kept at a constant temperature of 25 oC and had a 12-hour light/12-hour dark cycle. The test animals were randomly divided into eleven (11) groups, each with ten (10) rats. All of the experimental groups received a single intraperitoneal injection of aluminium chloride, 4.2 mg/kg body weight.

The experimental animals were given various R. hookeri fractions and crude extracts, with groups 4 and 5 receiving n-hexane fraction at 10 and 20 mg/kg body weight, respectively. Ethyl acetate fraction was given to groups 6 and 7 at doses of 10 and 20 mg/kg b. w. Aqueous fractions at 10 and 20

mg/kg b. w. were given to groups 8 and 9. While groups 10 and 11 got ethanolic crude extract daily for 21 days at doses of 200 mg/kg b. w. and 400 mg/kg b. w., respectively. While the negative control group received only aluminium chloride, the standard control group received 200 mg/kg b. w. of vitamin C. The experimental animals were fasted overnight, put to sleep with chloroform, and then killed after 24 hours of the last/final administration of the fractions and extracts of R. hookeri. A heart puncture was used to collect the blood sample, which was then divided into plain and EDTA bottles for the plasma and serum, respectively. The samples were centrifuged at 3000 rpm for 5 minutes to get the serum.

Tissue Homogenization

Wistar rats in each group had their liver, kidney, and testes removed aseptically and placed in normal saline. After being weighed, the organ tissues were homogenized in phosphate buffer (1:10 w/v) at pH 7.4, and the supernatants were used to measure the activities of catalase (CAT), superoxide dismutase (SOD), and thiobarbituric acid reactive substances (TBARS) in the organs.

Determination of in vivo Antioxidant activities in Wistar Rats

Estimation of Thiobarbituric Acid Reactive Substance (TBARS)

As a general gauge of lipid peroxidation in biological fluids, the Thiobarbituric Acid Reactive Substances (TBARS) assay has been extensively employed. With adequate handling and storage, it is frequently regarded as a reliable predictor of the degrees of oxidative stress present in biological samples [19]. A test tube was filled with one milliliter of 14% trichloroacetic acid, one milliliter of thiobarbituric acid (0.67%), and fifty microliters of the tissue homogenate. The mixture was then heated to 800C in a water bath for 30 minutes. After being quickly cooled in ice for 5 minutes, it was centrifuged at 3000 x g for 10 minutes. The amount of lipid peroxidation was determined using the molar extinction coefficient of malondialdehyde (MDA), which was measured spectrophotometrically at 535 nm.

Calculation

Molar extinction of MDA = 1.56 x 10^5 $M^{-1}cm^{-1}$

MDA concentration = Absorbance/1.56 x 10^5 $M^{-1}cm^{-}$

Estimation of Superoxide Dismutase (SOD) Activity

The technique outlined by [20] was used to test the activity of superoxide dismutase. A clean test tube was filled with 920 mL of phosphate buffer (0.05 M, pH 7.8) before 40 mL of the sample—tissue homogenate—was added. This procedure was repeated for Samples A2 through A7. By adding 40 L of assay buffer (phosphate buffer 0.05 M, pH 7.8) to another clean test tube, a reagent test (blank without sample) was also made.

The mixtures were shaken and allowed to sit at room temperature for two minutes. Additionally, 40 L of hematoxylin were added to the blank test tubes for the reagent and sample, respectively, and promptly mixed to initiate the auto-oxidation reaction.

A spectrophotometer was used to measure the absorbance of the sample and reagent test (blank) against distilled water at 560 nm every 30 seconds for 5 minutes after the addition of 40 μL 460 of hematoxylin. By comparing the auto-oxidation rates in the presence and absence of the sample, SOD activity was calculated.

SOD activity in the sample was calculated as follows:

Absorbance Reagent testAR=Absorbance Reagent test 2-Absorbance Reagent test 1

Absorbance Sample test AS=Absorbance Sample test 2-Absorbance Sample test 1

% SOD inhibition=1-ASAR $\times$100

SOD activity (U/ml) =1-ASAR$\times$100 $\times$1.25

Estimation of Catalase (CAT) activity

The method outlined by [21] was used to measure catalase activity. A cuvette was filled with working solution (50 mM potassium phosphate buffer, pH 7.0, 1000 L), which was used to calibrate the spectrophotometer at a wavelength of 240 nm.

950 ml of the working buffer (490 ml of 50 mM potassium phosphate buffer, pH 7.0) and 460 mL of 30 mM hydrogen peroxide (H_2O_2) combination were also monitored at 240 nm once every minute for five minutes.

The amount of catalase activity was measured and quantified as (U/mL) of the sample's decomposition rate, which was supplied as (A240 nm/min). ÄA240 nm/min = Change in absorbance per minute

Catalase (U/mL) = (ÄA240 nm/min)/Volume of reaction mixture.

Data analysis

One-way ANOVA was used to analyze the data using the SPSS Statistical Package Version 26.0 (SPSS Inc., IL, USA). Every piece of information was presented as Mean SD, and a difference between groups was deemed significant at p 0.05.

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RESULTS

Total Antioxidant Activity of ethanolic fruit extract of R. hookeri

Result for total antioxidant activity of R. hookeri shows that fraction 24b (Ethanol: Water) with the ratio of 00:100 revealed the highest antioxidant activity of (0.912 mg/mL) followed by fraction 20b (Methanol: Ethanol with ratio of 20:80 with a value of 0.904 mg/ mL, while fraction 15 b (Ethyl acetate: Methanol) with the ratio of 20:80 had the lowest antioxidant activity (0.009 mg/mL) as presented in Figure 1.

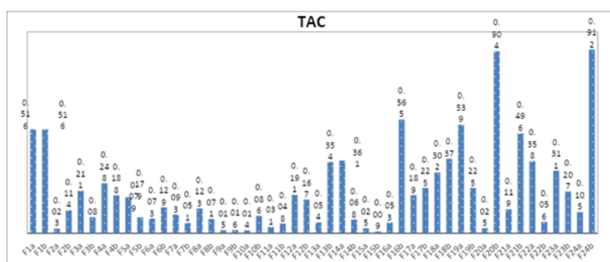

Figure 1: Total Antioxidant Activity of ethanolic fruit extract of R. hookeri

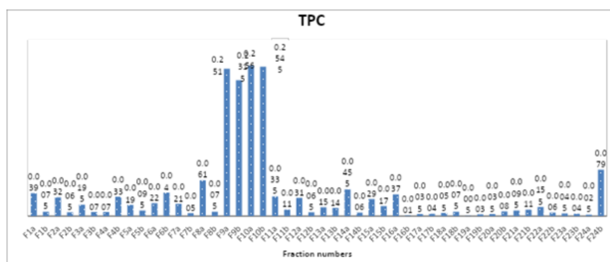

Figure 2: Total Phenolic Content of ethanolic fruit extract of R. hookeri

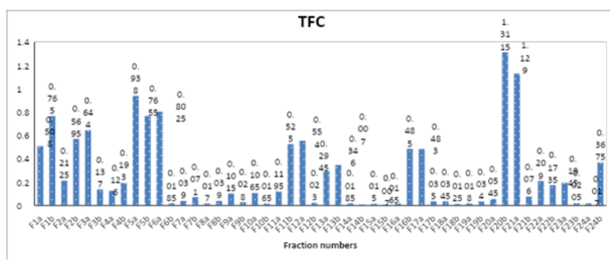

Figure 3: Total Flavonoid Content of ethanolic fruit extract of R. hookeri

In Figure 1, F1= n-hexane: chloroform (100:00), F2=n-hexane: chloroform (80:20), F3=n-hexane: chloroform (60:40) F4=n-hexane: chloroform (40:60) F5=n-hexane: chloroform (20:80), F6=chloroform: ethyl acetate (100:00), F=7 chloroform: ethyl acetate (80:20), F8= chloroform: ethyl acetate (60:40), F9=chloroform: ethyl acetate (40:60), F10= chloroform: ethyl acetate (20:80),

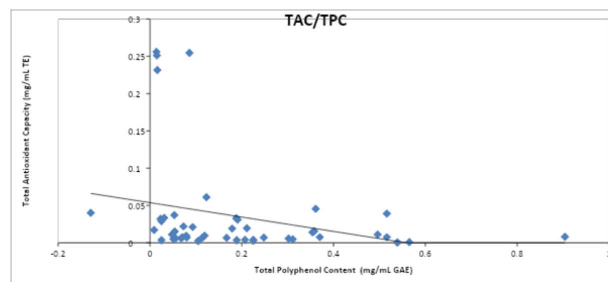

Figure 4: Linear correlation between total antioxidant capacity and total polyphenolic content of different fractions obtained from ethanol fruit extract of R. hookeri

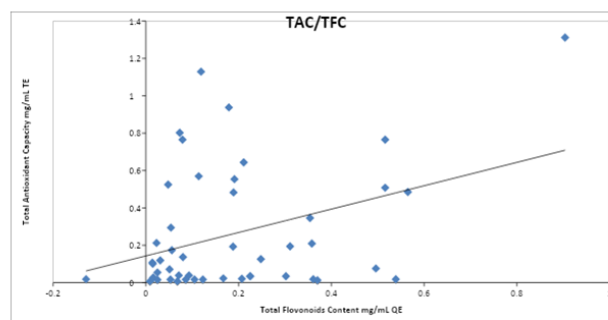

Figure 5: Linear correlation between total antioxidant capacity and total flavonoids content of different fractions obtained from ethanol fruit extract of R. hookeri

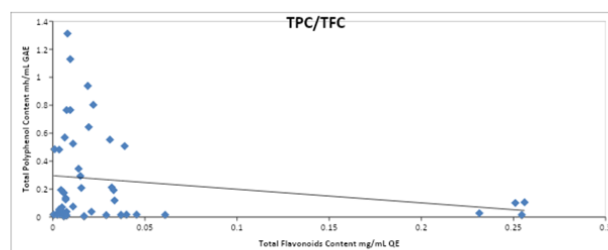

Figure 6: Linear correlation between total polyphenolic content and total flavonoid content of different fractions obtained from ethanol fruit extract of R. hookeri

F11=ethyl acetate: methanol (100:00), F12=ethyl acetate: methanol (80:20), F13=ethyl acetate: methanol (60:40), F14=ethyl acetate: methanol (40:60), F15=ethyl acetate: methanol (20:80), F16=Methanol: ethanol (100:00), F17=Methanol: ethanol (80:20), F18=Methanol: ethanol (60:40), F19=Methanol: ethanol (40:60), F20=Methanol: ethanol(20:80), F21=ethanol: Water (100:00), F22=ethanol: Water (80:20), F23=ethanol: Water (60:40), F24=ethanol: Water (00:100).

Total Phenolic Content of ethanolic fruit extract of R. hookeri

The total phenolic content of the fractions obtained from the ethanolic fruit extract of R. hookeri showed

Table 1: Compounds identified in ethanol fruit extract of Raphia hookeri

Name of Compound	Molecular formula	Molecular weight (g/mol)
Heptadecyl acetate	C19H38O2	298.5
Propanal, 2,3-dihydroxy-, (S)-	C3H6O3	90.08
6-Oxa-3,9-dithiaundecane-1,11-diol	C8H18O3S2	226.4
.beta.-copaene	C15H24	204.35
Benzenebutanal,.gamma.,4dimel-	C12H16O	176.25
Germacrene D	C15H24	204.35
1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*,S*)]-	C15H24	204.35
.beta.-Bisabolene	C15H24	204.35
Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-	C15H24	204.35
Tetradecane, 1-chloro-	C14H29Cl	232.83
2-Butenoic acid, 4-hydroxy-, methyl ester	C5H8O3	116.11
2-Pentadecanol	C15H32O	228.41
Oxalic acid, cyclobutyl tridecyl ester	C19H34O4	326.5
Imidazole, 2-fluoro-5-hydroxy-1-ribofuranosyl-	C8H11FN2O5	234.18
Hexadecanoic acid, methyl ester	C17H34O2	270.5
D-Galactonic acid, .gamma.-lactone	C6H10O6	178.14
Pentane-1,2,3,4,5-pentaol	C10H20O7	252.26
Xylitol	C5H12O5	152.15
Ribitol	C5H12O5	152.15
.beta.-D-Glucopyranoside, methyl 3,6-anhydro-	C7H12O5	176.17
1-Decanol, 2-ethyl-	C12H26O	186.33
5-Methyl-7-amino-s-triazolo(1,5-a)pyrimidine	C6H7N5	149.15
2-Heptadecanol	C17H36O	256.5
17-Pentatriacontene	C35H70	490.9
Supraene	C30H50	410.7
.alpha.-D-Glucose	C6H13O9P	260.14

that fraction 10a (Chloroform: Ethyl acetate) with ratio of 20:80 had the highest phenolic content (0.256 mg/mL), followed by fraction 10 b (0.2545 mg/mL) with the ratio of 40:60, while fraction 16b (Methanol: Ethanol) with ratio of 60:40 had the lowest phenolic content (0.001 mg/mL) as presented in Figure 2.

In Figure 2, F1= n-hexane: chloroform (100:00), F2=n-hexane: chloroform (80:20), F3=n-hexane: chloroform (60:40) F4=n-hexane: chloroform (40:60) F5=n-hexane: chloroform (20:80), F6=chloroform: ethyl acetate (100:00), F=7 chloroform: ethyl acetate (80:20), F8= chloroform: ethyl acetate (60:40), F9=chloroform: ethyl acetate (40:60), F10= chloroform: ethyl acetate (20:80), F11=ethyl acetate: methanol (100:00), F12=ethyl acetate: methanol (80:20), F13=ethyl acetate: methanol (60:40), F14=ethyl acetate: methanol (40:60), F15=ethyl acetate: methanol (20:80), F16=Methanol: ethanol (100:00), F17=Methanol: ethanol (80:20), F18=Methanol: ethanol (60:40), F19=Methanol: ethanol (40:60), F20=Methanol: ethanol(20:80), F21=ethanol : Water (100:00), F22=ethanol : Water (80:20), F23=ethanol : Water (60:40), F24=ethanol : Water (00:100)

Total Flavonoid Content of ethanolic fruit extract of R. hookeri

According to Figure 3, the total flavonoid content of the ethanolic fruit extract of R. hookeri was highest

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Table 2: Results for TBARS, SOD and Catalase in the livers of animals induced with $AlCl_3$ and treated with active fractions and crude ethanolic fruit extract of R. hookeri

Parameters	TBARS (nmol/mg protein)	SOD (u/mL)	CAT (u/mL)
Normal control	0.235 ± 0.007a	0.253 ± 0.002c	0.268 ± 0.038c
Negative control (AlCl3 only)	0.577 ± 0.019b	0.071 ± 0.002a	0.058 ± 0.019a
AlCl3+Vit. C (200 mg)	0.248 ± 0.001a	0.148 ± 0.004b	0.151 ± 0.004b
AlCl3+ n-h 10 mg	0.231 ± 0.006a	0.159 ± 0.005b	0.241 ± 0.003c
AlCl3+ n-h 20 mg	0.269 ± 0.011a	0.147 ± 0.015b	0.212 ± 0.004c
AlCl3+ Eth 10 mg	0.265 ± 0.013a	0.152 ± 0.011b	0.264 ± 0.002c
AlCl3+ Eth 20 mg	0.245 ± 0.004a	0.155 ± 0.004b	0.282 ± 0.003c
AlCl3+ 10 mg aq	0.232 ± 0.021a	0.161 ± 0.011b	0.228 ± 0.002c
AlCl3+ 20 mg aq	0.223 ± 0.006a	0.156 ± 0.021b	0.281 ± 0.001c
AlCl3+ Crude 200 mg	0.215 ± 0.009a	0.160 ± 0.010b	0.287 ± 0.006c
AlCl3+ Crude 400 mg	0.258 ± 0.006a	0.159 ± 0.013b	0.267 ± 0.003c

Each value represents mean ± SD. Result within a column with the same superscript indicate no levels of significance ($P \leq 0.05$), while result within the same column with different superscript indicate level of significance ($P \leq 0.05$)

Table 3: Results for TBARS, SOD and Catalase in the kidneys of animals induced with $AlCl_3$ and treated with active fractions and crude ethanolic fruit extract of R. hookeri

Parameters	TBARS (nmol/mg protein)	SOD (u/mL)	CAT (u/mL)
Normal control	0.313 ± 0.005b	0.152 ± 0.005b	0.266 ± 0.002c
Negative control (AlCl3 only)	0.608 ± 0.047c	0.067 ± 0.003a	0.071 ± 0.009a
AlCl3+Vit. C (200 mg)	0.317 ± 0.010b	0.191 ± 0.002b	0.187 ± 0.004b
AlCl3+ n-h 10 mg	0.309 ± 0.013b	0.156 ± 0.004b	0.165 ± 0.007b
AlCl3+ n-h 20 mg	0.308 ± 0.007b	0.154 ± 0.011b	0.168 ± 0.003b
AlCl3+ Eth 10 mg	0.243 ± 0.013a	0.125 ± 0.003b	0.160 ± 0.005b
AlCl3+ Eth 20 mg	0.212 ± 0.043a	0.163 ± 0.006b	0.174 ± 0.004b
AlCl3+ 10 mg aq	0.238 ± 0.006a	0.182 ± 0.005b	0.159 ± 0.002b
AlCl3+ 20 mg aq	0.263 ± 0.045a	0.191 ± 0.014b	0.161 ± 0.005b
AlCl3+ Crude 200 mg	0.267 ± 0.016a	0.128 ± 0.013b	0.178 ± 0.010b
AlCl3+ Crude 400 mg	0.267 ± 0.012a	0.133 ± 0.010b	0.174 ± 0.001b

Each value represents mean ± SD. Result within a column with the same superscript indicate no levels of significance ($P \leq 0.05$), while result within the same column with different superscript indicate level of significance ($P \leq 0.05$)

Table 4: Results for TBARS, SOD and Catalase in the testes of animals induced with $AlCl_3$ and treated with active fractions and crude ethanolic fruit extract of R. hookeri

Parameters	TBARS (nmol/mg protein)	SOD (u/mL)	CAT (u/mL)
Normal control	0.171 ± 0.003a	0.119 ± 0.036b	0.262 ± 0.002b
Negative control (AlCl3 only)	0.442 ± 0.004b	0.085 ± 0.013a	0.078 ± 0.010a
AlCl3+Vit. C (200 mg)	0.180 ± 0.002a	0.140 ± 0.027b	0.263 ± 0.004b
AlCl3+ n-h 10 mg	0.179 ± 0.010a	0.155 ± 0.005b	0.200 ± 0.005b
AlCl3+ n-h 20 mg	0.185 ± 0.004a	0.162 ± 0.024b	0.292 ± 0.002b
AlCl3+ Eth 10 mg	0.192 ± 0.008a	0.158 ± 0.033b	0.268 ± 0.008b
AlCl3+ Eth 20 mg	0.181 ± 0.002a	0.143 ± 0.128b	0.244 ± 0.007b
AlCl3+ 10 mg aq	0.169 ± 0.013a	0.148 ± 0.166b	0.246 ± 0.003b
AlCl3+ 20 mg aq	0.177 ± 0.002a	0.146 ± 0.110b	0.275 ± 0.006b

Every value corresponds to mean SD. Results within the same column withthe same superscript indicate that there is no level of significance (P 0.05), whereas results within the same column with a different superscript show that there is a level of significance (P 0.05)

in fraction 20b (Methanol: Ethanol) with a ratio of 20:80, followed by fraction 21a (Ethanol: Water), which had a concentration of 1.129 mg/mL with a ratio of 100:00, and lowest in fraction 14b (0.007 mg/mL with a ratio of 40:60).

In Figure 3, F1= n-hexane: chloroform (100:00), F2=n-hexane: chloroform (80:20), F3=n-hexane: chloroform (60:40) F4=n-hexane: chloroform (40:60) F5=n-hexane: chloroform (20:80), F6=chloroform: ethyl acetate (100:00), F=7 chloroform: ethyl acetate (80:20), F8= chloroform: ethyl acetate (60:40), F9=chloroform: ethyl acetate (40:60), F10= chloroform: ethyl acetate (20:80), F11=ethyl acetate: methanol (100:00), F12=ethyl acetate: methanol (80:20), F13=ethyl acetate: methanol (60:40), F14=ethyl acetate: methanol (40:60), F15=ethyl acetate: methanol (20:80), F16=Methanol: ethanol (100:00), F17=Methanol: ethanol (80:20), F18=Methanol: ethanol (60:40), F19=Methanol: ethanol (40:60), F20=Methanol: ethanol(20:80), F21=ethanol : Water (100:00), F22=ethanol : Water (80:20), F23=ethanol : Water (60:40), F24=ethanol : Water (00:100).

Figure 4 illustrates the weakly negative linear connection between total antioxidant capacity and total polyphenol (R2 = -0.255). Total antioxidant capacity and total flavonoids content were linearly correlated, and the association between the two was only marginally positive (R2 = 0.0399) as shown in Figure 5. Figure 6 shows a substantially negative (R2 = -0.602) connection between total polyphenol and total flavonoids based on a linear association between total phenolic content and total flavonoids content (Table 1).

The results of the in vivo antioxidant activities of TBARS, SOD, and CAT of the groups induced with AlCl3 and treated with active fractions and crude ethanolic fruit extract of R. hookeri were shown in Table 2. The outcomes demonstrated a measurable rise in TBARS in all groups exposed to AlCl3. However, when compared to the control, the activity of the enzymes catalase (CAT) and superoxide dismutase (SOD) considerably increased in the livers of all treatment groups.

The results of the groups that were stimulated with AlCl3 and treated with active fractions and crude ethanolic fruit extract of R. hookeri were displayed in Table 3 for the in vivo antioxidant activities of TBARS, superoxide dismutase (SOD), and catalase (CAT). The outcomes demonstrated a measurable rise in TBARS in all groups exposed to AlCl3. However, when compared to the control, the activity of the enzymes catalase (CAT) and superoxide dismutase (SOD) considerably increased in the kidneys of all treatment groups.

The results of the in vivo antioxidant activities of TBARS, SOD, and CAT of the groups induced with AlCl3 and treated with active fractions and crude ethanolic fruit extract of R. hookeri were shown in Table 4. The outcomes demonstrated a measurable rise in TBARS in all groups exposed to AlCl3. However, when compared to the control, the activity of the enzymes catalase (CAT) and superoxide dismutase (SOD) considerably increased in the testes of all treatment groups.

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DISCUSSION

The fruit extract of R. hookeria was subjected to column chromatography using a solvent system of progressively increasing polarity, starting with n-Hexane, Chloroform, Ethyl acetate, Methanol, Ethanol, and ultimately Water. Because it is polar and can extract both hydrophilic and lipophilic materials, ethanol was chosen as the extraction solvent. Due to its high volatility, ethanol is easily removed from materials at room temperature. This is consistent with research on the fractionation and assessment of the total antioxidant capacity, total phenolic, and total flavonoid contents of Vitex doniana leaf extracts in water, ethanol, and n-hexane. Asserted that the optimum solvents for extracting chemicals with antibacterial activity and antioxidant activity, respectively, were ethanol and acetone. This might imply that the selection of solvent is solely based on the substances of interest.

Reactive oxygen species (ROS) are an inevitable byproduct of aerobic metabolism. Free radicals like the superoxide anion (O2●), the hydroxyl radical (● OH), and non-radical molecules like hydrogen peroxide (H_2O_2), singlet oxygen (1O2), and other molecules are examples of reactive oxygen species (ROS). ROS can cause cellular damage to DNA, proteins, lipids, and other macromolecules, impairing physiological function and increasing the risk of developing diseases like cancer, cardiovascular disease, and neurological illnesses as well as the aging process. Asserts that medicinal plants are excellent providers of naturally occurring antioxidants that offer defense against harm brought on by free radicals. Over time, there has been an upsurge in study on medicinal herbs to clarify the mode of action and support claims made by conventional healers. The impact of the active components of the ethanolic fruit extract of R. hookeri has been a focus of this research.

To increase the useful lifetime of industrial products including polymers, fuels, and lubricants, antioxidants are commonly added. For its antioxidant activities, R. hookeri fruit extract was studied using ethanol. According to the results shown in Figure 4 above, fraction 24b (Ethyl acetate: Methanol) exhibits the highest antioxidant activity (0.912 mg/mL), whereas fraction 10 b (n-hexane: Chloroform) exhibits the lowest antioxidant activity (0.014 mg/mL). The plant has a mild antioxidant property, according to the findings of the current study, which can be attributed to the presence of polyphenolic components. Although the ethanolic fruit extract of R. hookeri may have antioxidant properties independent of phenolic components, other substances with antioxidant properties include catechins, vitamins A, C, and E, beta-carotene, lycopene, lutein, selenium, and manganese. The functions of vitamin E in human health and several illnesses were discussed. Vitamin C protects against oxidative stress-induced cellular damage by scavenging reactive oxygen species, vitamin E-dependent neutralization of lipid hydroperoxyl radicals, and by shielding proteins from alkylation by electrophilic lipid peroxidation products, according to in their work on vitamins C and E: Beneficial effects from a mechanistic perspective. The antioxidant functions of R. hookeri's fruits may be demonstrated by their high vitamin content.

A wide class of naturally occurring chemical substances known as polyphenols is distinguished by numerous phenol units. Plants produce a variety of them, and they are widely distributed. Flavonoids, tannic acid, and ellagitannin are some of the polyphenols that have historically been employed as colors and for tanning. Phenolic compounds have the ability to behave as antioxidants and free radical scavengers. Using the Folin-Ciocalteu reagent and the colorimetric method described, the Total Phenolic Content was calculated and represented as a galic acid equivalent (GAE). According to Figure 2 above, the total phenolic content of the ethanolic fruit extract of R. hookeri ranges between 0.256 mg/mL, with fraction 10a having the highest amount and fraction 16 b having the lowest. This demonstrates that n-hexane and chloroform make the optimal solvent combination for its extraction. Reported greater results for the R. hookeri leaf extract's total phenolic content, while the mesocarp had a lower value. The differences between the two experiments' extraction techniques and solvents, as well as potential ecological variances in the plants used, may be to blame. Using High Performance Liquid Chromatography-Mass Spectroscopy (HPLC-MS), Microwave Assisted Extraction (MAE), Pressurized Liquid Extraction (PLE), and Supercritical C02 Extraction (SC- CO2), more than 100 phenolic constituents with an array of biological activities can be thoroughly identified in plants.

Plants contain a group of secondary polyphenolic compounds known as flavonoids, which are frequently included in human diets. One of the most significant natural phenolics is flavonoids, which are frequently present in natural goods. Aluminium chloride (AlCl3) was used to estimate the amount of total flavonoids present in the ethanolic fruit extract of R. hookeri. Flavonoids were measured in milligrams per millilitre of quercetin equivalents. The ethanolic fruit extract of R. hookeri contained total flavonoids in concentrations ranging from 1.3115

mg/mL to 0.015 mg/mL, with fraction 20 b having the highest and fraction 14 b having the lowest value. Different solvent mixtures with varying degrees of polarity were employed for the elution, but chloroform: ethyl acetate produced the best results. This could be the cause of the changes in total flavonoid concentration among the fractions.

The above-mentioned result indicates that there is a positive correlation between total antioxidant capacity and total flavonoid content in Figure 5 (R2 = 0.0399), whereas there is a negative correlation between total antioxidant capacity and total polyphenol content in Figure 4. This suggests that flavonoids, rather than phenols, are entirely responsible for the antioxidant activity displayed by the plant. Figure 6 shows a negative connection (R2= -0.602) between the total polyphenol content and flavonoids content.

The sample's Gas Chromatography-Mass Spectrometry (GC-MS) profile identified twenty-seven (27) different bioactive chemicals. Beta is the chemical with the second-highest peak percent, followed by 1, 3-Cyclohexadiene, 5-(1, 5-dimethyl-4-hexenyl)-2-methyl.Cyclohexene, 3-(1, 5-dimethyl-4-hexenyl)-6-methylene, and bisabolene. In nature,.beta.-Bisabolene is mostly utilized in fragrances and citrus flavors for beverages. It has anti-cancer properties and can be utilized to examine breast cancer. An antibacterial and antifungal agent is cyclohexene, 3-(1, 5-dimethyl-4-hexenyl)-6-methylene. Other bioactive substances include Xylitol (used as a sugar alternative and food additive), Germacrene D (a natural essential oil having antioxidant action and cytotoxicity on tumor cells), and others. Middle ear infections can be avoided by taking xylitol as a medication. It is a sweetener found in chewing gums and other dietetic products; ribitol (a water-soluble vitamin involved in the synthesis of flavin mononucleotide-FMN and flavin adenine dinucleotide-FAD that provides protection against oxidative damage); and other dietetic products. Chewing gums of this type are highly recommended by many dentists; Supraene, a chemical that occurs naturally in people, animals, and plants, is a part of various adjuvants that are included in vaccinations to boost the immune response; -D-glucose, which is employed in a variety of cellular metabolic activities and is predominantly stored as starch in plants and glycogen in animals; 17-pentatriacontene, which has been shown to have anti-inflammatory, anticancer, antibacterial, and antiarthritic properties. In IUPAC nomenclature, hexadecanoic acid is also known as palmitic acid. It has a 16-carbon chain and is a fatty acid. It is the most prevalent saturated fatty acid in all living things, including humans, animals, and plants. The biological action of hexadecanoic acid includes anti-oxidants, hypocholesterolemia, nematicides, and pesticides. A treatment for ischemic heart disease is 5-methyl-7-diethylamino-s-triazolo-1, 5-a) pyrimidine (trapidil, Rocornal). Cyclohexanol is a crucial raw material for the polymer industry because it may be used to make various plasticizers and nylons, as well as in tiny quantities, as a solvent.

Free radicals are unfavorable substances that are created naturally by a number of biological processes in our bodies, including breathing, digestion, alcohol and drug metabolism, and the conversion of lipids into energy. The natural antioxidant mechanism in our bodies normally eliminates free radicals. Free radicals can start a chain reaction in the body that can damage the cell membrane, obstruct the activity of key enzymes, prevent cellular processes essential for proper body function, inhibit normal cell division, destroy deoxyribonucleic acid (DNA), and prevent the production of energy if the system is unable to handle them properly. A number of metabolic, chronic diseases, or malignancies have been linked to oxidative stress.

In many different applications, including water purification, food additives, electrical equipment, and fuel additives, aluminum (Al) compounds, alloys, and powders are utilized, all of which have negative effects on both human and animal health. The liver is a vital organ that serves as a hub for many bodily metabolic processes, the breakdown of waste products, and the removal of hazardous chemicals from the body. The elimination of active oxygen species is done at a fundamental level and with consideration for both their beneficial physiological activities and their detrimental effects. Liver diseases are still a severe threat to human health. Redox equilibrium is moved to create oxidation states in the body when there is an excessive buildup of free radicals or when the body is unable to eliminate them. All mammalian tissues, including the kidneys, liver, heart, blood, bones, and brain, collect aluminum. Rat liver dysfunction is brought on by al buildup in the liver, which also results in aberrant bile acid deposition, microsomal cytochrome P450 enzyme system suppression, pro-oxidant/antioxidant imbalance, and inflammatory responses. The modification in their levels is a sign of the alterations in the histological structure of hepatocytes. Plasma enzymes are well-known hepatic biomarkers. The presence of phenols and flavonoids may have contributed to the fact that all treated groups significantly (P 0.05) lowered the quantities of TBARS (nmol/mg protein) in the liver as compared to the positive control

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(0.577 0.019). Alcl3)-induced thiobarituric acid-reactive substances (TBARS) formation, promoting lipid ordering at the water-lipid interface, and preventing AlCl3-induced membrane disruption; (b) all the investigated compounds inhibited lipid oxidation induced by the water-soluble oxidant 2,2′-azobis (2-amidinopropane) (AAPH), with no family-related effects noted. The hydrophilicity of the compounds, the degree of flavonoid oligomerization, and the quantity of hydroxyl groups in the molecule were primarily responsible for the protective actions of the investigated phenolics on membranes. The current findings are consistent with the theory that phenolics' chemical structure influences how they interact with membranes. MDA is the byproduct of lipid peroxidation and a valuable indicator of oxidative stress. Thus, a measure of the antioxidant capacity of exogenously delivered substances is the reduction in the amount of MDA. The increase in SOD activities (u/mL) across all treatment groups further demonstrated the fractions' and crude extracts' antioxidant activity, which was further encouraged by the fact that SOD serves as an essential antioxidant that serves as the first line of defense against damage caused by reactive oxygen species (ROS) in almost all living things. Superoxide radical (O2-) dismutation into oxygen and hydrogen peroxide is catalyzed by SOD. This enzyme is in charge of neutralizing hydrogen peroxide through its breakdown, so preserving an ideal level of the molecule in the cell, which is also necessary for cellular signaling. Strong antioxidant potentials were revealed by the significantly elevated SOD and CAT activities found in the active fractions and ethanol fruit extract of R. hookeri treated groups. When cells experience oxidative stress, free radicals that have escaped from the oxidant system attack the unsaturated fatty acids, changing the structure of the cell membrane. One of the main effects of oxidative stress is a phenomenon known as lipid peroxidation. This research supports that of about the antioxidant potentials of vitex doniana fruit extract and fractions.

CONCLUSION

The ethanol: water fraction had the maximum antioxidant activity, according to the results for R. hookeri's overall antioxidant activity. ethanol had the highest total flavonoid concentration, whereas methanol had the lowest. The optimum solvent mixture for developing medications to treat oxidative stress disease is methanol: water because the ethanol fruit extract of R. hookeri showed the best association between total antioxidant capacity, total phenol, and flavonoid content. When applied to animal systems, these phytochemicals, which are recognized antioxidants, are anticipated to have antioxidant capacity and consequently prevent oxidative stress.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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