Regular Edition - PNgAS. Vol 17, No 2, 2024

https://doi.org/10.57046/ATXA8815

Original Research Article

Antifungal effect of *Euphorbia prostrata* ethyl acetate extract on *Microsporum audounii*-induced skin dysfunction diseases in male rats

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Abstract

The search for medicinal plants as viable substitutes for antifungal drug development is crucial due to the increasing threat of multiple drug resistance in global health. However, some of these medicinal plants have become prominent candidates due to reported biological functions such as antimicrobial properties, especially when it comes to treating drug-resistant skin infections with no or little side effects. Therefore, this study was designed to assess the topical application of ethyl acetate fraction of *Euphorbia prostrata* leaf extract on haematological parameters of rats infected with fungi. Cold maceration was employed to extract *Euphorbia prostrata* leaves, followed by vacuum liquid chromatography to fractionate the extract into n-hexane, ethyl acetate, chloroform, and methanol fractions. Phytochemicals and antioxidants assays determined the most active fraction, while antimicrobial susceptibility testing (AST) gauged its efficacy. Cream formulations ranging from 0.05% to 0.4% of ethyl acetate fraction were developed as antifungal agents and tested on rat model. The results indicated that the ethyl acetate fraction's dominance in flavonoid and phenolic content, alongside potent antioxidant activity. Treatment with both standard medication and the plant extract significantly elevated haemoglobin concentrations, with the 0.2% plant extract exhibiting the most pronounced effect. Monocyte counts increased notably in rats treated with varying concentrations of the plant extract, particularly in the 0.2% group. Eosinophil proportions were highest in rats treated with 0.4% ethyl acetate fraction cream, highlighting its efficacy against fungal infections. Overall, the study suggests the potential of *Euphorbia prostrata* extract in modulating erythropoiesis, immune function, and coagulation, thereby offering a promising avenue for addressing fungal infections and associated haematological disturbances.

Biological: Microbiology

Keywords: *Medicinal plants, Microsporum audounii, phytochemicals*

Introduction

A distinct class of plants known as "medicinal plants" are those with therapeutic qualities and are used in herbalism [1]. They are widely used in traditional medicine and over 3.3 billion people regularly utilise them in underdeveloped countries [2]. Throughout history herbal medicine has been used for medical purposes and has developed in tandem with the times. Indigenous plants and herbs have been used for millennia by traditional healers all over the world to cure a wide range of illnesses, and these have been shown to have pharmacological activity. Traditionally, herbal medications were used as powders, tinctures, teas, and poultices. Afterwards, they were formulated and then used as pure compounds [3,4]. *Euphorbia prostrata*, referred to as prostrate

https://doi.org/10.57046/ATXA8815

spurge, is a weed that grows next to grass in gardens. *E. prostrata* is a member of the vast dicotyledonous angiosperm family Euphorbiaceae. The morphology of the Euphorbiaceae family, which spans both hemispheres, varies from massive desert succulents to trees and even tiny herbaceous species [5]. It is a tiny prostrate annual herb that grows widely in Asia and Africa [6]. The species *Euphorbia* has a wide range of therapeutic uses. According to the published literature, most species are used to treat a wide range of illnesses, including microbial illnesses, cellular tissue ailments, nutritional diseases, injuries, endocrine disorders, pregnancy/birth/puerperium disorders, digestive issues, blood syndromes, inflammatory infections, body pain, genitourinary syndromes, and infections of the respiratory system and skin [7].

The skin is the biggest organ in the human body, making up 15% of the adult body weight. Many vital functions are carried out by the skin, including keeping the body from losing too much water and shielding it from outside dangers that could be chemical, biological, or physical. Globally, skin infection is ranked the fourth most frequent skin disease [8]. The prevalence of skin fungal infections is expected to reach 20–25% of the world's population, and its incidence continues to increase [9]. This increase may be as a result of the usage of antibiotics and immunosuppressive drugs [10]. Fungi have symbiotic interactions with bacteria and plants and can reproduce both sexually and asexually. Nevertheless, they are accountable for certain diseases in plants and animals. Pathogenic fungi are the source of fungal infection, commonly referred to as mycosis [11].

Fungi particularly dermatophytes are the main cause of skin fungal infections [9]. *Trichophyton spp., Microsporum spp*., and *Epidermophyton spp.* are the 3 genera that comprise the genus dermatophytes [12]. It has been reported that skin fungal infections are less frequently caused by non-dermatophyte fungi (e.g., *Malassezia in* tinea versicolor) [9]. However, dermatophytosis is a cutaneous skin disorder that is caused by dermatophytes. The prevalent fungal infection that affects keratin-rich areas of the human body including the skin, hair, and nails, is the hallmark of this disease [13]. The non-inflammatory fungal infection *Microsporum audouinii* typically affects the skin and scalps of children and young adults. It is common in developing countries, mostly affecting the impoverished [14]. Although nail infections can also result from it, children are primarily affected by tinea capitis (scalp ringworm) and tinea corporis (body ringworm) [15]. This study was designed to investigate the threat of antifungal medication resistance, raising the death rate from systemic fungal infections. As the need for safe, effective, and alternative antifungal treatments has increased due to this circumstance; numerous medicinal plants have shown promise in the treatment of fungal skin infections [16, 17]. More so, *Euphorbia prostrata* has drawn attention because of its antibacterial and anti-inflammatory effects [18, 19]. Therefore, in order to produce efficient remedies for fungal infections, this study intends to explore the therapeutic potential of extracts from *Euphorbia prostrata*.

Materials and Methods

Plant materials

Euphorbia prostrata leaves used for this study were obtained from Botanical Garden, University of Ibadan, Ibadan, Oyo State and were authenticated at Department of Botany, Faculty of Sciences, University of Ibadan, Oyo State with voucher number; UIH No.: 23211.

Preparation of leaf extract

The leaves were washed and air-dried until it was dried. The dried leaves were pulverized into

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coarse powder. 1000g of the powder was extracted in 6 litres of methanol for 72 hours. It was filtered using a muslin cloth and washed again with 1 litre of methanol, the two filtrates were pulled together and filtered using Whatman filter paper (1 mm). The filtrates collected were then concentrated using a rotary evaporator that was set at 40 ^{o}C with a pressure of 700 mmHg and then 106.5 g of the obtained extract was placed in an amber bottle and kept in the refrigerator till further use. The obtained extract was in a chocolate colour with an aromatic odour [20].

Vacuum liquid chromatography (VLC)

To make the crude extract from the *Euphorbia prostrata* plant easier to mix with thin-layer chromatography grade silica gel powder, it was dissolved in 10 mL of methanol and then allowed to dry. For the easy separation of these various fractions from the crude plant extraction, this combination was ground into a powder. Before the crude extract mixture was packed and pressed under pressure, 150 g of silica gel was weighed, put into the Buckner sintered glass funnel, crushed under pressure from the vacuum pump, rinsed with the first solvent to be used, drained, and dried under pressure. The extract bed was set up by pre-adsorbing, drying, and using the crude methanol extract weight of 106.5 g. The solvents were added from the less polar to the more polar and drained until a very clear solvent was observed on each elution. The fractions were concentrated using a rotary evaporator and the concentrates were further allowed to dry the contents were then stored in glass containers and stored at 4 ^{o}C until ready for use. The fractions obtained were the n-hexane fraction (nHFEP) (0.1 g), the chloroform fraction (CFEP) (5.3 g), the ethyl acetate fraction (EFEP) (2.9 g), and the methanol fraction (MFEP) (14.1 g).

Extract preparation/preliminary phytochemical screening

1.0 g of the nHFEP, CFEP, EFEP, and MFEP fractions of *Euphorbia prostrata* were dissolved in 100 mL DMSO solvent to obtain a stock of concentration 1% (w/v). The solutions obtained were subjected to preliminary phytochemical screening following the methodology of [21]. The preliminary phytochemicals detected are; alkaloids, carbohydrates, flavonoids, proteins, anthraquinone, cardiac glycosides, saponins, steroids, phenols, tannins, and terpenoids.

Determination of antioxidant activity

Determination of total phenolic content (TPC)

TPC of the four (4) fractions was done using the method modified by [22]. 1.0 mL of the sample is mixed with 1.0 mL (10%) of Folin-Ciocalteu phenol reagent. After 5 min, 5.0 mL of 7% sodium carbonate was added followed immediately by the addition of 5.0 mL of distilled water and shaken thoroughly. The mixture was kept in the dark for 90 min at room temperature. The absorbance was read at 750 nm and the TPC was evaluated from the gallic acid standard curve and expressed as gallic acid equivalent (mg GAE/100 g).

Determination of total flavonoid content (TFC)

According to Park *et al*. [23] method, 0.3 mL of the sample was mixed with 3.4 mL (30%) of methanol, 0.15 mL (0.5 M) of sodium nitrite, and 0.15 mL (0.3 M) of aluminium chloride successively. After 5 min, 1 mL of 1M sodium hydroxide was added and mixed well. The absorbance was read at 506 nm and the flavonoid content was evaluated from the quercetin standard curve and expressed as quercetin equivalent (mg QUE/100 g).

Total antioxidant capacity phosphomolybdate assay (TAC)

The total antioxidant capacity of each of the four fractions was calculated using the Prieto *et al.*

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[24] approach. A complete mixture was achieved by mixing 0.4 mL of the sample with 4.0 mL of the phosphomolybdate reagent (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The mixture was capped and was left to boil using a water bath at 95 ^{o}C for 90 mins. After boiling, the mixture was cooled at room temperature, and the absorbance of the sample was taken at 695 nm. Using the ascorbic acid calibration curve, the total antioxidant capacity was determined and represented as ascorbic acid equivalent (mg AAE/100 g).

Determination of ferric-reducing antioxidant potential (FRAP)

Benzie and Szeto's [25] approach was used for the FRAP of the four fractions. Freshly prepared FRAP working reagent was heated to 37 ^{o}C before use by combining solutions of 25 mL acetate buffer, 2.5 mL TPTZ solution, and ferric chloride in a ratio of 10:1:1. 0.2 mL of sample and 2.80 mL of the FRAP reagent were mixed, and the solutions were allowed to sit at room temperature for 30 minutes in the dark. The absorbance was measured at 593 nm, and the ferrous sulphate standard curve was used to calculate the FRAP, which was then reported as mg Fe^{2}E/100 g.

Determination of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity

Gyamfi *et al*. [26] method was used, albeit with some modifications, to measure the DPPH radical scavenging activity of the four fractions. 4 mL of DPPH solution (30 mg/L) mixed with methanol with suitable dilutions of 1 mL (0.1–0.4 mg/ml) sample. After well blending the samples, they were placed in the dark for 30 minutes. The absorbance was read at 520 nm. The inhibition percentage was calculated as.

$$\boldsymbol{Inhibition\ \%\ of\ DDPH} = \{\frac{\boldsymbol{Abs\ control - Abs\ Sample}}{\boldsymbol{Abs\ control}}\} * \mathbf{100}$$

Determination of ABTS {2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonate

The method of Chaves *et al*. [27] was employed to evaluate the plant extracts antioxidant properties against ABTS. By oxidizing ABTS with potassium persulfate, radical ABTS was created. A 1:1 v/v combination of potassium persulfate (4.95 mM) and ABTS (7 mM) was made and allowed to stand at room temperature for 16 hours in the dark. After that, methanol was added to the mixture to dilute it until the mixture's absorbance at 734 nm was between 1 and 1.5. 3.9 mL of the ABTS dilution was added to aliquots of 0.1 mL of each sample of the methanol extract (at four different concentrations: 0.1, 0.5, 1, and 2 mg/mL; two replicates per sample and concentration). At 734 nm, the absorbance drop was recorded using a UV-visible spectrophotometer. ABTS was used to prepare the blank. The findings were presented as quercetin milligram equivalents per milligram of dry weight. The concentrations of quercetin that were used to establish the calibration line were 0.00062, 0.00125, 0.0025, 0.005, 0.01, and 0.032 mg/mL.

Determination of the functional groups

A small quantity of the sample was grounded with purified salt of potassium bromide pellet. The mixture was then crushed in a Perkin Elmer mechanical die press to form a translucent pellet which was then placed in the Perkin Elmer FT-IR spectrophotometer and the spectra of the synthesized compound were taken.

Antimicrobial susceptibility test

Antimicrobial susceptibility testing (AST) of the plant extract was done using the agar well

diffusion method [28]. Sterile swabs were dipped into 1 in 100 dilutions of an overnight broth culture of *Micropronium audunii* and *Trichophyton rubrum* and swabbed uniformly on a prepared Mueller Hinton Agar plate. 8 mm size of cork borer was used to make holes on congeal agar for 100 mg/mL and 50 mg/mL concentrations respectively. The well was filled with different concentrations of the plant extract with the positive control ketoconazole at the centre of the petri-plate. The plates were left on the bench for 45 minutes for pre-diffusion thereafter the plates were incubated at 24 ^{o}C for 48 hrs. The zones of inhibitions were taken in mm with a graduated ruler.

Minimum inhibitory concentration

The test organisms (*M. audunii* and *T. rubrum*) were subculture into nutrient broth medium to make overnight broth culture and thawed then the extracts were weighed. The antibacterial was dissolved to the concentration desired in the test medium to 2 × the concentration desired in the test. The desired concentration in the first column was 50 mg/ml, but 100 mg/ml of the extract was dispensed. Multi-pipettor 100 ml of TSB was dispensed into the wells of each of the microliter plates respectively the lids were labelled with the names of organisms, 100 µL appropriate 2 × extract and positive control was dispensed into wells in row one using the multi-pipettor set at 100 µL, the extracts were mixed in row one by sucking up and down without splashing. 100 µL was withdrawn from row 1 and row 2. This makes row 2, a twofold dilution of row one, 100 µL was transferred to row 3 and the procedure was repeated down to row 7.5 µL of 10^5 bacterial and fungi cultures were dispensed into wells in rows 7 to 1 in the order. The organism was not added to column 8 which serves as broth sterility control. The plates were incubated at 37 ^{o}C and 25 ^{o}C for 24 hrs and 48 hrs. The lowest concentration of the extract which showed no colour change (red colouration) was taken as MIC.

Minimum bactericidal concentration

The concentration which showed no colour change (red colouration) were streaked on solidified nutrient agar plates and incubated at 37 ^{o}C for 24 hrs and the concentration which showed no growth on nutrient agar medium was taken as MBC.

Preparation of the cream

Reagents/materials

Shear butter and standard drug (ketoconazole antifungal cream). The formulary for antifungal cream is shown in Table 1.

Table I: Antifungal cream formularies

Formulation code	Active ingredient (ethyl acetate fraction)	Vehicle (shear butter)
1	0.05%	99.95%
2	0.1%	99.90%
3	0.2%	99.80%
4	0.4%	99.60%

Preparation of sub-culture of micro-organism

Loops of well-grown nutrient agar micro-organisms of clinical isolates of *Microsporum audounii* obtained were suspended in 30 ml of saline water in a plain bottle and kept at a temperature of 4 ^{o}C in the laboratory fridge for 48 hours. The well-grown fresh sub-cultured organisms were used for inoculation during experimental investigation.

Setting up of the experimental study

Animal handling/care and ethical protocol

Forty (40) male albino Wistar rats, weighing 120 – 150 g were purchased from the University of Ibadan, Faculty of Veterinary Medicine Experimental Animal Unit and used for this study. Nine plastic cages (60 cm by 30 cm) were used to store the rats during the duration of the study. The top of the cages was well perforated to ensure proper ventilation. These cages were kept in the Animal House of the Department of Biochemistry, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, Ibadan, Oyo State. Wood shavings were used as bedding for the cages. The animals were fed with standard rat chow (Breedwell Feeds Limited) and water *ad libitum* and maintained under constant temperature (23±2 ^{o}C) and photoperiod (12L:12D). The study was carried out according to the guidelines provided by the University of Ibadan Animal Care and Use Research Ethical Committee (UI-ACUREC) following the principle laid down in the Declaration of Helsinki (1964), as revised in 2013 and National Institute of Health (NIH) Principles of Laboratory Animal Care. No human participants were involved in the study. The study proposal was submitted and subsequently approved (approved number: UI-ACUREC/090-0923/29).

Experimental selection and grouping of animals

The animals were separated into eight groups (5 rats per group). Forty rats were purchased and grouped into 8 groups of 5 rats each.

Group A: Positive control (Uninfected rats)
Group B: Negative control (Infected rats but not treated)
Group C: Infected rats treated with vehicle (shear butter)
Group D: Infected rats treated with standard drugs (ketoconazole)
Group E: Infected rats treated with 0.05% formulation alone.
Group F: Infected rats treated with 0.1% formulation alone.
Group G: Infected rats treated with 0.2% formulation alone.
Group H: Infected rats treated with 0.4% formulation alone.

Procedures

Week one: The Animals were fed with starter feed (65 g) and water twice a day in each cage and their wood shavings were changed every two days. This was done to ensure that the animals were in a clean environment, and to curb the spread of infections and left to acclimatize to the new environment.

Week two: The Animals continued to be fed with starter feed (65 g) and water twice daily in each cage and their wood shavings changed every two days. The acclimatization ended at the end of this week.

Week three: The albino rats were re-weighed, and each rat hair was scrapped at the side of the belly in the dimension of approximately 2 cm^2 by 2 cm^2 and was left for 24 hours after which inoculation of the animals with the dermatophytes was carried out. The inoculation was done by injecting 2 mL of subculture *Microsporum audounii*, followed by routine cleaning and feeding, and the animals were left for the fungal infection to develop.

Week four: After seven days from inoculation, treatment with the formulated cream of different

concentrations, standard drugs, and vehicle started.

Week five: At the end of seven days of treatment, the animals were euthanized by cervical dislocation.

Skin sensitivity test

Five (5) male Wistar rats (weighing 120 – 150 g) were given treatments on their shaved skin using all the cream formulations that included natural plant and shear butter, following the OECD guidelines 404 [29], with slight modification by Ankomah *et al*. [30]. The test samples were applied early in the day and left there till the next day. After applying, water was used to rinse, and absorbent paper was used to pat dry the treated surface. A day later, the region was checked for any indications of discomfort.

Haematological studies

Blood samples were taken from the euthanized rats and taken to the laboratory for a haematological study. Complete blood counts were determined using the Coulter HmX Haematology Analyzer according to the manufacturer's protocols, this was in line with the method of Dasofunjo *et al*. [31]. The parameters include Packed cell volume (PCV), Red blood count (RBC), white blood count (WBC), haemoglobin (Hb), platelet (PT), lymphocyte (LYM), neutrophils (NEUT), monocytes (MON), and eosinophil (EOS).

Statistical analysis

Graph Pad Prism was used to assess the differences between the groups. Data were expressed as mean ± standard deviation. Statistical analysis was carried out using one-way analysis of variance (ANOVA). Values of $p < 0.05$ were considered to be significant and post-hoc tests were carried out using the least significant difference (LSD).

Results

Phytochemical screening

The phytochemical groups in the solvent fractions of methanol, ethyl acetate, n-hexane, and chloroform of *Euphorbia prostrata* are depicted in Table II. The presence of phytochemicals such as carbohydrate, flavonoids, protein, saponins, steroids, phenol, terpenoids, tannins, alkaloids, anthraquinone, and cardiac glycoside were observed. It was reveals that the methanol fraction exhibits all phytochemicals investigated, the n-hexane fraction shows the presence of carbohydrate only. However, the ethyl acetate fractions show the presence of flavonoids, protein, saponins, steroids, phenol, terpenoids, alkaloids, anthraquinone, and cardiac glycoside, while, the chloroform fraction contained flavonoids, protein, saponins, tannins, alkaloids, anthraquinone, and cardiac glycoside.

Table II: Phytochemical components of *Euphorbia prostrata* fractions

S/N	Compounds	Ethyl acetate	Chloroform	Methanol	Hexane
1	Carbohydrate	-	-	+	+
2	Flavonoids	+	+	+	-
3	Protein	+	+	+	-
4	Saponins	+	+	+	-
5	Steroids	+	-	+	-
6	Phenol	+	-	+	-
7	Terpenoids	+	-	+	-
8	Tannins	-	+	+	-
9	Alkaloids	+	+	+	-
10	Anthraquinone	+	+	+	-
11	Cardiac glycoside	+	+	+	-

Antioxidant screening

The results below depict the *in vitro* antioxidant activities of fractions of *E. prostrata* leaf. The phenolics and flavonoids contents shown in Figures 1 and 2 respectively revealed that ethyl acetate fraction has the maximum contents of phenolics and flavonoids than the other fractions assayed. More so, the total antioxidant capacity (TAC), ferric reducing antioxidant power (FRAP) scavenging activity, and % inhibition 1, 1-diphenyl-2-picrylhydrazyl (DPPH) are displayed in Figures 3, 4 and 5 respectively. It was shows that the ethyl-acetate fraction exhibits the highest total antioxidant capacity (TAC), highest FRAP scavenging activity, and the highest percentage inhibition compared to other fractions. Furthermore, n-hexane fraction has the highest ABTs scavenging activity as shown in Figure 6, followed by chloroform.

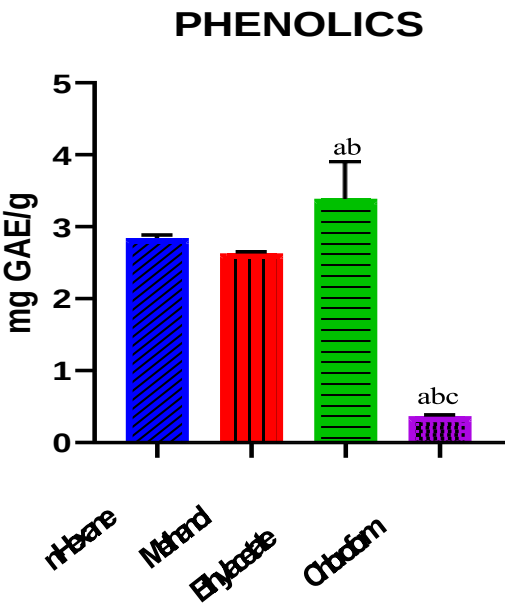

Figure 1: Total phenolic content of fractions of methanol extract of *Euphorbia prostrata*. Values represent Mean ± SD. n = 6. ($^{a}p < 0.05$ vs. n-hexane, $^{b}p < 0.05$ vs. methanol, $^{c}p < 0.05$ vs. ethyl acetate)

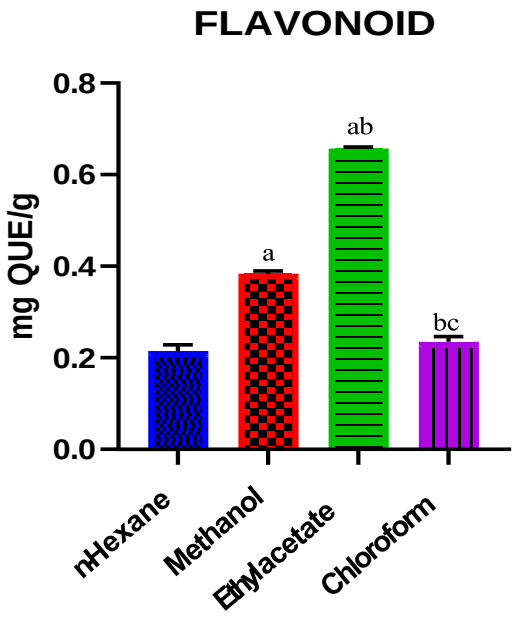

Figure 2: The flavonoid content of fractions of methanol extract of *Euphorbia prostrata*. Values represent Mean ± SD. n = 6. ([a]p < 0.05 vs. n-hexane, [b]p <0.05 vs. methanol, [c]p <0.05 vs. ethyl acetate)

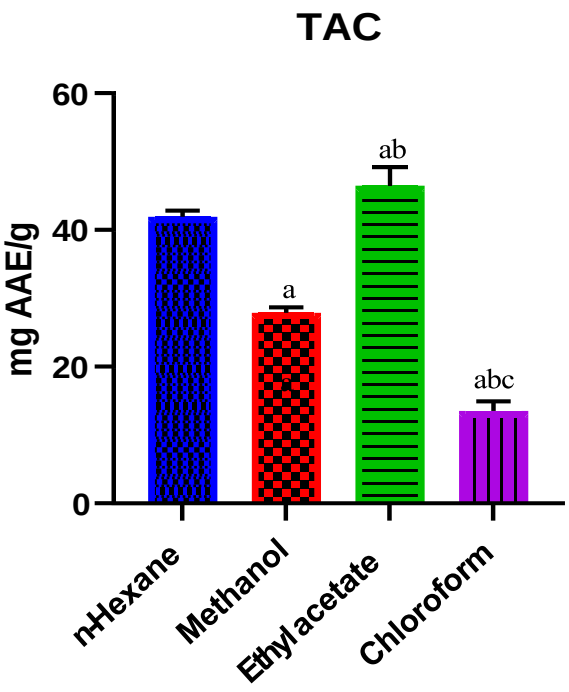

Figure 3: Total antioxidant capacity of fractions of methanol extract of *Euphorbia prostrata.* Values represent Mean ± SD. n = 6. ([a]p < 0.05 vs. n-Hexane, [b]p <0.05 vs. methanol, [c]p <0.05 vs. ethyl acetate)

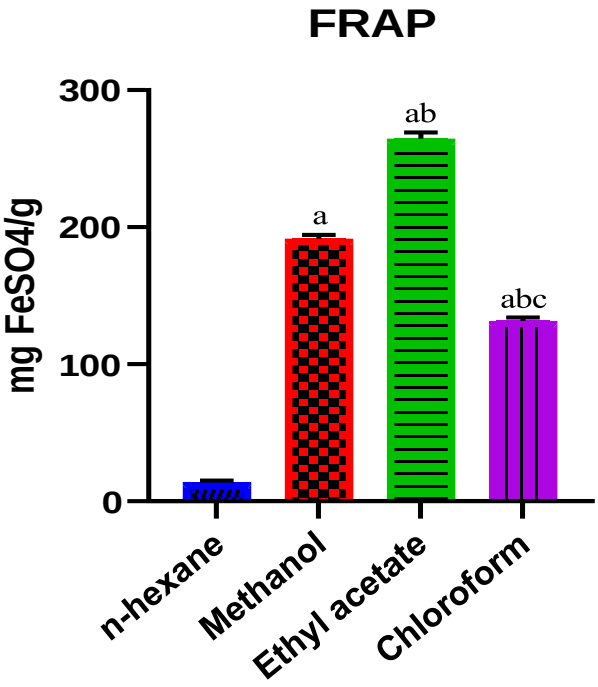

Figure 4: FRAP scavenging activity of fractions of methanol extract of *Euphorbia prostrata.* Values represent Mean ± SD. n = 6. ($^{a}p < 0.05$ vs. n-hexane, $^{b}p < 0.05$ vs. methanol, $^{c}p < 0.05$ vs. ethyl acetate)

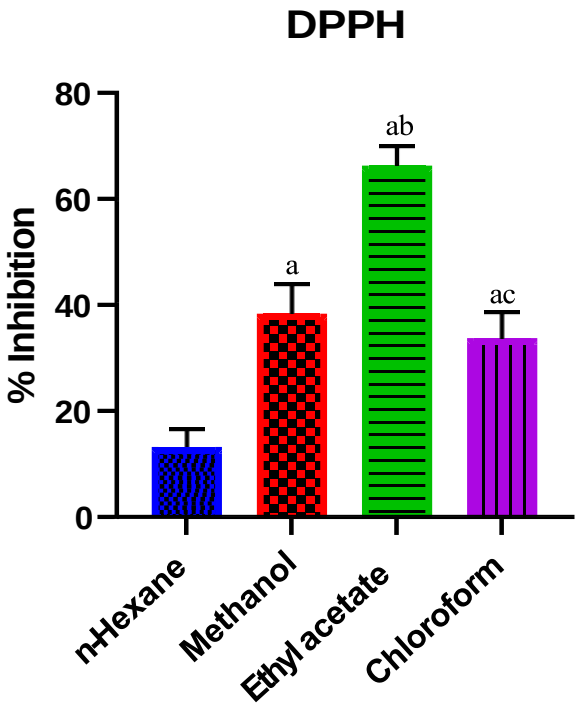

Figure 5: Percentage inhibition of fractions of methanol extract of *Euphorbia prostrata* against DPPH radicals. Values represent Mean ± SD. n = 6. ($^{a}p < 0.05$ vs. n-hexane, $^{b}p < 0.05$ vs. methanol, $^{c}p < 0.05$ vs. ethyl acetate)

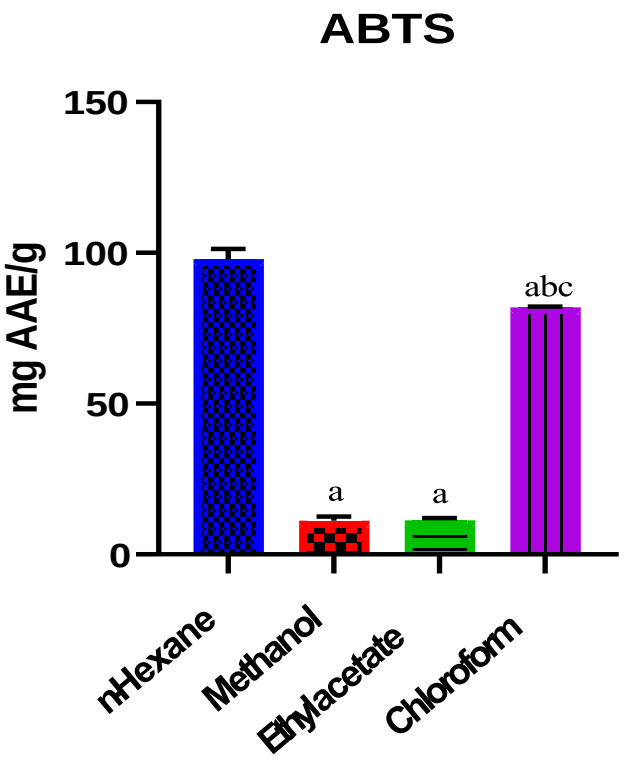

Figure 6: ABTS scavenging activity of fractions of methanol extract of *Euphorbia prostrata.* Values represent Mean ± SD. n = 6. ([a]p < 0.05 vs. n-hexane, [b]p <0.05 vs. methanol, [c]p <0.05 vs. ethyl acetate)

Thin layer chromatography

The TLC result is displayed in Figure 7, with the mobile phase of 70% for n-hexane fraction and 30% for ethyl-acetate fraction. It was observed that the n-hexane fraction has 2 compounds with Rf 0.86 and 0.94 respectively, while the chloroform fraction has 5 compounds with Rf 0.63,0.74,0.84,0.89 and 0.93 respectively. More so, the TLC mobile phase of 30% for n-hexane fraction and 70% for ethyl acetate fraction shows the ethyl-acetate fraction has 6 compounds with Rf values of 0.13, 0.26, 0.34, 0.40, 0.49, and 0.88 respectively, while, methanol fraction has only 1 compound with Rf of 0.11.

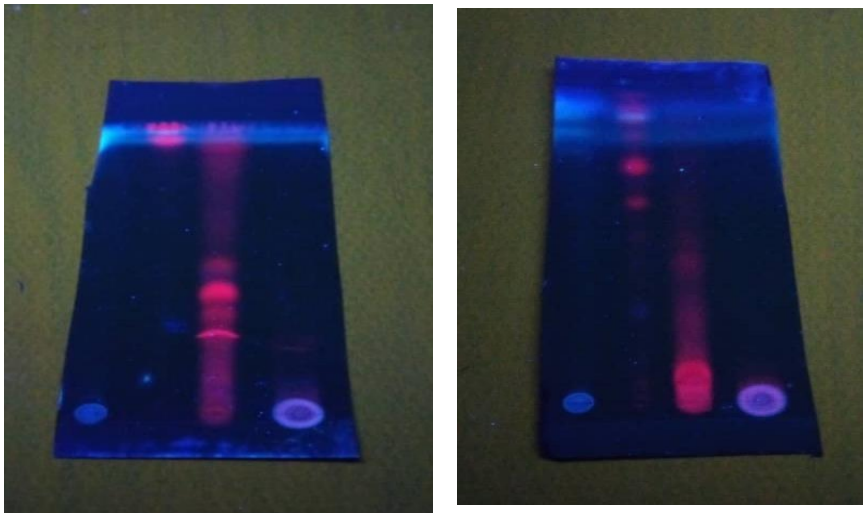

Figure 7: Thin layer chromatography (TLC) plates showing fractions of *Euphorbia prostrata* extract

Determination of functional group

The infrared spectra (IR) analysis of ethyl acetate fraction in Table III shows OH (bonded), sp^3C-H (stretch) and C=C vibrations at the peak of 3449.78 cm^{-1}, 2923.1 cm^{-1}, 2855.11 cm^{-1} and 1695.99 cm^{-1} respectively. However, Table IV of the methanol fraction revealed the presence of OH (hydroxyl), sp^3 CH, C=C (alkene), aromatic ring and carbonyl compounds showing at the peak 3449.2 cm^{-1}, 3018 cm^{-1}, 2925.02 cm^{-1}, and 2856.5 cm^{-1} respectively. More so, the IR analysis of chloroform fraction displayed in Table V revealed the presence of OH (hydroxyl), sp^3 CH, C=C (alkene), aromatic ring and carbonyl compounds at the peak of 3450.83 cm^{-1}, 3076 cm^{-1}, 2725.5 cm^{-1}, 1454.86 cm^{-1}, and 1584.79 cm^{-1}.

Table III: IR analysis of the ethyl acetate fraction

ῡ (cm^{-1})	% T	Nature of peak	Inference
3449.78	~34	Broad	O-H (bonded)
2923.41	~32	Sharp	sp^3 C-H stretch
2855.11	~32	Sharp	Sp^2 C-H stretch
1695.99	~28	Sharp	C=C

Table IV: IR analysis of the methanol fraction

ῡ (cm^{-1})	% T	Nature of peak	Inference
3449.02	~38	Broad	O-H (bonded)
3018	~42	Sharp	sp^2 C-H stretch
2925.02	~40	Sharp	sp^3 C-H stretch
2856.5	~42	Sharp	Sp^2 C-H stretch
1724.5	~40	Sharp	C=O
1638	~38	Sharp	C=C
1451	~42	Sharp	Aromatic CH

Table V: IR analysis of the chloroform fraction

ῡ (cm^{-1})	% T	Nature of peak	Inference
3450.83	~25	Broad	O-H (bonded)
3076	~30	Broad	sp^2 C-H Stretch
2923.29	~20	Sharp	sp^3 C-H stretch
2856.07	~20	Sharp	sp^3 C-H stretch
2725.5	~35	Sharp	Aldehyde CH
2671.6	~35	Sharp	Aldehyde CH
1731.27	~20	Sharp	C=O
1712.52	~20	Sharp	C=O
1584.79	~35	Sharp	C=C (aromatic)
1463	~20	Sharp	aromatic ring CH
1454.86	~20	Sharp	aromatic ring CH

Skin sensitivity test

After the application of the treatment to the exposed skin of the rats for twenty-four hours. There were no red spots (bumps) observed on the skin of the rats which indicates that the rats are not allergic to the treatment.

Haematological studies

The haemoglobin concentrations were significantly ($P<0.05$) higher in the treatment group receiving both the standard medication and the plant extract than in the control group as seen in Tables VI and VII, the treatment group treated with distilled water had the lowest concentration of Hb (15.53±0.15), while the 0.2% ethyl acetate plant extract produced the highest concentration (17.17±0.65 g/dl). There was no significant difference detected with respect to other indices including PCV, RBCs and WBCs. The monocyte counts significantly increased ($P<0.05$) in the group treated with 0.05, 0.1 and 0.2% ethyl acetate fraction formulated cream as compared with the *Microsporum audounii* infected rats and the positive control. 0.2% ethyl acetate formulated cream gave the highest monocyte counts (2.27±0.58) as compared with the control and all other groups. Both 0.4% ethyl acetate fraction of *Euphorbia prostrata* formulated cream and ketoconazole gave the highest proportion of eosinophil as compared with the *Microsporum audounii* infected rats and the positive control in Table VII.

Table VI: Effect of administration of Euphorbia prostrata on haematological indices in Wistar albino rats

Treatments	PCV (%)	Hb (g/dl)	RBC ($x10^6$uL)	WBC ($x10^3$ul)
Control	47.33±2.83^a	15.33±0.70^c	8.12±0.63^a	3933.30±494.97 ^{a}
MA infected	48.00±0.00^a	15.53±0.15^{bc}	7.80±0.04 ^{a}	4666.7±850.41 ^{a}
MA + vehicle	51.67±3.51 ^{a}	16.77±0.87^{abc}	8.39±0.57 ^{a}	5016.7±765.39 ^{a}
MA + Ket	51.00±2.65 ^{a}	16.33±1.10^{abc}	8.35±0.61 ^{a}	5233.3±2542.31 ^{a}
0.05% EEP + V+ MA	52.33±3.22 ^{a}	16.93±0.57^{ab}	8.67±0.16 ^{a}	4983.3±725.14 ^{a}
0.1% EEP + V+ MA	50.33±2.52 ^{a}	16.40±0.88^{abc}	8.37±0.62 ^{a}	4700±754.98 ^{a}
0.2% EEP + V+ MA	53.00±2.65 ^{a}	17.17±0.65^a	8.61±0.12 ^{a}	4483.3±682.52 ^{a}
0.4% EEP + V+ MA	50.67±5.03 ^{a}	16.40±1.15^{abc}	8.27±0.71 ^{a}	4216.7±202.07 ^{a}

Legends: MA = *Microsporum audounii*, Ket = Ketoconazole, EEP = Ethyl acetate *Euphorbia prostrata*, V = Vehicle, PCV = Packed cell volume, Hb = Haemoglobin, RBC = Red blood cell, WBC = White blood cell.

Mean values with the same alphabet are not significantly different ($p<0.05$).

Table VII: Effect of administration of *Euphorbia prostrata* on leukocyte differential count in Wistar albino rats

Treatments	PLATELET (%)	LYM (%)	NEUT (%)	MON (%)	EOS (%)
Control	88667±11313.71^{a}	74.00±00^{a}	22.33±0.71^{a}	1.67±0.71^{abc}	1.33±0.71^{ab}
MA infected	91667±18770.54^{a}	72.67±0.58^{a}	23.67±2.08^{a}	2.00±1.0^{abc}	1.67±1.53^{ab}
MA + vehicle	107667±42500.98^{a}	73.33±1.53^{a}	23.33±1.16^{a}	1.33±0.58^{abc}	2.00±1.00^{ab}
MA + Ket	108667±17243.36^{a}	73.67±0.58^{a}	23.00±2.65^{a}	1.67±1.16^{abc}	1.67±0.58^{a}
0.05% EEP + V+ MA	77667±10785.79^{a}	75.33±1.53^{a}	21.33±3.06^{a}	2.33±0.58^{ab}	1.00±1.00^{ab}
0.1% EEP + V+ MA	103000±18083.14^{a}	74.33±2.08^{a}	23.00±1.00^{a}	1.00±1.00^{bc}	1.67±0.58^{ab}
0.2% EEP + V+ MA	97000±5196.15^{a}	75.00±2.60^{a}	20.67±2.50^{a}	2.67±0.58^{a}	1.67±0.58^{ab}
0.4% EEP + V+ MA	93000±17578.40^{a}	74.33±2.31^{a}	21.67±3.21^{a}	1.67±1.15^{abc}	2.33±0.58^{a}

Legends: MA = *Microsporum audounii*, Ket = Ketoconazole, EEP = Ethyl acetate *Euphorbia prostrata*, V = Vehicle, EOS = Eosinophil, MON = Monocytes, LYM = Lymphocytes, NEU = Neutrophils.

Mean values with the same alphabet are not significantly different ($p<0.05$).

Antimicrobial assay

The results in Tables VIII and IX showed the antimicrobial activities of ethyl acetate, methanol, and chloroform fractions of *euphorbia prostrata* plant that was examined by determination of minimum inhibitory concentration (MICs) and minimum bactericidal concentration (MBCs) against *Microsporum audounii* and *Tricophyton rubrum* organism. This result shows that ethyl-acetate fraction had the highest activities against *Microsporum audounii* and *Tricophyton rubrum* organisms at 100 mg/ml (18 mm and 19 mm) and 50 mg/ml (15 mm and 19 mm) respectively, followed by the methanol fraction, and then the chloroform fraction. The organisms were tested at 6.25 mg/ml which kills at 6.25 mg/ml and 12.5 mg/ml which made it the most active fraction. Methanol also had its MIC at 25 mg/ml and 25 mg/ml for its MBC, while chloroform had its MIC at 50 mg/ml against the organism and it was not unable to kill at 50 mg/ml.

Table VIII: The antimicrobial susceptibility test of *Euphorbia prostrata* fractions

Organism	Chloroform (mg/mL)		Ethyl acetate (mg/mL)		Methanol (mg/mL)		DMSO (1%)		Ketoconazole (1%)
	100	50	100	50	100	50			
M. audounii	10	0	18	15	12	10	-	-	18
T. rubrum	10	110	19	15	14	11	-	-	20

Table IX: Minimum inhibitory concentration and minimum bactericidal concentrations of *Euphorbia prostrata* against *Microsporum audounii*

Organism	Chloroform (mg/ml)		Ethyl Acetate (mg/ml)		Methanol (mg/ml)		Ketoconazole	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC %	MBC %
M. audounii	50	>50	6.25	6.25	25	25	0.5	0.5
T. rubrum	50	>50	6.25	12.5	12.5	25	1.25	0.5

MIC = Minimum inhibitory concentration, MBC = Minimum bactericidal concentration.

Discussion

Phytochemical screening is an essential process that helps to understand the chemical composition of medicinal plants and evaluates their potential health benefits [32]. They are majorly responsible for the biological activities of medicinal plants such as antioxidant, hormonal action, stimulation

of enzymes and antibacterial effect among others [33]. The phytochemical result of the studied fractions of *E. prostrata* reveals that the methanol fraction is rich in all the phytochemicals studied, however, n-hexane was observed to possess carbohydrates only (Table II). Numerous studies have focused on the phytochemical analysis of *Euphorbia prostrata*, a medicinal plant with various traditional uses. For instance, in a study by [34], the authors conducted a comprehensive phytochemical screening of *Euphorbia prostrata* extracts using various solvents, including methanol, and reported the presence of several bioactive compounds, such as alkaloids, flavonoids, phenolic compounds, and tannins.

Furthermore, *in vitro* antioxidant activities of *E. prostrata* were also investigated to assess its potential health-promoting effects. Antioxidants are essential for scavenging dangerous free radicals and shielding the body from illnesses linked to oxidative stress [32]. In this study, *in vitro* antioxidant activity was examined using various assays like ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid), total antioxidant capacity (TAC), ferric reducing antioxidant power (FRAP), and % inhibition 1, 1-diphenyl-2-picrylhydrazyl (DPPH). The study reveals that the ethyl acetate fraction has the maximum contents of phenolics and flavonoids, the total antioxidant capacity (TAC), ferric reducing antioxidant power (FRAP) scavenging activity, and % inhibition 1, 1-diphenyl-2-picrylhydrazyl (DPPH) compared to other fractions (Figures 2 to 5). Furthermore, the n-hexane fraction has the highest ABTs scavenging activity, followed by chloroform (Figure 6). However, this study also highlighted the presence of various phytochemicals, like phenolics and flavonoids (Figures 1 and 2), which contributed to the *in vitro* antioxidant effect of *E. prostrata*. It has been demonstrated that *E. prostrata* exhibits a diverse range of phytochemicals and possesses notable antioxidant capacity [34, 35]. The presence of bioactive compounds and their antioxidant potential suggests that *E. prostrata* holds promise as a medicinal plant with potential health benefits. This study's findings align with a study conducted by [35], which demonstrated the significant *in vivo* antioxidant potential of *E. prostrata* and, hence, the correlation between the phytochemical composition and antioxidant capacity of *E. prostrata*.

Wound healing is a natural occurrence, which deals with tissue regrowth and regeneration [30]. This study reveals the treatment of excised wounds of Wistar rats with the ethyl acetate fraction of *E. prostrata* cream formulation accelerated the wound healing process after 24 hours. Furthermore, there were no red spots (bumps) observed on the skin of the rats which indicates that the rats are not allergic to the treatment. This agrees with Ankomah *et al*. [30] and Yakubu *et al*. [36] studies. *Euphorbia prostrata* was also studied for its potential effects on haematological parameters (Tables VI-VII). The scientific measurements of blood components, such as red blood cells, white blood cells, and platelets, are referred to as haematological parameters [31]. Understanding the impact *of Euphorbia prostrata* on haematological parameters is essential for evaluating its potential health benefits. In this study, a significant increase in red blood cell counts and haemoglobin levels was observed, indicating a potentially positive effect on erythropoiesis. Additionally, the extract was found to increase total leukocyte count, suggesting potential immune-stimulatory effects. However, a study by [35] explored the impact of *E. prostrata* extract on haematological parameters in rats and reported a significant increase in red blood cell count and haemoglobin levels, along with an increase in total leukocyte count, suggesting a potential positive influence on erythropoiesis and immune function. Moreover, a study by [37] investigated the effects of *E. prostrat*a extract on haematological parameters in albino rats, and observed an increase in red blood cell count, haemoglobin levels, and platelet count, indicating a potential positive influence on erythropoiesis and coagulation. This suggests that *E. prostrata* extract may

have positive effects on haematological parameters.

In many experiments, it is important to be able to separate a mixture into its chemical components in order to isolate one compound or to assess the purity of the mixture. Thin layer chromatography (TLC) is one of the easiest and most versatile methods of doing this because of its low cost, simplicity, quick development time, high sensitivity, and good reproducibility [38]. It is used by many industries and fields of research, including pharmaceutical production, clinical analysis, industrial chemistry, environmental toxicology, food chemistry, water, inorganic, and pesticide analysis, dye purity, cosmetics, plant materials, and herbal analysis [38]. In this study, TLC with a mobile phase of 70% n-hexane and 30% ethyl-acetate reveals an n-hexane fraction of 2 compounds, while the chloroform fraction has 5 compounds. Furthermore, TLC with a mobile phase of 30% n-hexane and 70% ethyl acetate reveals an ethyl acetate fraction of 6 compounds, while the methanol fraction has only 1 compound (Figure 7). These findings indicate that more than one active ingredient may account for *E. prostrata* fraction effective inhibition against *Microsporum audounii.*

The IR analysis of the ethyl acetate fraction in Table III suggests that the fraction contains unsaturated compounds with a hydroxyl functional group. Table IV of the methanol fraction suggest the extract contains aromatic compounds with alkyl side chains, compounds with hydroxyl functional groups and compounds with carbonyl groups. The IR analysis of chloroform fraction in Table V suggests that the extract contains aromatic compounds with alkyl side chain, compounds with hydroxyl functional groups and compounds with carbonyl groups. Thus, the effectiveness of ethyl acetate fraction may be due to the presence of OH and aromatic functional groups. This could be attributed to strong binding interaction between the active site of the fungal and OH, aromatic structure of the active ingredient. As reported by [39], the extracts exhibit distinct, identifiable peaks with a frequency range that corresponds to the O-H group's stretching vibration or O-H wagging of phenolic compounds. It is well known that phenolic chemicals have strong antioxidant properties. Furthermore, the N–H stretch can potentially be responsible for the band that appears in the extracts at approximately 3200–3400 cm^{-1} [40]. By using FT-IR, the different peaks and their corresponding functional groups; which included alkene, ester, imine/oxime, aromatic compound, carboxylic acid, amine salt, alkene, aldehyde, nitro compound, alkyl halide, halogenated compound, alkyne, and ester were identified. This implies that several kinds of chemical species may be present in the plant extracts, which may provide resources for the development of new drugs. Lastly, *E. prostrata* was investigated for its antimicrobial activity against *Microsporum audounii* and *Tricophyton rubrum* (Tables VII and IX). The findings suggest that ethyl-acetate fraction had the highest activities against *Microsporum audounii* and *Tricophyton rubrum* organisms at 100 mg/ml and 50 mg/ml respectively. However, several studies have explored the potential of *E. prostrata* extracts in inhibiting the growth of pathogenic bacteria and fungi [34, 37, 41].

Conclusion

The results presented in this study conclusively demonstrated the antifungal potential effect of the ethyl acetate fraction of *Euphorbia prostrata*. This fraction has been shown to be fungicidal to *Microsporum audoiunii*, varying in different concentrations of the effect in the treatment of skin infection. The varying dose cream formulation used was effective but 0.2% of the cream formulation paste showed a higher effectiveness on the fungal infection and the body weight of the treated rats. There was an increase in red blood cell count, haemoglobin levels, and platelet

count, indicating a potential positive influence on erythropoiesis and coagulation. In conclusion, the effectiveness of the ethyl acetate fraction may be due to the presence of phenolic OH and aromatic functional groups and the effectiveness could be attributed to multiple or synergistic effects of active ingredients in the fraction.

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