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Phytochemical investigation and antioxidant analysis of the ethyl acetate and methanol extracts of *Euphorbia polycnemoides* aerial parts

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ABSTRACT

Background: This study aimed to investigate the phytochemical composition and *in vitro* antioxidant potential of ethyl acetate and methanol extracts, from the aerial parts of *Euphorbia polycnemoides*.

Methods: Aerial parts of the plant were collected and extracted using ethyl acetate and methanol. The extracts were subjected to qualitative and quantitative phytochemical screening to identify and quantify secondary metabolites. Their antioxidant activity was assessed using ferric reducing, DPPH, nitric oxide, lipid peroxidation, and hydrogen peroxide scavenging assays.

***Results*:** Qualitative screening revealed the presence of tannins, phenols, alkaloids, flavonoids, saponins, steroids, anthraquinones, terpenoids, cardiac glycosides, and reducing sugars in both extracts while phlobatannin was absent. Quantitative analysis showed tannins to be the most abundant phytochemical, with methanol and ethyl acetate extracts containing 58.66 ± 0.28 mg/100 g and 50.95 ± 2.63 mg/100 g, respectively. The ethyl acetate extract had a higher concentration of alkaloids (57.22 ± 0.35 mg/100 g), while the methanol extract showed greater content of cardiac glycosides (47.92 ± 0.08 mg/100 g). Both extracts demonstrated a strong, dose-dependent antioxidant capacity. The ethyl acetate extract consistently exhibited superior potency, achieving 81.97 ± 0.38% DPPH scavenging activity at 100 µg/mL suggesting its efficiency in extracting key antioxidant.

Conclusion**:** These findings demonstrate that *E. polycnemoides* aerial parts are rich in bioactive compounds with significant antioxidant potential, supporting their traditional use and suggesting their promise as a source of natural therapeutic agents with applications in the prevention and treatment of oxidative stress-related diseases.

Keywords: *Euphorbia Polycnemoides,* Antioxidant activity, Methanol extract, Ethyl acetate extract**,** phytochemical screening

1. INTRODUCTION

In recent years, the global pursuit of safer, eco-friendly, and sustainable healthcare strategies has renewed interest in medicinal plants and their therapeutic potential. Since the late 1990s, the application of plant-based remedies in both human and veterinary medicine has expanded significantly [1]. Oxidative stress, characterized by an imbalance

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between the production of reactive oxygen species (ROS) and antioxidant defenses, plays a pivotal role in the pathogenesis of various human disorders. ROS-mediated damage to biomolecules compromises cellular function, contributing to disease progression. To mitigate oxidative stress-related pathologies, innovative antioxidant therapeutic strategies have been proposed, aiming to restore redox homeostasis and prevent disease exacerbation. Phytochemicals derived from medicinal plants have garnered significant attention for their potential to modulate immune responses, attenuate oxidative stress by scavenging reactive oxygen species (ROS), thereby offering a promising alternative to synthetic therapeutics. Ethnomedicine, rooted in centuries of indigenous knowledge, continues to provide a valuable reservoir of therapeutic agents. Phytochemicals isolated from plants have long been utilized in the treatment of a wide range of ailments such as respiratory and gastrointestinal disorders, skin conditions, inflammation, and microbial infections. Ethnopharmacology, an interdisciplinary field that validates traditional remedies through scientific inquiry, is essential in discovering novel therapeutic compounds and advancing drug development [2]. Among the most pharmacologically significant plant families is *Euphorbiaceae,* commonly known as the spurge family. This large and diverse family comprises more than 300 genera and over 8,000 species, with the genus *Euphorbia* alone encompassing more than 2,000 species [3]. Members of this genus are distributed globally, especially in tropical and subtropical regions, and are well-known for their morphological diversity and characteristic milky latex. *Euphorbia* species are rich in secondary metabolites such as sesquiterpenes, diterpenoids, triterpenoids, flavonoids, and essential oils, many of which possess documented anti-inflammatory, antimicrobial, cytotoxic, antiviral, and antitumor activities [4]. The genus *Euphorbia* has a rich history of traditional medicinal use across continents. Various species have been employed in the treatment of ailments ranging from skin diseases, gastrointestinal disorders, respiratory infections, microbial diseases, to pain relief from snakebites and scorpion stings ([5][6]. In India, *E. hirta* is used for gastrointestinal and respiratory conditions, while in Australia, it is utilized for managing hypertension, asthma, and edema. The latex, stems, leaves, and flowers of many *Euphorbia* species are traditionally prepared as decoctions, infusions, powders, or lotions for both human and veterinary use [7]. In Nigeria, *E. polycnemoides* is valued in traditional medicine for treating skin and respiratory ailments, highlighting its therapeutic and cultural significance [8]. The choice of solvent plays a crucial role in extracting bioactive compounds from plant materials. Different solvents can yield varying amounts and types of phytochemicals, impacting the antioxidant and biological activities of the extract. [9] reported the antioxidant and antibacterial activity of the aqueous and ethanol extracts of *E. polycnemoides* aerial parts. Methanol has been shown to be an effective solvent for extracting phytochemicals with high antioxidant activity. Studies have shown that methanolic extracts of plants possess significant antioxidant properties, attributed to the presence of phenolics and flavonoids [10]. Methanol extracts have yielded high amounts of flavonoids and phenols, correlating with high antioxidant activity. Ethyl acetate extracts have also shown promising antioxidant activity, with some studies indicating higher radical scavenging potential compared to other solvents. For instance, ethyl acetate extracts of *Ceiba pentandra* (L.) Gaertn. and Cleome viscosa have exhibited potent antioxidant effects [11][12]. This study investigates the phytochemical composition and *in vitro* antioxidant activity of ethyl acetate and methanol extracts from the aerial parts of *Euphorbia polycnemoides,* with the aim of validating its ethnomedicinal relevance and pharmacological potential, thereby contributing to the expanding knowledge on the therapeutic and economic value of Euphorbia species.

2. 0 MATERIALS AND METHODS

2.1 Materials

2.1.1 Biological Materials

Fresh aerial parts of the plant species *Euphorbia polycnemoides*.

2.1.2 Chemicals and Reagents

Distilled water, Ethyl acetate, Methanol ,Tannin reagent, Tannic acid (Standard), acetic acid in ethanol, Concentrated ammonium hydroxide (NH_4 OH), sodium nitrite ($NaNO_2$),aluminum chloride ($AlCl_3$), Sodium hydroxide (NaOH),Quercetin (Standard), ethanol ,Diethyl ether, n-butanol, aqueous sodium chloride, Folin–Ciocalteu (Folin C) reagent, Sodium carbonate solution, Gallic acid , KOH, Scillaren A (Standard),3,5-dinitrosalicylic acid (DNSA) reagent, Sodium-potassium tartaric acid, NaOH, D-glucose (Standard),Phosphate buffer, Potassium ferricyanide [K_3 $Fe(CN)_6$],Trichloroacetic acid (TCA),Ferric chloride ($FeCl_3$)1,1-diphenyl-2-picrylhydrazyl (DPPH), Sodium nitroprusside, Phosphate buffered saline (PBS),Griess reagent (containing sulphanilamide, naphthylethylene diamine dihydrochloride, and H_3 PO_4),Hydrogen peroxide

(H_2 O_2),Thiobarbituric acid (TBA),Ascorbic acid .All the other chemicals were of analytical grade and were obtained from standard commercial suppliers

2.1.3 Equipment and Apparatus

Electric blender (Turbocrush High Performance Blender BX250. Ziploc® polyethylene bags, Whatman 1 filter paper, Water bath (Digital 6 Hole water bath HH-S6), Spectrophotometer (Spectrumlab 23a), 250 mL separatory funnel, Hot air oven (Zenith Lab DHG-9070A), Centrifuge (Cgoldenwall 80-2 Electric Lab Centrifuge), Test tubes

2.2 Methods

2.2.1 Sample Collection and Identification

Fresh aerial parts of *E. polycnemoides* were collected from multiple sites in Dukai village, Gande, Silame Local Government Area, Sokoto State, Nigeria. Plant identification was performed at the Botany Unit, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, and assigned voucher number UDUS/ANS/0876.

2.2.2 Collection and Identification of Plant material

Collected plant material was rinsed with distilled water to remove dust, air-dried under shade at ambient temperature, and pulverized into fine powder using an electric blender. The powder was stored in Ziploc® polyethylene bags until analysis [13].

2.2.3 Preparation of the Extract

The aerial part of *E. polycnemoides* was shade-dried, pulverized into a fine powder, labeled, and stored at room temperature until use. A total of 250 g of the powdered aerial part was subjected to ethyl acetate and methanol extraction using the maceration method for 72 hours with intermittent shaking. The extracts were filtered with Whatman filter paper, the filtrate was then concentrated to dryness in water.

2.2.4 Phytochemical Investigation

The extracts were subjected to preliminary phytochemical screening to detect the presence of secondary metabolites, following standard qualitative procedures.

The resulting extracts were subsequently subjected to both qualitative and quantitative phytochemical screening using standard procedures to detect the presence of the following secondary metabolites: Tannin, phenol, phlobatannin alkaloid, saponin flavonoid, steroid anthraquinone terpenoid cardiac glycosides, and reducing sugars.

***2**.2.4 Phytochemical Screening*

The extracts were subjected to phytochemical screening using the standard procedure described by [14] and [15], to identify the phytoconstituents present in the extracts. Test for alkaloids (Dragendorff's test), Tannins (Braymer's Test) Flavonoids, Saponins, Cardiac Glycosides, Phenols (Using Ferric Chloride) and reducing sugars (Fehlings test)

2.2.4.1 Quantitative Phytochemical Investigation

2.2.4.1a Estimation of Total Tannins

A volume of 1 mL of the extract was mixed with 10 mL of distilled water, incubated for 1 hour, and then filtered. The volume of the filtrate was adjusted to 25 mL with distilled water. To 1 mL of the filtrate, 4 drops of tannin reagent and 5 mL of distilled water were added. The mixture was left to react, and the absorbance was measured at 720 nm after 10 minutes using tannic acid as the standard [16].

2.2.4.1b Estimation of Alkaloids (Harborne Method)

An aliquot of 1 mL of the extract was added to 20 mL of 10% acetic acid in ethanol. The mixture was covered and allowed to stand for 4 hours, then filtered. The filtrate was concentrated on a water bath to one-fourth of its original volume. Concentrated ammonium hydroxide (NH_4OH) was added dropwise to precipitate the alkaloids. The precipitate was collected on a pre-weighed filter paper, rinsed with 1% NH_4OH, and dried in an oven at 60 °C for 30 minutes. The filter paper was then reweighed to determine the weight of alkaloids [17]. The percentage of alkaloids was calculated using the formula:

$$\% \, Alkaloid = \frac{Final\ weight\ of\ the\ sample}{Weight\ of\ the\ sample} \, x\,100 \qquad \textbf{(1)}$$

2.2.4.1c Estimation of Total Flavonoid Content

The method of [18] was adapted. An aliquot of 0.5 mL of the extract was mixed with 0.5 mL of distilled water, and 0.3 mL of 5% sodium nitrite ($NaNO_2$) and allowed to incubate for 5 minutes. Then followed immediately with addition 0.3 mL of 10% aluminum chloride ($AlCl_3$). Then, 2 mL of sodium hydroxide (NaOH) was added. Then, 2 mL of sodium hydroxide (NaOH) was added, and the volume was brought up to 10 mL with distilled water .The absorbance was measured at 510 nm using quercetin as a standard[18].

2.2.4.1d Estimation of Saponins

The method described by [19] was employed with slight modifications. A quantity of 1 g of the extract was mixed with 25 mL of 20% ethanol and heated in a water bath at approximately 55 °C for 2 hours with continuous stirring. The mixture was filtered, and the residue was re-extracted with another 50 mL of 20% ethanol. The two filtrates were combined and concentrated over a water bath to a volume of 40 mL at about 90 °C. The concentrated extract was transferred into a 250 mL separatory funnel, followed by the addition of 20 mL of diethyl ether. The mixture was shaken vigorously, and the ether layer was discarded. Next, 60 mL of *n*-butanol was added to the aqueous layer. The *n*-butanol phase was washed twice with 10 mL portions of 5% aqueous sodium chloride to remove any residual impurities. The remaining solution was heated on a water bath until evaporation was complete. The final residue was dried in a hot air oven to a constant weight and recorded as the total saponin content.

2.2.4.1e Estimation of Total Phenolic Compounds

The determination of total phenolic content was performed following the method described by [16],with slight modifications. A quantity of 0.5 g of the extract was weighed and dissolved in 50 mL of distilled water. From this solution, 0.5 mL was transferred into a test tube and mixed with 0.1 mL of Folin–Ciocalteu (Folin C) reagent. The mixture was incubated at room temperature for 15 minutes. Subsequently, 2.5 mL of sodium carbonate solution (7.5% w/v Na_2CO_3) was added to the reaction mixture and further incubated for 30 minutes at room temperature. The absorbance of the resulting blue-colored solution was measured at 760 nm using a spectrophotometer. Gallic acid was used as the standard, and the total phenolic content was expressed in milligrams of gallic acid equivalents (mg GAE/g dry extract).

2.2.4.1f Estimation of cardiac glycosides

The cardiac glycoside content was quantified using a spectrophotometric method Heftmann as described by [20]. An aliquot of 50 µl the extract reacted with 150 µl 1 N methanolic KOH for 2 hrs at room temperature. The resulting bufadienolides was measured at 355 nm. A calibration curve was established using Scillaren A as a standard at concentrations of (20, 40 , 60, 80 ,100 µg)).

2.2.4.1g Estimation of reducing sugars

The reducing sugar content (RSC) was quantified using the 3,5-dinitrosalicylic acid (DNSA) assay, adapted from the method of [21]. The DNSA reagent was prepared by dissolving 1 g DNSA and 30 g of sodium-potassium tartaric acid in 80 mL of 0.5 N NaOH at 45°C. The reaction mixture consisted of 1 mL of plant extract (1 mg/mL) and 2 mL of DNSA reagent, heated at 95°C for 5 minutes. After cooling,7 mL of the distilled water was added to the solution and the absorbance was measured at 540 nm, and the reducing sugar content was calculated from a calibration curve generated using D-glucose standards (200-1000 mg/L).

2.2.4.2 Antioxidant Assays

2.2.4.2a Invitro antioxidant analysis

Reducing power

The reducing power of each extract was determined according to the method of [22]. 1mL of the sample water was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN_6]$ (2.5 ml, 1%). The mixture was incubated at 50ºC for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 g for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%) and the absorbance was measured at 700 nm. Ascorbic acid was used as the reference material. All the tests were performed in duplicate and the graph was plotted with the average of two observations.

DPPH radical scavenging activity

The free radical scavenging activity of each extract was measured by 1,1-diphenyl-2-picryl- hydrazil (DPPH) using the method of [23]. An aliquot of 0.1 mM solution of DPPH in methanol was prepared and 1ml of this solution was added to 3 ml of various concentration of sample and the reference compound (10-125µg/ml). After 30 min, absorbance was measured at 517 nm. Ascorbic acid was used as the reference material. All the tests were performed in duplicate and the graph was plotted with the mean values. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples.

$$\text{Percentage inhibition} = \frac{(\text{Absorbance control} - \text{Absorbance test}) \times 100}{\text{Absorbance control}} \quad (2)$$

Nitric oxide scavenging activity:

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which were measured by the Griess reaction [24][25].

The reaction mixture (3 ml) containing sodium nitroprusside (10 mM) in phosphate buffered saline (PBS) and each extract and the reference compound in different concentrations (25-100 µg/ml) were incubated at 25°C for 150 min. Each 30 min, 0.5 ml of the incubated sample was removed and 0.5 ml of the Griess reagent (1% sulphanilamide, 0.1% naphthylethylene diamine dihydrochloride in 2% H_3PO_4) was added. The absorbance of the chromophore formed was measured at 546 nm. Ascorbic acid was used as a positive control compound. All the tests were performed in duplicate and the results averaged. The percentage inhibition of nitric oxide generated was measured by comparing the absorbance values of control and test samples using Eq. (2).

Hydrogen peroxide scavenging activity

Scavenging activity of hydrogen peroxide (H_2O_2) by each extract was determined by the method of [26]). Sample (4 ml) at various concentration was mixed with 0.6 ml of 4 mM H_2O_2 solution prepared in phosphate buffer (0.1 M pH 7.4) and incubated for 10 min. The absorbance of the solution was taken at 230 nm. Ascorbic acid was used as a positive control compound. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples using Eq. (2).

Determination of lipid peroxidation

Lipid peroxidation was estimated by thiobarbituric acid (TBA) reaction with malondialdehyde (MDA) 19. To 1 mL of supernatant, 0.5 mL of 30% trichloroacetic acid (TCA) was added followed by ,0.5 mL of 0.8% TBA. The tubes were kept in a shaking water bath for 30 min at 80 °C. After 30 min of incubation the tubes were taken out and kept in ice-cold water for 10 min. These were then centrifuged at 800 g for 15 min. The amount of MDA was assessed by measuring the absorbance of supernatant at 540 nm at room temperature against an appropriate blank. The percentage inhibition of Lipid peroxidation was calculated using the equation:

$$\text{Percentage inhibition} = \frac{A_{control} - A_{sample}}{1} \times \frac{100}{1} \quad (3)$$

$A_{control}$ and A_{sample} define the absorbance of the control (without the antioxidant) and of the sample (with the antioxidant) respectively.

2.3 Statistical Analysis

Data collected for each parameter were analyzed for their central tendencies (mean) using descriptive statistics; values were expressed as mean ± standard deviation of the observations**.**

3.0 RESULTS

3.1 Phytochemical Screening

The qualitative phytochemical analysis of the aerial part extracts of *Euphorbia polycnemoides* confirmed the presence of diverse secondary metabolites in both ethyl acetate and methanol. Table 1 summaries the detected phytoconstituents which include alkaloids, flavonoids, phenols, anthraquinones, tannins, saponins, steroids, terpenoids, cardiac glycosides, and reducing sugars.

Table 1: Qualitative Phytochemical Screening of *E. polycnemoides* aerial part Extracts

Phytochemicals	Ethyl acetate	Methanol
Tannins	+	+
Phlobatannins	-	-
Alkaloids	+	+
Saponins	+	+
Flavonoids	+	+
Steroids	+	+
Anthraquinone	+	+
Terpenoids	+	+
Cardiac glycosides	+	+
Reducing Sugar	+	+

Key: + indicates presence - indicates absence

3.2 ***Quantitative phytochemical analysis***

Quantitative analysis (Table 2) revealed marked differences in the concentrations of specific phytochemicals between the two solvent extracts. Tannins were the most abundant compounds overall, with methanol extract recording a higher concentration (58.66 ± 0.28 mg/100 g) than ethyl acetate (50.95±2.63 mg/100 g) extract. Similarly, phenols, reducing sugars, flavonoids, and cardiac glycosides were significantly more concentrated in the methanol extract, suggesting its higher efficiency in extracting polar compounds. For example, phenol content in methanol (42.44 ± 0.32 mg/100 g) was nearly double that of ethyl acetate (21.70 ± 0.52 mg/100 g). Conversely, alkaloids and saponins were extracted in slightly higher amounts with ethyl acetate, though differences were relatively small. The ethyl acetate extract contained 57.22 ± 0.35 mg/100 g alkaloids compared to 56.72 ± 0.49 mg/100 g in methanol, while saponins were also higher in ethyl acetate (9.75 ± 0.08 mg/100 g vs. 8.09 ± 0.06 mg/100 g,

Table 2: Quantitative Phytochemical Analysis of *E. polycnemoides* aerial part Extracts

Phytochemicals (mg/100g)	Ethyl acetate	Methanol
Tannin	50.95±2.63	58.66±0.28
Phenol	21.70±0.52	42.44±0.32
Reducing sugar	38.34±0.25	46.34±0.07
Alkaloid	57.22±0.35	56.72±0.49
Flavonoid	25.51±1.14	31.76±0.20
Saponin	09.75±0.08	08.09±0.06
Cardiac Glycoside	25.01±0.62	47.92±0.08

Values represent mean ± SD

3.3 Antioxidant Activity

The antioxidant potential of *E. polycnemoides* extracts was assessed using five in vitro assays (Tables 3–7). Across all assays, both extracts demonstrated strong, dose-dependent antioxidant activity, though with notable differences between solvents.

3.3.1 Ferric Reducing Antioxidant Power (FRAP)

Both extracts exhibited concentration-dependent increases in reducing power. Ethyl acetate extract showed superior ferric ion-reducing capacity (0.54 ± 0.003 at 100 µg/mL) compared to methanol (0.43 ± 0.004). Although ascorbic acid remained the strongest reducer (0.69 ± 0.003), the performance of ethyl acetate was comparatively close. The details are presented in Table 3

Table 3: FRAP Scavenging Activity of *E. polycnemoides* aerial parts

Conc. (µg/mL)	Ethyl acetate	Methanol	Ascorbic acid
20	0.12±0.004	0.10±0.004	0.13±0.002
40	0.18±0.004	0.14±0.001	0.14±0.004
60	0.32±0.002	0.26±0.001	0.38±0.002
80	0.37±0.004	0.34±0.001	0.45±0.006
100	0.54±0.003	0.43±0.004	0.69 ±0.003

Values represent mean ± SD

Key: FRAP = Ferric Reducing Antioxidant Potential

3.3.2 DPPH Radical Scavenging

In Table 4, the DPPH assay confirmed the superior radical scavenging activity of the ethyl acetate extract, which reached 81.97 ± 0.38% inhibition at 100 µg/mL compared to 63.86 ± 0.65% for methanol. These values closely approached the standard ascorbic acid (86.67 ± 0.47%), highlighting the potency of the ethyl acetate extract in neutralizing free radicals.

Table 4: DPPH Scavenging Activity of *E. polycnemoides* aerial parts extracts

Conc. (µg/mL)	**Ethyl acetate**	**Methanol**	**Ascorbic acid**
20	28.85±0.47	9.82±0.56	24.08±0.09
40	38.60±0.19	27.06±0.37	50.60±0.28
60	64.19±0.57	49.21±0.37	70.42±0.75
80	73.54±0.28	59.15±0.57	77.33±0.37
100	81.97±0.38	63.86±0.65	86.67±0.47

Values represent mean ± SD

Key: DPPH = 2, 2- Diphenyl-1-picrylhydrazyl.

3.3.3 Nitric Oxide Scavenging

Ethyl acetate extract also demonstrated greater nitric oxide inhibition (70.88 ± 0.16% at 100 µg/mL) than methanol (63.74 ± 0.62%). This suggests a capacity to modulate reactive nitrogen species, which are implicated in inflammation and oxidative damage. The details are presented in Table 5.

Table 5: Nitrogen Oxide Scavenging Activity of *E. polycnemoides* Aqueous Extract

Conc. (µg/mL)	Ethyl acetate	Methanol	Ascorbic acid
20	11.43±0.62	8.68±0.47	14.07±0.93
40	20.22±0.62	13.08±0.16	21.76±0.62
60	42.31±1.40	34.29±0.62	38.35±2.02
80	59.23±0.47	50.55±1.24	62.75±0.47
100	70.88±0.16	63.74±0.62	79.34±0.31

Values represent mean ± SD

3.3.4 Lipid Peroxidation Inhibition

Table 6 presents the result lipid peroxide scavenging activity of *E. polycnemoides* aerial parts extract.At 100 µg/mL, ethyl acetate achieved 69.02 ± 0.70% inhibition compared to 63.12 ± 0.70% for methanol, while ascorbic acid recorded 72.30 ± 0.23%.

Table 6: Lipid Peroxide Scavenging Activity of *E. polycnemoides* Aerial parts Extract

Conc. (µg/mL)	Ethyl acetate	Methanol	Ascorbic acid
20	13.44±0.47	11.64±0.69	17.54±1.16
40	28.86±0.93	25.09±0.23	39.67±0.47
60	39.51±1.16	43.77±1.16	52.46±1.39
80	57.05±0.93	53.44±0.47	63.61±0.46
100	69.02±0.70	63.12±0.70	72.30±0.23

Values represent mean ± SD

3.3.5 Hydrogen Peroxide Scavenging

In Table 5, the hydrogen peroxide assay further confirmed the superior activity of ethyl acetate extract (69.58 ± 0.47% at 100 µg/mL), compared to methanol (60.74 ± 0.63%). Although lower than ascorbic acid (83.67 ± 0.31%).

Table 7: Hydrogen Peroxide Scavenging Activity of *E. polycnemoides* aerial parts extracts

Conc. (µg/mL)	Ethyl acetate	Methanol	Ascorbic acid
20	17.34±0.32	8.62±0.63	24.95±0.32
40	30.71±0.23	23.72±0.47	39.88±0.23
60	47.49±0.23	36.13±0.31	55.32±0.23
80	56.94±0.47	51.51±0.55	63.03±0.71
100	69.58±0.47	60.74±0.63	83.67±0.31

4.0 **DISCUSSION**

The qualitative phytochemical screening of *E. polycnemoides* aerial part extracts, prepared using ethyl acetate and methanol revealed a diverse array of secondary metabolites Important phytochemical classes, including alkaloids, flavonoids, phenols, anthraquinones, tannins, saponins, steroids, terpenoids, cardiac glycosides, and reducing sugars, were detected in both extracts, indicating a rich and complex phytochemical profile. These results align with previous findings on *Euphorbia* species, which have been reported to contain over 80 distinct phytoconstituents [27]. The presence of these compounds suggests a broad spectrum of potential therapeutic activities, notably antioxidant, antimicrobial, and cardio protective effects. Phlobatannins were not identified in the both extracts, though [9] reported the presence of phlobatannins in the aqueous and methanol extracts of the plant reflecting their selective solubility in highly polar solvents. The detection of flavonoids, tannins, and phenols in both extracts are particularly noteworthy, given their well-documented antioxidant properties. Cardiac glycosides present in the extracts have been associated with antimicrobial and anticancer activities [28], while phenolic compounds are recognized for their antimicrobial, antifungal, and antioxidant potential [29]. Thus, the observed phytochemical composition of *E. polycnemoides* underscores its promise as a source of natural bioactive agents with potential pharmaceutical applications. The quantitative phytochemical analysis of *Euphorbia polycnemoides* aerial part extracts revealed varying concentrations of important bioactive compounds in both solvents used (Table 2). Tannins were the most abundant phytochemical, with the methanol extract recording the higher concentration than ethyl acetate extract. The reducing sugars and alkaloids were also present in appreciable amounts, with methanol extracts exhibiting higher levels. Cardiac glycosides were present in both extracts, with the methanol extract containing the higher concentration. These findings highlight the phytopharmacological potential of *E. polycnemoides*, particularly in relation to cardiovascular and metabolic health. The variations in concentration illustrate the solvent-dependent solubility of phytochemicals, with methanol favoring the extraction of highly polar compounds such as phenolics and glycosides, while ethyl acetate efficiently extracted moderately polar alkaloids. Phytochemicals are non-nutritive plant compounds that play essential roles in disease prevention and treatment [30]. The bioactive constituents identified in this study have been widely reported to exhibit significant medicinal and physiological properties [31]. For instance, alkaloids are well-established precursors for several pharmacologically important drugs, including anticholinergics (e.g., atropine), analgesics (e.g., morphine), antiparasitics (e.g., quinine), anticholinesterases (e.g., galantamine), and chemotherapeutic agents (e.g., vincristine and vinblastine). Phenolic compounds are known to inhibit hydrolytic enzymes particularly proteolytic enzymes secreted by pathogens and have been associated with antimicrobial resistance mechanisms. The presence of glycosidic precursors that release toxic phenolics upon hydrolysis further supports their role in plant defense [32]. Collectively, these secondary metabolites contribute significantly to the pharmacological activities observed in medicinal plants [33], supporting

the ethnomedicinal relevance of *E. polycnemoides*. The FRAP assay results (Table 3) demonstrated a concentration-dependent increase in antioxidant activity of the *E. polycnemoides* aerial part extracts (ethyl acetate and methanol) studied with higher extract concentrations (20–100 µg/mL) yielding greater ferric-reducing ability. Between the two extracts, ethyl acetate exhibited a stronger reducing power, achieving a peak absorbance of 0.54 ± 0.003 at 100 µg/mL. This suggests a significant presence of moderately polar to nonpolar phytochemicals such as flavonoid aglycones and terpenoids that are efficiently extracted in ethyl acetate and contribute to the reducing capacity the extract [34]. The methanol extract showed moderate reducing capacity, with maximum values of 0.43 ± 0.004. Ascorbic acid, used as the positive control, exhibited the highest FRAP value (0.69 ± 0.003 at 100 µg/mL), consistent with its well-documented antioxidant potential. Although the extracts did not surpass the standard, the similarity in activity profiles supports their efficacy as natural antioxidants capable of donating electrons to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). The DPPH assay further confirmed the antioxidant potential of the extracts, revealing a similar dose-dependent trend. Ethyl acetate extract again showed a more potent radical scavenging activity consistent with its FRAP results. The methanol extract also demonstrated significant scavenging activity, though to a lesser extent. In the nitric oxide scavenging assay, the ethyl acetate extract exhibited a higher activity than methanol extract. This result indicates that the phytochemicals responsible for electron transfer and radical neutralization also effectively inhibit reactive nitrogen species, further validating the multifunctional antioxidant profile of the plant. The lipid peroxide and hydrogen peroxide scavenging assays reinforced the findings from the FRAP and DPPH assays. Ethyl acetate extract consistently exhibited a higher antioxidant activity. Ethyl acetate extract, though not as efficient as ascorbic acid showed more inhibition of lipid peroxidation and hydrogen peroxide. These results highlight that the moderately polar to nonpolar phytochemicals such as flavonoid aglycones and terpenoids that are efficiently extracted in ethyl acetate and contribute to the reducing capacity of the extract. Both extracts demonstrated significant, dose-dependent antioxidant activity across multiple in vitro models, including FRAP, DPPH, nitric oxide, lipid peroxidation, and hydrogen peroxide assays. The ethyl acetate extract consistently showed a stronger activity, than the methanol extract Thus showing contribution of moderately polar (e.g., flavonoids, terpenoids) compounds to the antioxidant potential of the plant. Remarkably, antioxidants have also been reported to exhibit direct antimicrobial effects by inhibiting the growth of pathogens such as bacteria, viruses, and parasites many of which are associated with gastrointestinal diseases such as diarrhea [35]., the strong antioxidant properties of *E. polycnemoides* extracts further support their therapeutic potential in managing oxidative stress related diseases as well as infectious diseases. Overall, while the methanol extract contained higher concentrations of key polar phytochemicals (tannins, phenols, flavonoids, cardiac glycosides), the ethyl acetate extract consistently exhibited stronger antioxidant activity across all assays. This suggests that the antioxidant efficacy of *E. polycnemoides* is not solely dependent on the total concentration of phenolics and flavonoids but also on the presence of moderately polar compounds—such as terpenoids, certain alkaloids, and flavonoid aglycones—that are preferentially extracted in ethyl acetate. The observed activities have important implications. First, they provide scientific validation for the traditional use of *E. polycnemoides* in treating oxidative stress-related ailments. Second, the dual presence of strong phytochemical diversity and high antioxidant capacity positions the plant as a promising candidate for the development of natural antioxidant formulations. Third, considering the global push toward plant-based therapeutic alternatives, these findings expand the pharmacological relevance of the genus *Euphorbia* and highlight the importance of solvent selection in maximizing bioactivity. Finally, since antioxidants are also known to modulate immune responses and exhibit antimicrobial effects [35], the extracts of *E. polycnemoides* may have additional therapeutic benefits beyond oxidative stress management, including potential applications in infectious disease treatment.

5. CONCLUSION

The study revealed that the aerial parts of *Euphorbia polycnemoides* are a rich source of diverse phytochemicals, including alkaloids, flavonoids, phenols, tannins, saponins, steroids, anthraquinones, terpenoids, cardiac glycosides, and reducing sugars, while phlobatannins were absent. Quantitative analysis showed that the methanol extract yielded higher concentrations of several polar phytochemicals such as phenols, flavonoids, and cardiac glycosides compared to the ethyl acetate extract. However, antioxidant assays consistently demonstrated that the ethyl acetate extract exhibited stronger activity across all tests, including FRAP, DPPH, nitric oxide, lipid peroxidation, and hydrogen peroxide scavenging, in some cases closely approaching the performance of standard ascorbic acid. These results suggest that moderately polar compounds extracted in ethyl acetate, such as terpenoids and flavonoid aglycones, make substantial contributions to the antioxidant efficacy of the plant. From these findings, it can be concluded that *E. polycnemoides* possesses considerable antioxidant potential, validating its ethnomedicinal use and

underscoring its pharmacological relevance. The contrasting results between phytochemical content and antioxidant performance highlight the importance of solvent selection, showing that biological activity may not always correlate directly with total phenolic or flavonoid concentration but also depends on the types of compounds extracted. It is recommended that further studies be conducted to isolate and characterize the specific bioactive constituents responsible for the strong antioxidant effects observed, particularly in the ethyl acetate extract. In vivo studies and toxicity evaluations are necessary to establish the safety and therapeutic applicability of the plant extracts. Additionally, future research should explore the antimicrobial, anti-inflammatory, and other pharmacological properties of *E. polycnemoides* to broaden its potential use in drug development and support its sustainable application as a natural source of therapeutic agents.

Declarations
We declare that this an original work and is not being considered for publication anywhere else .

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Conflict of interest
The authors declare no conflict of interest.

Contribution the Authors
UIA conceived the idea and wrote the final draft, HKA prepared the initial draft, OOO was in charge of the analysis and some literature search, JEO carried out the literature review. All the authors went through the final draft.

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