

Phytochemical Characteristics, *in-Vitro* and *in-Vivo* Antioxidant Potentials of Ethyl acetate Fraction from Apple-ring *acacia* (*Faidherbia albida* (*Delile*) A. Chev.) Leaves Extract on Albino Rats

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ABSTRACT: The objective of this paper as to investigate the phytochemical characteristics, *in-vitro* and *in-vivo* antioxidant potentials of ethyl acetate fraction from apple-ring acacia (*Faidherbia albida* (Delile) A. Chev.) Leaves Extract on Albino Rats using appropriate standard procedures. Phytochemical examination identified alkaloids, steroids, saponins, glycosides, phenols, and tannins in both extracts. The antioxidant potential evaluated using the Ferric Reducing Antioxidant Power (FRAP) assay, show ethyl acetate fraction exhibited dose-dependent activity akin to that of vitamin E (34.69, 40.28, 70.26). *In-vivo* investigations assessed the impact of the extracts on oxidative stress indicators, comprising malondialdehyde (MDA) [102.77±2.99], glutathione (GSH) [110.28±0.39], catalase (CAT) [1.93±0.12], superoxide dismutase (SOD) [3.58±0.14], and concentrations of vitamins A [61.02±0.48], C [555.99±1.66], and E [71.24±0.49] respectively. The ethyl acetate fraction dramatically (P<0.05) decreased MDA levels, signifying diminished lipid peroxidation, while preserving GSH, CAT, and SOD levels near baseline. The results indicate a significant antioxidant capacity of the ethyl acetate fraction, offering defense against oxidative The research indicates that the ethyl acetate fraction of Faidherbia albida leaf exhibits significant antioxidant activity, positioning it as a viable candidate for medicinal use in managing oxidative stress.

DOI: https://dx.doi.org/10.4314/jasem.v29i2.27

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Cite this Article as: HYELADZIRA, Y. B; MUHAMMAD, F; HUSSAINA, S. I; GACHI, J. A; JOSEPH, G(2025). Phytochemical Characteristics, In-Vitro and In-Vivo Antioxidant Potentials of Ethyl acetate Fraction from Apple-ring *acacia (Faidherbia albida (Delile) A. Chev.)* Leaves Extract on Albino Rats. *J. Appl. Sci. Environ. Manage.* 29 (2): 563-568

Dates: Received: 23December 2024; Revised: 27January 2025; Accepted: 09February 2025; Published: 28 February 2025

Keywords: Antioxidant; oxidative stress; Apple-ring acacia; Faidherbia albida; Phytochemicals; Fraction

Haraz tree is named Faidherbia albida or Acacia albida and belongs to a large family of flowering plants Fabaceae which is commonly known as legume or bean family (Leguminosae) kingdom Plantae (Tutu, 2002 and Mokgolodi *et al.*, 2011). The tree was originally a riverine tree of Eastern and Southern Africa which was introduced into West Africa through pastoralism and agriculture. In the Sudan, Haraz tree is distributed through the different vegetation zones from Semi-desert region to the Savannah and mountainous area. Also, the species occur along the River Nile and its tributaries, Strom banks, Valleys and on hilly slopes on the Blue Nile State, South Kordofan, Northern State and Khartoum State (Harrison and Jackson, 1958 and El-Amin, 1990; Bernard, 2002 and Moser, 2006). F. albida is used in folkloric medicine as a remedy for chills, bronchitis, pneumonia, cough, diarrhea, haemorrhage, postpartum complications and kidney diseases (Hammiche and Maiza, 2006 and Belayneh *et al.*, 2012). F. albida has been found to contain various phytochemicals such as alkaloids, tannins, saponins and terpenoids (Wurochekke *et al.*, 2013). The tree also contributes to soil conservation and soil fertility improvement (Dangasuk *et al.*, 2001).

Oxidative stress is defined as a "state in which oxidation exceeds the antioxidant systems in the body secondary to a loss of the balance between them." It not only causes hazardous events such as lipid peroxidation and oxidative DNA damage, but also physiologic adaptation phenomena and regulation of intracellular signal transduction. From a clinical standpoint, if biomarkers that reflect the extent of oxidative stress were available, such markers would be useful for physicians to gain an insight into the pathological features of various diseases and assess the efficacy of drugs (Lambeth, 2014).

To cope with the oxidative stress elicited by aerobic metabolism, animal and human cells have developed a ubiquitous antioxidant defense system, which consists of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase together with a number of low molecularweight antioxidants such as ascorbate, α -tocopherol and glutathione, cysteine, thioredoxin, vitamins, etc. However, this antioxidant defense system may be overwhelmed bv various pathological or environmental factors so that a fraction of ROS may escape destruction and form the far more reactive hydroxyl radicals (Raghuvanshi, 2007; Garrido, 2004). An increase in ROS- elicited oxidative damage to DNA and other biomolecules may impair normal functions of tissue cells and lead to human aging and disease (Yudoh, 2005; Vasavidevi, 2006). The objective of this paper is to investigate the phytochemical characteristics, in-vitro and in-vivo antioxidant potentials of ethyl acetate fraction from apple-ring acacia (Faidherbiaalbida (Delile) A. Chev.) Leaves Extract on Albino Rat

MATERIALS AND METHOD

Collection and Identification of Plant Sample: Applering acacia (*Faidherbiaalbida* (Delile) A. Chev.)leave were collected from Zuru, Zuru Local Government Area of Kebbi State. The plant was identity and authenticated by Dr. Hassan AjayiShindi of the Department of Crop Science, College of Agriculture, Federal University of Agriculture, Zuru. *Plant Preparation and Extraction:* Apple-ring acacia (*Faidherbiaalbida* (Delile) A. Chev.) *leave* was washed with water and allowed to dry under shade

for two weeks. It was then grinded to coarse powder using mortar and pestle.

Methanol Extraction of Apple-ring acacia (Faidherbiaalbida (Delile) A. Chev.) leave: One thousand grams (100g) of the powdered Stembark was dissolved in 2500mls of methanol for 72 hrs (Dupontet al., 2002). It was then filtered using muslin cloth and and Number 1 filter paper, then the filtrate was evaporated using an oven at 45°C. The dried extract was stored in an air tight container and kept in refrigerator at 4 °C.

Percentage yield =
$$\frac{\text{weight of extract}}{\text{weight of samlpe}} * \frac{100}{1}$$
 (1)

Solvent Fractionation of Apple-ring acacia (Faidherbiaalbida (Delile) A. Chev.)

LeaveMethanol Extract: Fifty grams (50g) of dried Apple-ring acacia (Faidherbiaalbida (Delile) A. Chev.) leave methanol extract was re-dissolved in water and subsequently partitioned with n-hexane 50:50 v/v in separating funnel. Solvent fractionation was conducted according to the method describes by Kupchan, (1962). Fifty grams (50g) of Apple-ring acacia (Faidherbiaalbida (Delile) A. Chev.) leave dried methanol extract (crude extract) was transferred into a separatory funnel using a small amount of the water mixture. Equal volume of hexane was added into the same separatory funnel, mixed well, and set aside for layer separation. The hexane layer was collected into a round bottom flask and the solvent was evaporated to dryness. The water portion was poured into the separatory funnel. Equal volume of ethyl acetate was added into the separatory funnel, mixed well, and set aside for layer separation. The ethyl acetate layer was collected into another round bottom flask and the solvent was evaporated to dryness. Equal volume of saturated butanol was added to the remaining water layer, mixed well in a separatory funnel, and left aside for layer separation. The butanol layer was collected into a round bottom flask and the solvent was evaporated to dryness. The remaining water layer was then dried to get Ethylacetate extract.

Percentage yield =
$$\frac{\text{weight of Fraction}}{\text{weight of samlpe}} * \frac{100}{1}$$
 (2)

Phytochemical Screening of Apple-ring acacia (Faidherbiaalbida (Delile) A. Chev.)

Methanol and Ethylacetate Fraction: General phytochemical screening of the F.albida leave was carried out to investigate the active constituents of the extracts (Harborne, 1984).

In vitro Antioxidant Activity

Determination of Antioxidant Potential by Ferric Reducing Antioxidant Power Assay (FRAP): Determination of Antioxidant Potential by Ferric Reducing Antioxidant Power Assay (FRAP): The ferric reducing power of extract was determined by using the potassium ferricyanide- ferric chloride method described by Oyaizu, (1986).

About 2mL of extract and standard drug vitamin C at various concentrations (250, 500 and 750) was added to 2.5mL of potassium ferricyanide in separate test tubes and the mixture was incubated at 50° C for 20 min. Then 2.5mL of trichloroacetic acid was added to the mixture then centrifuged at $650 \times \text{g}$ for 10 min. To 2.5mL of the supernatant, 2.5mL of distilled water and 0.5mL ferric chloride were added. The absorbance was read at 700 nm using spectrophotometer. Higher absorbance indicates greater reducing capacity which was calculated as follows.

Reducing power
$$= \frac{AM}{AC} \times 100$$
 (3)

Where: AM = Absorbance of reaction mixture, AC =Absorbance of control mixture (distilled water).

In vivo Antioxidant Assay: Induction of Oxidative Stress: Oxidative stress was induced in the rats by intraperitoneal injection of CCL_4 in a dose of 0.25 mL of CCl_4 in liquid paraffin (8%) 1.25 mL/kg (Chougale*et al.*, 2007). Then the rats were randomly divided into 6 groups (n=4) and treated as follows:

| Group 1 | Served as the normal control. No extract treatment. |
|---------|---|
| Group 2 | CCL ₄ treated (untreated control) |
| Group 3 | CCL ₄ induced rats treated with vitamin C (5mg/kg). |
| Group 4 | CCL ₄ induced rats treated with extracts (100mg/kg). |
| Group 5 | CCL ₄ induced rats treated with extracts (200mg/kg). |
| Group 6 | CCL ₄ induced rats treated with extracts (400mg/kg). |

Sampling: Blood samples were collected in clean tubes after sacrificed the rats under anesthesia at the end of experiment. Blood was centrifuged after clotting for 10 minutes at 2500rpm. Serum was separated and stored at - 20° C for biochemical analysis

Biochemical Assay: The activities of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) were measured according to the method of Reitman and Frankel (1957) and Sood (1999).GSH by method of Jollow*et al.* (1974).Catalase Activity the activity of catalase was assayed according to the method of Aebi (1983). (MDA) was described by Wallin*et al.*

(1993).Superoxide Dismutase (SOD) this was determined using the method of Xin *et al.*, (1991).

Data Analysis: The data generated from the study are presented as Mean \pm Standard error of mean (SEM) and subjected to one-way analysis of variance (ANOVA) and statistical difference between means were separated using Duncan multiple comparison test using statistical package for social science (SPSS) version 20. Values are considered statistically significant at P<0.05

RESULTS AND DISCUSSION

Percentage Yield: The extraction of *Faidherbiaalbida leaf* with methanol yielded 25.5%, while Ethylacetate Fraction yielded 6.65%. The extract easily dissolves in water and is light green in colour; sour in taste, nice smell and with a gummy texture.

Phytochemical Screening of Faidherbiaalbida leaf Methanol and Ethylacetate Fraction: The qualitative phytochemical screening of *Faidherbiaalbida leaf* methanol and Ethylacetate fraction is presented in Table 1.

The results for methanol extract revealed the present of alkaloids, steroids, saponins, glycoside, flavonoids, tannins and phenols. While *Faidherbiaalbida leaf*Ethylacetate fraction revealed the presences of alkaloids, steroids, saponins, glycoside, phenols and tannins.

| Table 1: Qualitative Phytochemical Constituents of |
|--|
| Faidherbiaalbida leaf Methanol and Ethylacetate Fraction |

| Phytochemicals | Methanol Extract | Ethylacetate Fraction | | | |
|------------------------------------|---------------------|--------------------------|--|--|--|
| Flavonoids | - | - | | | |
| Tannins | + | + | | | |
| Steroids | + | + | | | |
| Saponins | + | + | | | |
| Glycosides | + | + | | | |
| Alkaloids | + | + | | | |
| Anthraquinone | - | - | | | |
| Phenols | + | + | | | |
| KEY: + = Present, - = Not detected | | | | | |

Ferric Reducing Antioxidant Power (FRAP) of Ficusplatyphylla Ethylacetate Fraction: The ferric reducing antioxidant power (FRAP) of *Ficusplatyphylla* Ethylacetate fraction is presented in (Figure 1).

The result demonstrates a dose dependent increase in FRAP in vitamin E. while *Faidherbia albida leaf* Ethylacetate fraction revealed similar reducing powers at all concentration (5, 10 and 15mg/ml).



Fig 1: Ferric Reducing Antioxidant Power of *Faidherbiaalbida leaf* Methanol

Antioxdant Potential of Faidherbia albida Leaf of Ethylacetate Fraction: The antioxidant potential of faidherbia albda leaf ethylacetate fraction is presented in (Table 2). The result revealed a significant (p<0.05) increase in MDA concentration of negative control compare to normal control, positive control and all extracts treated group. Although the concentration of MDA in positive control and methanol extract 400mg/kg significantly (p< 0.05) increase compare to normal control, however MDA concentration of ethylacetate fraction and normal control shows no significant (p<0.05)difference. The GSH concentration of negative control decrease significantly (p<0.05) compared to normal control, positive control and all extract treated groups. However, GSH concentration of positive control methanol extract shows no significant (p<0.05) difference to the normal control, while a significant (p<0.05) decrease was observed in ethylacetate fraction compared to the normal control. The CAT concentration of negative control significantly (p<0.05) decrease compare to normal control, positive control and all extracts treated groups, however, there is no significant (p<0.05) difference in CAT concentration of aethylacetate fraction 400mg/kg, methanol extract, positive control compared to the normal control. The SOD concentration of negative control significantly (p<0.05) decrease compared to normal control, positive control and all extracts treated groups. However, SOD concentration of positive control, ethylacetate fraction shows no significant (p>0.05) difference compare to the normal control while the SOD concentration of methanol extract significantly

(p<0.05) decreased compared to normal control. The Vitamin A concentration of negative control significantly (p<0.05) increase compare to normal control, positive control and all extracts treated groups. However there is no significant (p<0.05)difference between Vitamin A concentration of positive control and acethylactate fraction 400mg/kg, although the methanol extract significantly (p<0.05)increase compare to the normal control. The Vitamin C. concentration of negative control significantly (p<0.05) decreased compare to normal control, positive control and all extracts treated groups. However there is significantly (p<0.05) increase in Vitamin C concentration in methanol extract 400mg/kg, compared to normal control. Although the ethylacetate fraction significant (p<0.05)decrease compare to the normal control and there is a significant (p<0.05) increase in Vitamin C concentration in positive control compare to the normal. The Vitamin E concentration of negative control significantly (p<0.05) increase compare to normal control, positive control and all extracts treated groups. Although the ethylacetate fraction significant (p<0.05) decrease compared to normal control. However, there is a significantly decrease in Vitamin E concentration in positive control compared to the normal control, while methanol extract significant (p<0.05) increase in Vitamin E concentration compared to normal control. The ALT concentration of negative control significant (p<0.05) increase compare to normal control and positive control. However, there is significant (p<0.05)increase of the ethylacetate fraction compared to normal control. Although, there is no significant (p>0.05) difference in ALT concentration methanol extract and negative control. The AST concentration of negative control significantly (p<0.05) increase compare to the normal control, positive control and all extracts treated groups. However, there is no significant (p>0.05) increase in AST concentration in methanol extracts 400g/kg compared to normal control. Although, there is a significant (p<0.05)increase of ethylacetate fraction compared to normal control. The ALP Concentration in negative control significantly (p<0.05) increase compare to normal control and positive control. However, there is significant (p<0.05) increased of ALP concentration in methanol extracts 400g/kg compared to normal control. Although, there is significant (p<0.05) increase of in ALP concentration in ethylacetate fraction compared to the normal control. Therefore, there is a significant deference of ALP. concentration in methanol extracts 400g/kg compared to negative control and there is significant (p<0.05) difference in ALP concentration in ethylacetate fraction compared to the negative control.

| Table 2. / | Antiovidant Potential | s of Faidharbiaal | hida loaf Ethylacetat | e Fraction |
|------------|-----------------------|--------------------------|-----------------------------|------------|
| Table 2: F | Anuoxidant rotentiai | s of <i>Falanerblaal</i> | <i>Juu ieur</i> Eurviacetai | e riacuon |

| Treatments | Normal | Negative | Positive | Methanol | Ethylacetate |
|--------------------------|--------------------------|--------------------------|--------------------------|---------------------------|--------------------------|
| | Control | control | control Vit C | Extract | Fraction |
| | | (CCL ₄) | (5mg/kg) | 400mg/kg | 400mg/kg |
| MDA (Nmole/L) | 104.48 ± 1.34^{a} | 245.29±1.54° | 119.23±1.11 ^b | 116.02±0.98 ^b | 102.77±2.99 ^a |
| GSH (mg/dl) | 123.34±0.25° | 40.23±0.39 ^a | 120.81±0.36° | 123.78±3.79° | 110.28±0.39 ^b |
| CAT (Unit /ml of enzyme) | 1.93±0.12 ^b | 0.83 ± 0.04^{a} | 1.62 ± 0.89^{b} | 1.86±0.11 ^b | 1.93±0.12 ^b |
| SOD (Unit /ml of enzyme) | 3.76±0.23° | $1.18{\pm}0.10^{a}$ | 3.37±0.25° | 2.20±0.11 ^b | 3.58±0.14° |
| VIT A (mg/dl) | 69.60 ± 0.69^{b} | 102.67±0.21 ^d | 59.76±0.41 ^a | 76.93±0.29° | 61.02 ± 0.48^{a} |
| VIT C (µmol/l) | 797.51±1.22 ^c | 41.56 ± 0.79^{a} | 922.18 ± 1.22^{d} | 1136.92±1.66 ^e | 555.99±1.66 ^b |
| VIT E (mg/dl) | 77.39±0.19° | 124.89±0.25 ^e | 53.86±0.37 ^a | 106.65 ± 0.37^{d} | 71.24±0.49 ^b |
| ALT (U/I) | 12.22±0.22 ^a | 19.50±0.73° | 17.17±0.27 ^b | 18.24 ± 0.28^{bc} | 27.69±0.35 ^d |
| AST (U/l) | 79.39±0.63ª | 147.46±0.63 ^e | 115.52±0.63° | 128.08 ± 0.46^{d} | 105.22 ± 0.80^{b} |
| ALP (U/l) | 31.93±3.35 ^a | 83.75±2.73 ^{cd} | 71.89±0.14 ^b | 88.32 ± 5.75^{d} | 76.36±2.43° |

Values are presented as mean \pm SEM (n = 3) value having similar superscript in rows are not significantly different at (P>0.05) analyzed using One-Way ANOVA, followed by Duncan multiple comparison test with SPSS version 20.0. SOD= superoxide dismutase, CAT= catalase, GSH= glutathione reductase and MDA= malondialdehyde, AST = aspartasetransferases, ALP = alkaline phosphatases, ALT, alanine amino transferases

The study investigated the antioxidant potential of Faidherbiaalbida leaf extracts, focusing on the differences between methanol and ethyl acetate fractions. The extraction process using methanol yielded a higher percentage (25.5%) compared to ethyl acetate (6.65%), which is consistent with other studies that highlight the efficiency of polar solvents like methanol in extracting a broad range of phytochemicals (Do et al., 2014). This suggests that methanol might be more suitable for extracting a diverse array of compounds from Faidherbia albida. Phytochemical screening revealed the presence of key compounds like alkaloids, saponins, steroids, phenols, and tannins in both extracts. The presence of these compounds aligns with earlier findings on Faidherbia albida's potential medicinal properties (Ibrahimet al., 2012). Phenolic compounds, in particular, are known for their role in neutralizing free radicals, which supports the observed antioxidant activity (Rice-Evans et al., 1997). The Ferric Reducing Antioxidant Power (FRAP) assav demonstrated a significant antioxidant capability of the ethyl acetate fraction, showing comparable activity to vitamin E across various concentrations. This is indicative of its potential as a natural antioxidant, which aligns with studies on other plantbased antioxidants (Kaur and Kapoor, 2001). The ethyl acetate fraction's ability to maintain stable levels of oxidative stress markers such as MDA (malondialdehyde), SOD (superoxide dismutase), and CAT (catalase) in albino rats further underscores its protective effects against oxidative stress. The in vivo results indicate that the ethyl acetate fraction effectively reduces lipid peroxidation and supports the body's antioxidant defense mechanisms, as seen in the stable GSH (glutathione) levels. These outcomes are consistent with the known effects of phenolic antioxidants in reducing oxidative damage (Halliwell and Gutteridge, 2015). However, the methanol extract showed increased MDA levels. suggesting that the ethyl acetate fraction may provide more stable antioxidant effects in vivo.

Conclusion: The findings of this study demonstrate that Faidherbiaalbida leaf extracts, particularly the ethyl acetate fraction, exhibit significant antioxidant potential. The presence of various bioactive compounds, such as phenols and tannins, is likely responsible for the observed antioxidant activity, a result that aligns with previous studies highlighting the role of these phytochemicals in combating oxidative stress (Rice-Evans et al., 1997; Ibrahim et al., 2012). The ethyl acetate fraction's ability to maintain antioxidant enzyme levels similar to vitamin E suggests it could be a valuable natural antioxidant for therapeutic applications. This study also highlights the importance of selecting appropriate solvents for extracting desired compounds, as the ethyl acetate fraction showed a better antioxidant profile than the methanol extract (Do et al., 2014).

Declaration of Conflict of Interest: The authors declare no conflict of interest

Data Availability Statement: Data are available upon request from the corresponding author

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