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GC-MS analysis, Elemental and Nutritional Composition and Biological Investigation of Medicinally Valued Fingerhuthia africana Lehm.

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GC-MS Analysis, Elemental and Nutritional Composition and Biological Investigation of Medicinally Valued *Fingerhuthia africana* Lehm.

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Abstract: *Fingerhuthia africana* Lehm belongs to family Poaceae, is an erect perennial grass used traditionally to cure different diseases. The current study was carried out to find the bioactive compounds by GC-MS, elemental analysis through atomic absorption spectrophotometry, proximate analysis by AOAC, genotoxic activity through comet assay, antioxidant activity through DPPH and antidiarrheal activity through castor oil induce diarrhea followed by charcoal meal on mice model. GC-MS chromatogram represents different compounds detected in the crude extract. Major pharmacological bioactive compounds are 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-; 4-Methyl itaconate; .alpha.-Terpineol; 1,2,3-Propanetriol, 1-acetate; Benzofuran, 2,3-dihydro-; Caryophyllene oxide; Hinesol; n-Hexadecanoic acid; 9,12-Octadecadienoic acid (Z,Z)-, methyl ester; Hexadecanoic acid and methyl ester. Elemental analysis of stem, leaves and roots of the plant show the presence of (macro and micro) nutrients such as Ca, Mg, K, Na, Cu, Pb, Zn, Mn, Fe, Cd and Co were absent in all parts. Proximate analysis of different parts revealed the presence of moisture contents (8.57%), ash (9.77%), fats contents (5.96%), fibers contents (45.03%), protein (3.62%), carbohydrates (39.71%) and Gross energy (Kcal/100g) (220.10). The ethanolic extract show dose-dependent genoprotective potential (127.38 ± 1.16), (83.28 ± 0.65), and (44.08 ± 1.40) at 50, 75, and 100 mg/100 ml, respectively, as compared to the methanolic extract which show genotoxic activity (85.13 ± 1.23) at high dose. After 90 minutes at a concentration of 300 (g/ml), methanolic extract demonstrated the greatest antioxidant potential (79.5%), followed by aqueous extract (76.5%). The methanolic extract of *F. africana* demonstrated antidiarrheal efficacy, with the maximum inhibition of charcoal movement (83.78%) occurring at a dosage of 300 mg/kg, followed by doses of 200 and 100 mg/kg, 73.26% and 59.56%, respectively. The current research highlights that the plant contains a substantial quantity of micro and macro nutrients, possesses high nutritional value, and exhibits pharmacological activities. These properties render the plant suitable for developing remedies to treat various therapeutic diseases.

Keywords: GC-MS; proximate analysis; genotoxic activity; DPPH; antidiarrheal activity

1. Introduction

In accordance with cultural heritage and traditional beliefs, individuals have utilized plants for medicinal use since ancient times. Local communities relied on their personal experiences to employ medicinal plants for various health remedies [1].

Medicinal plants serve as a diverse source of remedies in traditional and modern medicine, encompassing nutraceuticals, supplements, folk medicine, and pharmaceutical compounds [2]. Plants' natural derivatives are employed in medicines, supplements, and diverse healthcare products. They play a crucial role in identifying novel beneficial medicinal compounds, containing phytochemical constituents such as antioxidants, hypoglycemic, and hypolipidemic agents [3]. The use of natural plant-based products contributes to human well-being by minimizing side effects and

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offering cost-effectiveness. As a result, there is a growing demand for therapeutic products derived from plants [4]. The World Health Organization (WHO) reported that 80% of the emerging world's population relies on traditional medicine for therapy [5]. It is known that the plant species used for the treatment of diseases are around 70,000 and only about 15% of the plant species grown in the world have been investigated for their medical use. Despite this low rate, 25% of conventional medicines used in modern medicine today are of plant origin [6]. Pakistan has a rich reserve of medicinal plants with a species diversity of approximately 6,000 plant species, 400-600 of which are used for medicinal purposes. This vast biodiversity places the country in a unique position in the field of ethno-pharmacology [7].

Medicinal plants encompass a range of physiologically active elements, such as minerals and phytochemicals, which exhibit diverse physiological impacts on human health [8,9]. Plants contain various phytochemicals, referred to as secondary metabolites, which possess the potential to address specific disorders through their individual, additive, or synergistic mechanisms, thereby enhancing well-being [10]. Phytochemicals play a crucial role within the pharmaceutical industry, contributing to the creation of novel medications and the formulation of therapeutic agents [11]. The application of gas chromatography coupled with mass spectrometry (GC–MS) has become a crucial method for the identification and quantification of medically valuable compounds within medicinal plants. This approach offers a relatively rapid, precise, and efficient means of detecting a diverse array of bioactive substances like alkaloids, long-chain hydrocarbons, steroids, sugars, amino acids, and nitro compounds, using minimal extract volumes [12].

The human body requires essential minerals and trace elements for proper maintenance of metabolic and physiological functions. The main essential minerals for humans include calcium, phosphorus, potassium, sodium, and magnesium. Meanwhile, iron, copper, zinc, manganese, iodine, and selenium are categorized as trace elements. The presence of these minerals and trace elements is critical for the human body's health and overall well-being [13]. Minerals and trace elements play an important role in the activation of enzymes involved in cell metabolism and antioxidant systems [14]. The element content of plants can be influenced by a variety of factors, including the geochemical properties of the soil, the application of natural and artificial fertilizers, climatic conditions, the proximity of industrial activity and extensive agricultural activity, as well as the ability of herb species to accumulate elements. These factors can have both positive and negative effects on the nutrient content of plants and can affect the overall quality of the food produced [15]. The significance of wild plants as sources of secondary metabolites and micronutrients has been emphasized, particularly in the context of sustainable development and global food security, and their role in sustaining human and environmental health. Numerous studies have been conducted on wild plants to investigate their exceptional nutritional and therapeutic benefits [16].

Proximate analysis is a useful tool for determining the nutritional composition of plant-based products. This analysis typically includes measurements of crude protein, crude fiber, total ash, moisture content, and dry matter. However, it is important to note that the exact composition of food samples can be influenced by a wide range of factors, including the plant variety, climate, cultivar, maturity level, production conditions, handling, processing, and storage. By conducting proximate analysis, researchers and food processors can gain a better understanding of the nutrient content of different foods, which can help with nutrient labeling and data validation during the food processing stage [17].

Reactive oxygen species (ROS), produced during aerobic metabolism, have essential functions, but their overproduction damages biomolecules. ROS involvement is seen in conditions including Alzheimer's, atherosclerosis, diabetes, inflammation, and neurodegenerative diseases. They also influence cancer and aging. Antioxidants counter ROS by impeding oxidative reactions, reducing lipid peroxidation, minimizing free radicals, and chelating metal ions [18]. The influence of oxidative stress on human health and rising apprehensions regarding synthetic antioxidants have prompted the scientific community to explore secure and viable natural antioxidant alternatives [19–21]. Plant-based foods are rich sources of naturally occurring antioxidants such as vitamin C, tocopherols (vitamin E), carotenoids, phenolic compounds, and polyphenolic compounds. Additionally, research

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has shown that bioactive peptides derived from protein-rich foods of both animal and plant origin can also act as antioxidants through similar mechanisms [22].

The term genotoxicity is often used interchangeably with mutagenicity; however, while all mutagens are genotoxic, not all genotoxic substances are mutagenic. Various assays have been developed to assess the genotoxic effects and their relationship to changes in plant growth and development [23]. Genotoxic agents can cause damage to the genetic material, leading to gene mutation, chromosomal alterations, and DNA damage. Such genetic changes can result in cell death or malignancies that can impair the organism's function and potentially reduce its survival [24].

Gastrointestinal issues are a common health problem in humans, affecting a significant percentage of the global population, with estimates suggesting it may impact 15 to 20% of people worldwide [25]. The primary antispasmodic medications are anti-muscarinic substances, which are derived from the plant belladonna and their synthetic derivatives, and calcium channel blockers like otilonium and pinaverium [26].

Fingerhuthia africana Lehm belongs to family Poaceae commonly known as thimble grass and Zulu fescue, is an erect perennial grass (0.73 m long). They are distributed in Karak, DI Khan, Banu in Pakistan and widely distributed in Africa, United State, Afghanistan, Oman, and Saudi Arabia. Many members of the family Poaceae are not only used as fodder and forage but also as an anti-inflammatory, anticancer, antibacterial, antifungal and against eyes infection, malaria and skin allergies [27]. The Poaceae family, also known as grasses, is the most significant plant family consists of around 11,000 species, which are distributed among roughly 750 to 770 genera across the world, making it the fifth largest family of flowering plants. Grasses cover around forty percent of the earth's surface [28]. The Poaceae family is highly significant in Pakistan and comprises of 158 genera and 492 species. It holds great economic and medicinal value [29]. In past the members of the family Poaceae are investigated for phytochemicals screening [30], elemental analysis and antioxidant activity [31], proximate composition [32], genotoxic activity [33] and antidiarrheal activity [34]. There is no literature are present on pharmacognostic evaluation of the selected plant. Therefore, the current study was design to evaluate the pharmacognostic characters and pharmacological activities of crude extract of *F. africana*.

2. Results

2.1. GC-MS Analysis

The *F. africana* crude methanolic extract of whole plant was checked for phytochemicals. The plant extract showed several peaks, which exhibits different bioactive compounds present in the crude sample (Figure 1). These peaks were matched with the database of the spectrum of known components present in the GC-MS library. A total of 35 compounds were identified from the GC-MS analysis, while the chemical compounds with their retention time (RT), molecular formula (MF), molecular weight (MW) and concentration are presented in (Table 1). The following phytocompound with their %area are; 1,3-Dihydroxyacetone dimer (1.94), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (0.99), 4-Methyl itaconate (1.17), .alpha.-Terpineol (0.53), 1,2,3-Propanetriol, 1-acetate (0.93), Benzofuran, 2,3-dihydro- (2.52), 2-Cyclohexen-1-one, 3-methyl-6-(1-methylethyl)- (23.69), p-tert.-Butylcatechol (1.59), 6-Methyl-2,3-dihydropyran-2,4-dione (0.24), 2-Hydroxy-3-methylbenzaldehyde (0.64), Cyclohexanemethanol, 4-ethenyl-.alpha.,.alpha.,4-trimethyl-3-(1-methylethenyl)-, [1R-(1.alpha.,3.alpha.,4.beta.)]- (2.06), Caryophyllene oxide (039), .alpha.-epi-7-epi-5-Eudesmol (3.09), 2-((2S,4aR)-4a,8-Dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalen-2-yl) propan-2-ol (0.54), Cyclohexene, 6-ethenyl-6-methyl-1-(1-methylethyl)-3-(1-methylethylidene)-, (S)- (5.62), Hinesol (1.1), 2-Naphthalenemethanol, decahydro-.alpha.,.alpha.,4a-trimethyl-8-methylene-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]- (3.09), (1aR,3aS,7S,7aS,7bR)-1,1,3a,7-Tetramethyldecahydro-1H-cyclopropa[a]naphthalen-7-ol (5.29), 7-(2-Hydroxypropan-2-yl)-1,4a-dimethyldecahydronaphthalen-1-ol (1.2), 1H-Indene, 1-ethylideneoctahydro-7a-methyl-, cis- (2.26), Acetic acid, (3-fluorophenyl) methyl ester (14.92), 4-Methyl-1,2-bis(trimethylsilyloxy)pentane (0.92), 4-Methyl-1,2-bis(trimethylsilyloxy)pentane (0.65), 6-(2-Hydroxypropan-2-yl)-4,8a-dimethyl-

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2,3,4,6,7,8-hexahydro-1H-naphthalen-1-ol, 1-acetate (1.31), Hexadecanoic acid, methyl ester (1.87), 2,7:3,6-Dimethanonaphthalene, decahydro- (1.12), n-Hexadecanoic acid (2.24), trans-8-Methyl-1.beta.-acetyl-hydrindane (1.16), 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (1.64), 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (0.87), 9-Octadecenoic acid (Z)-, methyl ester (0.55), 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (3.39), Bis(2-ethylhexyl) phthalate (4.42), 1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (4.14) and .gamma.-Sitosterol (1.97).

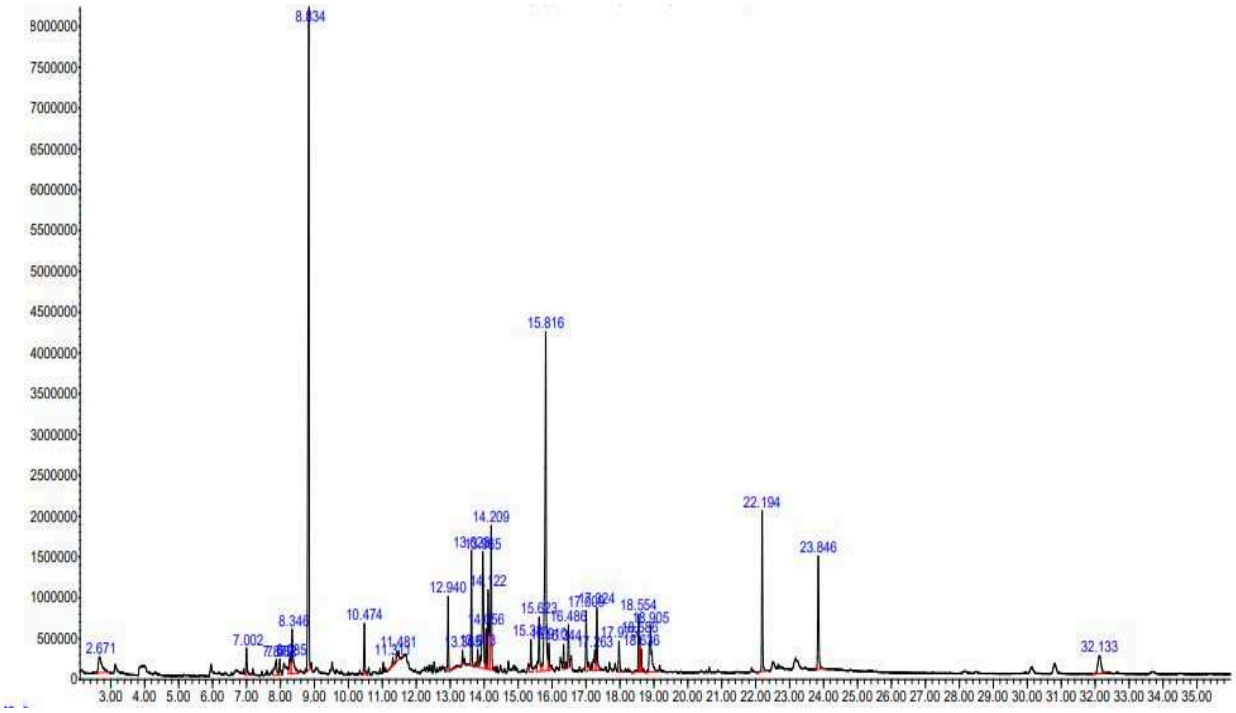

Figure 1. GC–MS chromatogram of methanolic extract of *F. africana* whole plant.

Table 1. List of important phytochemicals identified exclusively in the methanolic extract of *F. africana* using GC–MS analysis.

S. No	RT	%Area	SI	Name of Compound	MF	MW (g/mol)	Prob. %
1	2.671	1.94	55207	1,3-Dihydroxyacetone dimer	$C_3H_6O_3$	90.08	64
2	7.002	0.99	23823	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144.13	96
3	7.879	1.17	23777	4-Methyl itaconate	$C_6H_8O_4$	144.13	43
4	7.982	0.53	30998	.alpha.-Terpineol	$C_{10}H_{18}O$	154.24	91
5	8.285	0.93	17229	1,2,3-Propanetriol, 1-acetate	$C_5H_{10}O_4$	134.13	37
6	8.346	2.52	10680	Benzofuran, 2,3-dihydro-	C_8H_8O	120.15	95
7	8.834	23.69	29336	2-Cyclohexen-1-one, 3-methyl-6-(1-methylethyl)-	$C_{10}H_{16}O$	152.23	97
8	10.474	1.59	41104	p-tert.-Butylcatechol	$C_{10}H_{14}O_2$	166.22	70
9	11.311	0.24	12718	6-Methyl-2,3-dihydropyran-2,4-dione	$C_6H_6O_3$	126.11	15
10	11.481	0.64	18760	2-Hydroxy-3-methylbenzaldehyde	$C_8H_8O_2$	136.15	46
11	12.94	2.06	10211 1	Cyclohexanemethanol, 4-ethenyl-.alpha.,.alpha.,4-trimethyl-3-(1-	$C_{15}H_{26}O$	222.37	91

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				methylethenyl)-, [1R-(1.alpha.,3.alpha.,4.beta.)]-			
12	13.365	0.39	99194	Caryophyllene oxide	$C_{15}H_{24}O$	220.35	81
13	13.628	3.09	101977	.alpha.-epi-7-epi-5-Eudesmol	$C_{15}H_{26}O$	222.36	99
14	13.813	0.54	102063	2-((2S,4aR)-4a,8-Dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalen-2-yl) propan-2-ol	$C_{15}H_{26}O$	222.37	99
15	13.965	5.62	80415	Cyclohexene, 6-ethenyl-6-methyl-1-(1-methylethyl)-3-(1-methylethylidene)-, (S)-	$C_{15}H_{24}$	204.35	64
16	14.056	1.1	101915	Hinesol	$C_{15}H_{26}O$	222.37	91
17	14.122	3.09	102105	2-Naphthalenemethanol, decahydro-.alpha.,.alpha.,4a-trimethyl-8-methylene-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-	$C_{15}H_{26}O$	222.37	99
18	14.209	5.29	102072	(1aR,3aS,7S,7aS,7bR)-1,1,3a,7-Tetramethyldecahydro-1H-cyclopropa[a]naphthalen-7-ol	$C_{15}H_{26}O$	222.37	83
19	15.381	1.2	123744	7-(2-Hydroxypropan-2-yl)-1,4a-dimethyldecahydronaphthalen-1-ol	$C_{15}H_{28}O_2$	240.38	93
20	15.623	2.26	39830	1H-Indene, 1-ethylideneoctahydro-7a-methyl-, cis-	$C_{12}H_{20}$	164.29	89
21	15.816	14.92	44358	Acetic acid, (3-fluorophenyl) methyl ester	$C_9H_9FO_2$	168.16	25
22	15.91	0.92	150160	4-Methyl-1,2-bis(trimethylsilyloxy)pentane	$C_{12}H_{30}O_2Si_2$	262.54	22
23	16.344	0.65	99301	7R,8R-8-Hydroxy-4-isopropylidene-7-methylbicyclo [5.3.1] undec-1-ene	$C_{15}H_{25}O$	220.35	86
24	16.486	1.31	173460	6-(2-Hydroxypropan-2-yl)-4,8a-dimethyl-2,3,4,6,7,8-hexahydro-1H-naphthalen-1-ol, 1-acetate	$C_{17}H_{28}O_3$	280.40	53
25	17.009	1.87	161203	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45	99
26	17.263	1.12	37996	2,7:3,6-Dimethanonaphthalene, decahydro-	$C_{12}H_{18}$	162.27	25
27	17.324	2.24	143511	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	99
28	17.975	1.16	54896	trans-8-Methyl-1.beta.-acetyl-hydrindane	$C_{12}H_{20}O$	180.29	38
29	18.554	1.64	191917	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294.47	99
30	18.586	0.87	189451	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292.46	99
31	18.636	0.55	194438	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296.49	99

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32	18.905	3.39	171210	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278.43	99
33	22.194	4.42	295608	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.55	91
34	23.846	4.11	295779	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	$C_{24}H_{38}O_4$	390.56	95
35	32.133	1.97	310599	.gamma.-Sitosterol	$C_{29}H_{50}O$	414.70	98

RT: Retention Time; SI: Similarity Index; MF: Molecular Formula; MW: Molecular weight; Prob: Probability.

2.2. Macro and Micro-Nutrients Composition

The Nutrients Composition of Leaves, Stem and Roots of *F. africana* were analyzed through atomic absorption spectrometry. The results (Table 2) showed that calcium was highest (37.80 mg/L) in Leaves following Stem (33.75 mg/L) and lowest in Roots (10.56 mg/L), magnesium was highest (6.727 mg/L) in stem and lowest (2.952 mg/L) in roots, Potassium was highest in stem (19.02 mg/L) following leaves (18.65 mg/L) and lowest in roots (6.506 mg/L), Sodium was maximum (32.51 mg/L) in stem and minimum in leaves (13.16 mg/L), Copper was highest (0.013 mg/L) in roots and lowest (0.059 mg/L) in leaves, lead was highest in leaves (0.08 mg/L) whereas lowest in roots (0.015 mg/L), manganese was found greater (0.281 mg/L) in roots and lesser (0.975 mg/L) in leaves, Iron was found highest (3.350 mg/L) in leaves and lowest (0.377 mg/L) in stem, Zinc was high (1.185 mg/L) in stem and low (0.737 mg/L) in leaves, Cadmium was highest (0.013 mg/L) in stem and absent in roots and cobalt was absent in all parts of the selected plants.

Table 2. Macro and Micro-Nutrients Composition of Leaves, Stem and Roots of *F. africana*.

P.Parts	**Macro nutrients (mg/L)**				**Micro nutrients (mg/L)**						
	Ca	Mg	K	Na	Cu	Pb	Zn	Mn	Fe	Co	Cd
Leaves	37.80±0.12	5.645±0.29	18.65±0.19	13.16±1.87	0.059±0.09	0.08±0.06	0.737±0.02	0.975±0.03	3.350±0.06	0.00±0.00	0.034±0.02
Stem	33.75±0.58	6.727±0.49	19.02±0.26	32.51±1.23	0.055±0.05	0.13±0.11	1.185±0.01	0.498±0.05	0.377±0.09	0.00±0.00	0.013±0.01
Roots	10.56±0.20	2.952±0.73	6.506±0.27	18.32±1.73	0.013±0.03	0.15±0.03	0.153±0.01	0.281±0.02	0.295±0.04	0.00±0.00	0.00±0.00

Valeus indicated are Mean± standard deviation.

2.3. Proximate/Nutritional Analysis

The proximate analysis of stem, leaves and roots of Fingerhuthia africana was investigated to evaluate its nutritional significance (Table 3 and Figure 2). In the current research, moisture content was found to be highest in roots (8.57%) and lowest in leaves (7.70%); ash contents were maximum in root (9.77%) and minimum in leaves (5.00%); crude fats were found highest (5.96%) in roots and lowest (3.39%) in stem; crude fiber contents were highest in stem (45.03%) and lowest in leaves (39.00%); crude Proteins were highest (3.62%) in roots and lowest (2.92%) in stem; crude carbohydrates were higher in leaves (39.71%) while lower in roots (30.31%) and gross energy was highest in leaves (220.10Kcal/100g) and lowest in stem (180.34 Kcal/100g).

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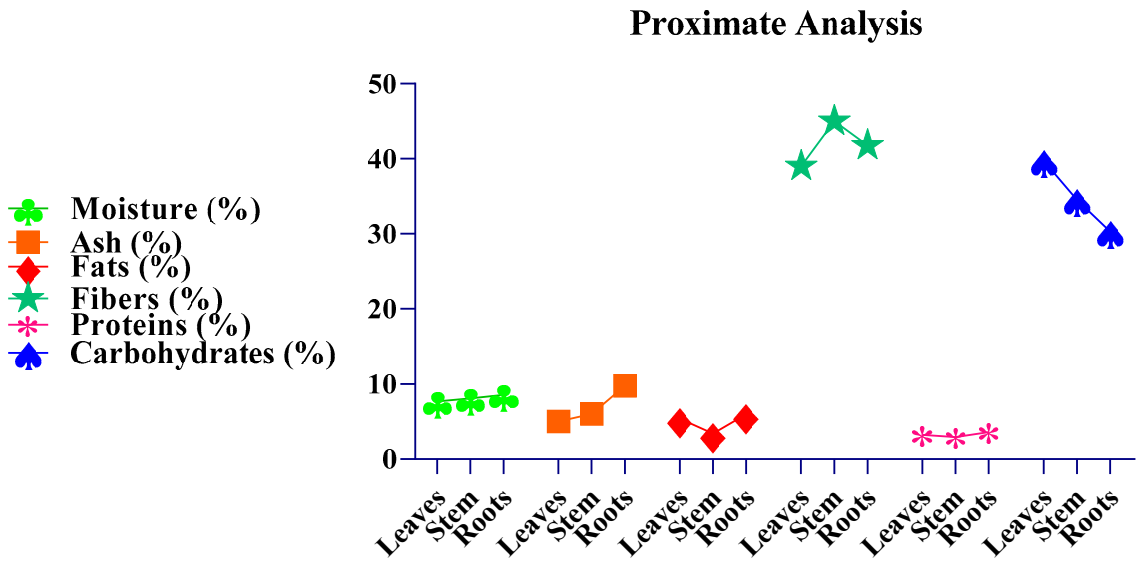
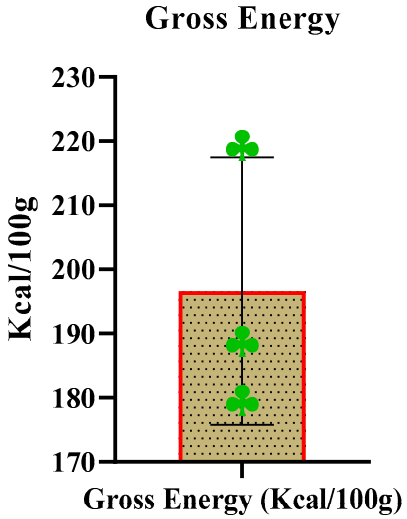
Table 3. Proximate analysis of Leaves, Stem and Roots of *F. africana*.

P. Parts	Moisture (%)	Ash (%)	Fats (%)	Fibers (%)	Proteins (%)	Carbohydrates (%)	Gross Energy(Kcal/100g)
Leaves	7.70	5.00	5.38	39.00	3.21	39.71	220.10
Stem	8.09	6.03	3.39	45.03	2.92	34.54	180.34
Roots	8.57	9.77	5.96	41.77	3.62	30.31	189.40
Mean	**8.12**	**6.93**	**4.91**	**41.93**	**3.25**	**34.85**	**196.61**



(a)



(b)

Figure 2. (a): Proximate analysis of Leaves, Stem and Roots of *F. africana*; (b): Gross energy (Kcal/100g) of Leaves, Stem and Roots of *F. africana*.

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2.4. Genotoxic Activity

2.4.1. Genotoxic Activity of methanol extract of F. africana.

The genotoxic potential of methanolic extract of *F. africana* on human lymphocytes show that the negative control exhibited a high percentage of cells in Class 0, indicating no DNA damage. On the other hand, the positive control exhibited a high proportion of cells in Classes 2 and 3, indicating significant DNA damage. The treatment groups showed a dose-dependent increase in the percentage of cells in Classes 1, 2, and 3, indicating DNA damage. The highest dose of 100mg/100ml showed the highest percentage of cells in Classes 2 and 3. Findings of this research suggest that crude methanolic extract of *F. africana* has genoprotective potential in human lymphocytes. The increase in the percentage of cells in Classes 1, 2, and 3 with increasing doses of the extract indicates a dose-dependent response (Table 4 and Figure 3).

Table 4. Comet assay of genomic DNA of human lymphocytes exposed to methanolic extract of *F. africana*.

Classes	Negative Control (Only Lymphocytes)	Positive Control (Lymphocytes + H_2O_2)	50mg/100ml	75mg100ml	100mg/100ml
Class 0	91.43 ± 1.52	38.00 ± 2.30	88.42 ± 1.37	85.47 ± 2.37	75.67 ± 1.53
Class 1	11.30 ± 0.60	55.43 ± 1.07	13.53 ± 2.27	18.27 ± 1.57	15.66 ± 0.50
Class 2	2.56 ± 0.47	13.37 ± 0.48	3.43 ± 0.63	1.56 ± 0.27	26.44 ± 1.02
Class 3	1.30 ± 0.00	5.53 ± 1.07	4.38 ± 1.15	3.26 ± 0.37	5.53 ± 1.06
TCS	**20.32±0.65**	**98.76±2.18**	**33.53±1.52***	**31.17±0.76***	**85.13±1.23***

TCS: Total comet score. Values are expressed as mean ± standard deviation (S.D). Difference significant relative to positive control at *P <0.001 (One-way ANOVA, Tukey Test).

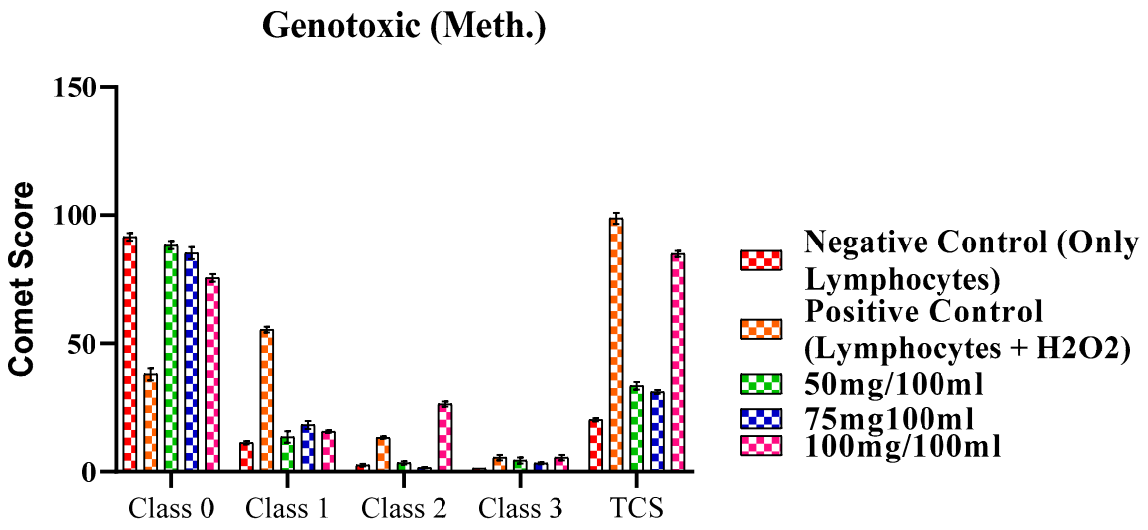

Figure 3. Comet assay of genomic DNA of human lymphocytes exposed to Methanol extract of *F. africana.*

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2.4.2. Genotoxic Activity of ethanolic extract of *F. africana*

The genotoxic activity of ethanolic extract of *F. africana* are revealed in the (Table 5). The negative control group (lymphocytes only) had a mean score of (88.0 ± 2.30) in class 0, indicating no DNA damage. The positive control group (lymphocytes + H_2O_2) had a significantly higher mean score of (35.63 ± 2.5) in class 0, indicating significant DNA damage. The treatment groups with the ethanolic extract of *F. africana* at concentrations of 50mg/100ml, 75mg/100ml, and 100mg/100ml showed dose-dependent effects on DNA damage.

In the treatment groups, class 0 mean scores decreased as the concentration of the extract increased. At the maximum concentration (100mg/100ml), the mean score was (56.31 ± 2.13), indicating significant DNA damage. Similarly, for class 1 and class 2, mean scores decreased with increasing concentration of the extract. Class 3 mean scores also decreased, but to a lesser extent. These results suggest that *F. africana* crude ethanolic extract has genotoxic potential, and the effect is dose-dependent (Figure 4). The total comet score (TCS) for negative control was (34.53 ± 2.32), while for the positive control it was (143.8 ± 2.05). The ethanolic fraction of plant at 50, 75, and 100mg/100ml doses showed mean values of (127.38 ± 1.16), (83.28 ± 0.65), and (44.08 ± 1.40) for TCS, respectively. Treatment with the ethanolic extract of *F. africana* resulted in a dose-dependent increase in TCS values, indicating DNA damage.

Table 5. Comet assay of genomic DNA of human lymphocytes exposed to ethanolic extract of *F. africana*.

Classes	Negative Control (Only Lymphocytes)	Positive Control (Lymphocytes + H_2O_2)	50mg/100ml	75mg100ml	100mg/100ml
Class 0	88.0 ± 2.30	35.63 ± 2.5	84.34 ± 2.5	66.52 ± 3.2	56.31 ± 2.13
Class 1	8.54 ± 1.43	40.50 ± 3.25	35.53 ± 1.41	23.40 ± 2.0	20.42 ± 3.6
Class 2	6.35 ± 1.5	27.26 ± 2.17	30.43 ± 2.5	18.63 ± 2.5	15.47 ± 2.1
Class 3	4.43 ± 2.0	16.30 ± 1.20	10.33 ± 2.5	7.54 ± 1.1	4.38 ± 1.0
TCS	**34.53±2.32**	**143.8±2.06**	**127.38±1.16**	**83.28±0.65**	**44.08±1.40**

TCS: Total comet score. Values are expressed as mean ± standard deviation (S.D). Difference significant relative to positive control at *P <0.001 (One-way ANOVA, Tukey Test).

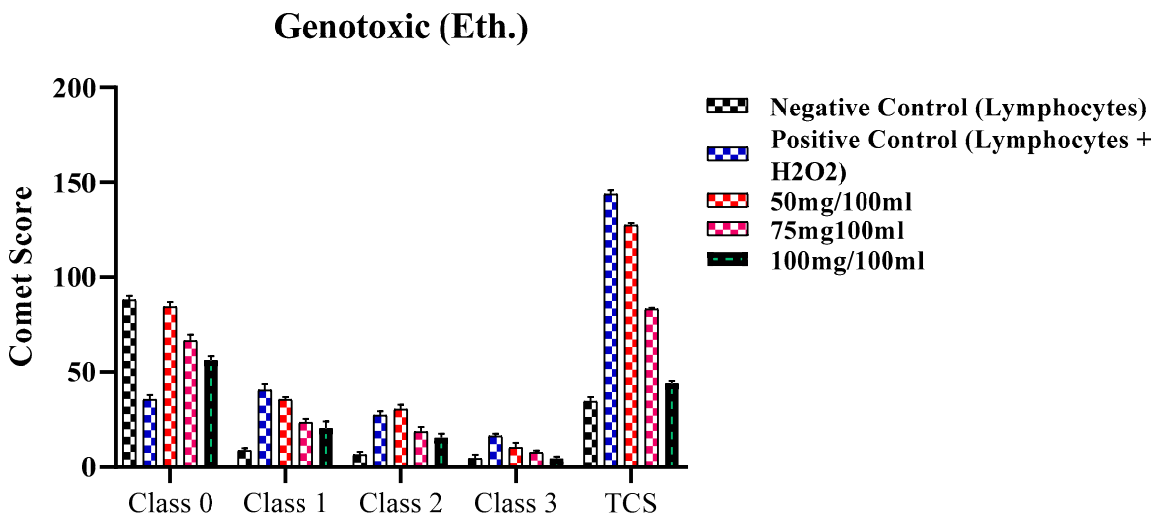

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Figure 4. Comet assay of genomic DNA of human lymphocytes exposed to ethanolic extract of *F. africana.*

2.5. Antioxidant Activity via DPPH

The results of the DPPH radical scavenging activities of both the methanolic and aqueous extracts of *F. africana* showed in (Table 6). The radical scavenging ability of each of the plant extracts was shown to increase in a manner that was dependent on both time and concentration. According to the findings, the methanolic extract of *F. africana* demonstrated the highest level of antioxidant activity (79.5%), in comparison to the aqueous extract (76.5%), at 300 mg/ml after 90 minutes; nevertheless, this level of antioxidant activity was much lower than that of the conventional medication ascorbic acid (91.5%). The IC_{50} value for the aqueous extract was found to be the lowest (IC_{50} = 81.62 g/ml), in contrast to the IC_{50} value for the methanolic extract, which was found to be (IC_{50} = 150.78.62 g/ml) (Figure 5).

Table 6. Mean±SD values of Antioxidant activity of methanolic and aqueous extracts of *F. africana*.

Plant Extract	Conc. (µg/ml)	(%) DPPH radical scavenging activity			
		30 min	60 min	90 min	IC_{50} (µg/ml)
Ascorbic acid	100	49.47±1.58	55.52±1.23	60.2±1.34	65.84
	200	56.09±1.53	61.13±1.11	71.3±2.32	
	300	72.47±1.88	83.38±1.03	91.5±2.34	
Methanol	100	35.66±1.29	45.14±1.20	52.5±1.07	150.78
	200	45.77±1.62	57.07±0.94	66.3±2.32	
	300	55.71±0.57	65.92±1.19	79.5±2.54	
Aqueous	100	43.53±0.74	51.66±0.69	56.2±1.51	81.62
	200	53.57±2.10	62.57±1.47	67.5±2.35	
	300	61.30±2.29	72.30±1.13	76.5±1.36	

Conc: Concentration; Valeus indicated are Mean± standard deviation.

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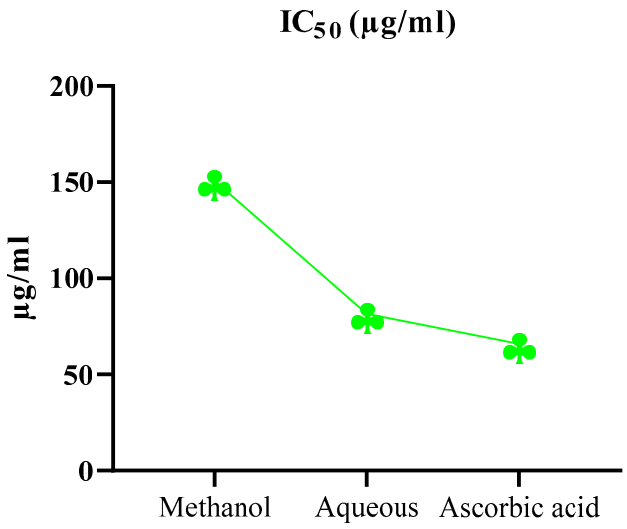

Figure 5. IC_{50} Values of methanolic and aqueous extracts of *F. africana*.

2.6. Antidiarrheal Activity

To find the antidiarrheal effect of F. africana the methanolic extract of the plant were evaluated for antidiarrheal activity by charcoal meal test. The results show that the methanolic extract of the selected plant possess highly significant antidiarrheal activity in dose depended manner as compared to standard group. The maximum antidiarrheal activity was observed at the dose of (300 mg/kg) which was (16.21%) followed by (26.73%) at the dose of (200 mg/kg). The percent (%) inhibition of charcoal movement of the plant extract was 59.63%, 73.26% and 83.78% at the doses of 100, 200 and 300 mg/kg while the percent (%) inhibition of standard drug Atropine sulphate (10 mg/kg) was (67.37%). The maximum reduction of charcoal movement was caused by the plant methanolic extract at the doses of 300 mg/kg and 200 mg/kg which was (8.3788±3.16 and 13.7300±9.72 respectively) followed by standard atropine sulphate (10 mg/kg) which was (18.0820±3.93) show in (Table 7).

Table 7. Antidiarrheal activity of crude methanolic extract of *F. africana*.

Treatment	Antidiarrheal activity of crude methanolic extract of *F. africana*.				
	Dose (mg/kg)	Mean length of intestine	Mean distance travelled by charcoal	Transit of intestine Percent (%)	Percent inhibition %
Normal saline	10ml/kg	54.8	49.2740±4.25	89.90%	10.09%
Atropine sulphate	10mg/kg	55.42	18.0820±3.93	32.62%	67.37%
Methanolic Extract	100 mg/kg	51.83	20.9240±2.54	40.36%	59.63%
	200 mg/kg	51.35	13.7300±9.72	26.73%	73.26%
	300 mg/kg	51.68	8.3788±3.16	16.21%	83.78%

Valeus indicated are Mean± standard deviation.

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3. Discussion

Throughout history, medicinal plants have served as remedies for a variety of human ailments. In our modern industrialized society, the utilization of medicinal plants can be attributed to the extraction and advancement of numerous drugs, building upon their traditional applications in folk medicine [35]. Medicinal flora contains powerful phytoconstituents that serve as a significant reservoir of antibiotic compounds, contributing to their therapeutic characteristics. These phytoconstituents confer upon them their valuable medicinal attributes [36,37]. About 35 compounds were identified in the methanolic fraction of *F. africana* through GC-MS. From the identified compounds some were found to have significant pharmacological activities. For example 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- has Anti-oxidant, anti-microbial, laxative and anti-cancer activities [38]. 4-Methyl itaconate has anti-inflammatory and anticancer potential [38,39]. .alpha.-Terpineol are reported for antioxidant, anticancer, anticonvulsant, antiulcer, antihypertensive and anti-nociceptive [40], It additionally restrains growth and triggers cell death in tumor cells via a mechanism that includes the suppression of NF-κB activity [41]. 1,2,3-Propanetriol, 1-acetate has antibacterial activity [42]. Benzofuran, 2,3-dihydro- have antibacterial, antifungal, anti-inflammatory, analgesic, antidepressant, anticonvulsant, antitumor, imaging, Anti-HIV, antidiabetic, antitubercular, antioxidant and miscellaneous activities [43]. Caryophyllene oxide are reported for Anti-inflammatory, antibiotic, antioxidant, anti-carcinogenic and local anesthetic activities [44]. Hinesol have antitrypsin activity, cytotoxicity, anthelmintic activity, inflammatory, anti-allergic, anti-viral, antinociceptive and neuroprotective effects [45]. n-Hexadecanoic acid possess Anti-inflammatory, antispasmodic, anticancer and antiviral activities [38]. 9,12-Octadecadienoic acid (Z,Z)-, methyl ester are used as Urine acidifier and increase zinc bioavailability [46]. Hexadecanoic acid, methyl ester have antioxidant, antitumor, immunostimulant, Anti-inflammatory, cytotoxic, antiarthritic, nematicide, antihistaminic and cancer preventive potential [44,47] and .gamma.-Sitosterol have anticancer, anti-inflammatory, antibacterial, antidiabetic, cytotoxic, Anti-tumor, chemo preventive, Antioxidant and free radical scavenging properties [38,44,48,49]. Previously Riaz et al. [50] investigated *Mazus goodenifolius* for GC-MS analysis which showed that the essential oil has some phytochemicals which may have antimicrobial and antioxidant activity.

The connection between mineral levels in the body and specific health disorders suggests that plants containing these minerals could help prevent illness. Therefore, the elemental composition of plants plays a vital role in enhancing their effectiveness against various diseases [51]. Minerals play a crucial role in nutrition, with key essential elements being provided through well-balanced diets. These minerals serve important functions in the body, encompassing structural, physiological, and metabolic processes [52]. Calcium is an essential for maintaining neuromuscular function, blood coagulation, metabolic processes, and skeletal strength. Calcium also plays a role in the formation of strong bones and acts as a mediator of hormonal effects on target organs by means of intracellular signaling pathways. The maximum amount of calcium that should be consumed on a daily basis is between 750 and 800 milligrams [53]. The calcium content was greatest in the leaves (37.80 mg/L) and was lowest in the roots (10.56 mg/L) shown in (Table 2). It is consistent with the findings of Chawla et al. [54] who found similar information from the blossom of *Rhododendron arboretum*.

Magnesium is an essential for various critical functions like control of muscle contraction, blood pressure, and insulin metabolism, and it participates as a cofactor in over 300 different enzymatic activities. In addition, DNA, RNA, and protein synthesis all need magnesium in order to proceed [55]. The daily recommended dosage range for children aged 1-8 years is between 80 mg/day and 130 mg/day, while the recommended dosage range for adults is between 320 mg/day and 420 mg/day [56]. According to the finding magnesium was highest (6.727 mg/L) in stem and lowest (2.952 mg/L) in roots. The results are similar with the findings of [57].

Potassium plays vital roles in managing hypertension, enhancing cardiac efficiency, and overseeing physiological processes, which encompass the regulation of water balance [52]. The suggested daily intake of potassium for adults is 4700 mg according to the recommended daily allowance (RDA) [58]. According to the findings Potassium was highest in stem (19.02 mg/L)

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following leaves (18.65 mg/L) and lowest in roots (6.506 mg/L). According to findings of Valdez-Solana et al. [59] the value of potassium in the leaves of *Moringa oleifera* was comparable.

Sodium is an important electrolyte that is an extremely important component of blood. It is necessary in order to keep the body's water levels stable. Numerous regulatory systems in the body need sodium in addition to potassium in order to function properly [60]. It is well knowledge that consuming an excessive quantity of salt may have a negative impact on blood pressure (BP), and increased risk of stroke and renal dysfunction. The World Health Organization (WHO) suggests consuming no more than 5 grams of salt on a daily basis [61]. Sodium was maximum (32.51 mg/L) in stem and minimum in leaves (13.16 mg/L). Both the leaf and the fruit of *Myrica esculenta* were examined by Kabra & Baghel, [62] and yielded comparable findings.

Copper is a very effective enzyme catalyst; yet, it is also a potentially harmful reactant that produces hydroxyl radicals, having insufficient copper levels may cause glucose intolerance, a reduced insulin response, higher glucose sensitivity, and an overall rise in glucose responsiveness [63]. The World Health Organization (WHO) has set a maximum limit of 40 g/L for Cu in order to maintain proper metabolic function [64]. According to the findings the level of copper was highest (0.013 mg/L) in roots and lowest (0.059 mg/L) in leaves. This coincides with the findings of Ozyigit et al. [65] who found a comparable amount of copper in the leaves of *Alchemilla alpine* L.

Lead has the potential to build up in a number of the plant's tissues, and this may have a detrimental effect on a variety of physiological activities, including photosynthesis, respiration, mineral nutrition, membrane structure, characteristics, and gene expression [66]. In humans, it may be harmful to the digestive tract, the kidneys, and the central nervous system. The World Health Organization (WHO) has determined that the safe daily consumption of lead should be 10 mg/Kg [15]. The concentration of lead was highest in leaves (0.08 mg/L) whereas lowest in roots (0.015 mg/L).

Manganese is a crucial element in redox processes, and essential for oxygen evolution during photosynthesis. The concentration of Mn in leaves of various medicinal herbs ranges from 20 to 300 mg/kg [67]. The amount of manganese on a daily basis is dependent on age and gender. Children as young as 8 years old need 1.2 to 1.5 mg per day, whereas adult men of any age up to 70 years old need 1.9-2.3 mg per day. Females up to the age of 70 need 1.6-1.8 mg per day [68]. The quantity of manganese discovered was found greater (0.281 mg/L) in roots and lesser (0.975 mg/L) in leaves.

Iron is essential component of different biological molecules including cytochromes, myoglobin, peroxidase, hemoglobin, and electron transport [69]. Iron has several important functions in the body, including the synthesis of hemoglobin and myoglobin as well as energy production. If there is a severe deficiency of iron, it can lead to hypochromic anemia. However, high amounts of iron may be harmful to the body and can be caused by a number of different things, including hereditary or metabolic abnormalities, repeated blood transfusions, or overconsumption. The optimal daily consumption of iron is between 8 and 18 mg [70]. According to the results the level of iron was found highest (3.350 mg/L) in leaves and lowest (0.377 mg/L) in stem. These findings are supported by Mashkoor Hussein [71] who also describe the presence of iron in the *cardaria draba*.

Zinc is an essential component in a variety of biological activities, including protein synthesis, the differentiation and replication of cells, immunological function, and the development of sexual characteristics [72]. Zinc insufficiency are cause diarrhea and pneumonia in children. Other symptoms of zinc shortage include eye and skin lesions, as well as weight loss, stunted growth, poor development in babies and adolescents, decreased immunological function, delayed sexual maturation, and hypogonadism in males [73]. The World Health Organization (WHO) has established a recommended limit for zinc in plants of 50 mg/kg [74]. Zinc was high (1.185 mg/L) in stem and low (0.737 mg/L) in leaves. Hameed & Hussain [75] found comparable findings in the stem and leaves of *Solanum surattense* plant.

Cobalt is a vital element for the human body which assists the absorption and processing of vitamin B12, and functioning as a therapy for disorders such as anemia and some viral diseases [69]. Prolonged exposure to high levels of cobalt may results adverse health effects, including toxicity to the liver, dermatitis, as well as endocrine and reproductive damage [76]. According to the results cobalt was not detected in any of the chosen plant sections. These findings are in agreement with

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those that were reported by Sara et al. (2022) who also found comparable information in the leaves and stem bark of *Diospyros mespiliformis*. According to WHO, the maximum amount of cobalt that may be present in the body at one time is 1.5 mg/kg [78]. The existing plant components had a concentration that was lower than the permissible upper limit.

Cadmium is a toxic heavy metal harmful to plants and animals. It can accumulate in their tissues and lead to reduced growth and yield in plants, while in animals, it can cause health problems such as kidney damage, osteoporosis, and cancer. Cadmium can also interfere with hormone function and negatively impact the reproductive system of both plants and animals [79]. The World Health Organization (WHO), China, and Thailand have decided that a cadmium concentration of 0.3 ppm or below is an acceptable level for medicinal plants [15]. According to the results Cadmium was highest (0.013 mg/L) in stem and absent in roots. The fluctuation in the elemental composition may be linked to a mix of climatological variables, edaphic factors, and the preferred absorbability of elements [80].

Proximate analysis is a widely used method for food analysis that involves the assessment of five key constituents: ash, moisture, proteins, fats, and carbohydrates [81]. The amount of moisture present in food plays a crucial role in determining its shelf life. The results of nutritional composition showed that moisture contents were high (8.57%) in roots followed by stem (8.09%) and lower (7.70%) in leaves. This results are closely related to Idris et al. [82] who also obtain similar amount of moisture contents from the leaf and root of *Rumex crispus* L. Studies have indicated that plant materials containing moisture levels above 8% create a favorable environment for insect infestation while if exceeds 15%, there is an increased risk of bacterial and fungal contamination [83]. Our findings demonstrate that *F. africana* possesses low moisture content, making it possible to preserve the plant with minimal risk of microorganism and insect infestation. Ash is the inorganic substance comprised of oxides and salts that include a variety of anions, including phosphates, sulfates, chlorides, and halides, as well as cations, such as sodium, potassium, calcium, magnesium, and manganese. The amount of ash in food is a good indicator of the number of minerals that are present in it [84]. According to the findings, the highest levels of ash were found in the roots (9.77%), while the lowest levels were found in the leaves (5.00%). Based on these findings, it is likely that the roots of plants might be used as a useful source of minerals. Fat is regarded as a key source of energy in the human diet. The eating of specific vegetables that contain fat is suggested for those who are battling obesity [85]. The results of this study demonstrate that the highest concentration of fats was found in the roots (5.96%), while lowest in the stem (3.39%). Ezeabara & Egwuoba, [86] discovered that both the leaves and roots of *Oldenlandia corymbosa* yielded comparable results. Fats not only act as a form of energy storage in living organisms but also play an essential role as structural components of biological membranes, namely in the form of phospholipids and sterols [87]. The recommended daily allowance (RDA) for fat in food is at least 2.0 mg per 100 grams [88]. The quantity of fats present in *F. africana* might play a role in its medicinal effectiveness.

Dietary fiber is a crucial element of a balanced diet as it promotes healthy digestion and facilitates regular bowel movements. Additionally, it plays a role in weight management, reducing the risk of cardiac conditions, and supporting gastrointestinal health [52]. Our results indicates that stem had the highest fiber content (45.03%), whereas the leaves had the lowest fiber content (39.00%). According to the recommended daily allowance (RDA), males should strive to eat between 31.00 and 38 g of fiber each day, while women should aim for between 25 and 26 g of fiber each day. Pregnant women should take 28 grams of fiber per day, while breastfeeding moms should consume 29 grams of fiber per day [89]. Protein is an important component of human nutrition, and a lack of it may lead to growth retardation, muscle wasting, edema, abdominal distension, and fluid accumulation, especially in children. Protein shortage can also cause edema, abdominal distension, and fluid accumulation [90]. The standards for the recommended daily allowance (RDA) children should take 28.0 g/100 g of protein, while adult males and females should consume 63.0 g/100 g and 50.0 g/100 g of protein, respectively [91]. Protein content was highest (3.62%) in the roots, whereas it was lowest (2.92%) in the stem. The findings of this research are in agreement with the findings of Ezeabara & Nwafulugo, [92] who found similar findings from the leaves and stems of *Cleome ciliate*.

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Carbohydrates are an extremely important component and play an important role in the brain, which is an organ that operates most effectively when it receives a sufficient amount of glucose [52]. The recommended dietary allowance (RDA) for both children and adults are 130 g per day. Pregnant and nursing moms have an increased need for carbs, with an RDA of 175 g and 210 g per day, respectively [93]. The findings showed that carbohydrates in leaves were greater (39.71%) than those found in the roots, which were (30.31%). Based on these observations, it seems that the leaves are the most important source of carbohydrates. Due to the large concentration of sugars that plants possess, they have the potential to be a substantial source of energy. The daily energy requirements of an adult male normally vary from 2300-2900 Kcal/day, whereas for adult female typically range from 1900-2200 Kcal/day [94]. Our findings showed that stem had a gross energy content that was much lower than that of the leaves (180.34 Kcal/100g).

Genotoxicity is a critical property of toxic substances that can cause changes in hereditary traits or DNA strand breakage, resulting in various effects such as apoptosis, carcinogenesis, or alterations in phenotype [95]. Oxidative stress can result in DNA damage, leading to various diseases such as cancer, mutations, and aging. Therefore, it is crucial to identify compounds that may cause or prevent DNA damage and to assess their biological consequences [96]. The comet assay is a widely used and versatile method for measuring various types of DNA damage. The genotoxic effects of hydrogen peroxide (H_2O_2) are often employed as a model system to assess the protective properties of different compounds against DNA damage [97]. The genotoxic activity of the plant indicates that the ethanolic fraction show dose-dependent genoprotection at 50, 75, and 100 mg/100 ml, respectively. Whereas the methanolic fraction of the plant possess genotoxic activity at high concentration (100 mg/100 ml). Mattana et al. [98], Andrade et al. [99], Al-Faifi et al. [100] are only few of the researchers that have researched the genotoxic effects of medicinal plants and have discovered similar results. The genotoxic potential of selected plant is due to the presence of several phytochemicals such as 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- ; 4-Methyl itaconate [38], Benzofuran, 2,3-dihydro- [43] and Hexadecanoic acid, methyl ester [44].

Plants are rich in various antioxidants that provide protection against diseases linked to free radicals. These antioxidant compounds are primarily synthesized as secondary metabolites in plants. They have the ability to halt chain reactions and prevent oxidation reactions by removing radical intermediates and undergoing oxidation themselves [101]. The results of the DPPH radical scavenging activities of both the methanolic and aqueous extracts of *F. africana* shows that the methanolic extract of demonstrated the highest level of antioxidant activity (79.5%), in comparison to the aqueous extract (76.5%), at 300 mg/ml after 90 minutes; nevertheless, this level of antioxidant activity was much lower than that of the conventional medication ascorbic acid (91.5%). IC_{50} value for the aqueous extract was found to be the lowest (IC_{50} = 81.62 g/ml), in contrast to the IC_{50} value for the methanolic extract, which was found to be (IC_{50} = 150.78.62 g/ml). These findings are in agreement with those that were published by Jahanban-Esfahlan et al. [102] who investigated the effects of methanolic and aqueous extracts of the *Juglans regia* L (walnut species). A significant amount of natural antioxidants, including polyphenols (such as flavonoids, lignans, phenolic acids, anthocyanins, and stilbenes), carotenoids (including carotenes and xanthophylls), and vitamins, particularly vitamin C and vitamin E, can be found in medicinal plants, and these plants are regarded as a valuable source of antioxidants [103,104].

Diarrhea is commonly characterized by the presence of unusually watery or loose stools, accompanied by an increased frequency of bowel movements and abdominal discomfort [105]. Castor oil is frequently employed as a diarrhea inducer in mice models, with its active metabolite, ricinoleic acid, being the primary cause of its diarrhea-inducing effects which is liberated by the action of lipases in the upper part of the small intestine [106]. The findings show that the methanolic extract of the plant demonstrated antidiarrheal efficacy, with the maximum inhibition of charcoal movement (83.78%) occurring at a dosage of 300 mg/kg, followed by doses of 200 and 100 mg/kg, 73.26% and 59.56%, respectively. The findings shown here are in accordance with those discovered by Ferede et al. [107] and Kifle et al. [108] who observed that the methanolic extract displayed a comparable suppression of castor oil-induced diarrhea. The anti-diarrheal properties of medicinal

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plants are associated with their phytochemical components, such as saponins, terpenes, flavonoids, sugars, tannins and sterols [109]. Flavonoids possess the capability to hinder intestinal motility and hydro-electrolytic secretions, while tannins cause the precipitation of proteins, thereby reducing secretion and peristaltic movements [110]. Asrie et al. [111] reported that Saponins demonstrate the inhibition of histamine release in vitro; terpenoids impede the release of autacoids and prostaglandins; phenols enhance the resilience of the intestinal mucosa, decrease secretion and intestinal transit, and exhibit astringent properties.

4. Materials and Methods

4.1. Collection and Identification of Plant

Plant will be collected from village Mohabati Killa, district Karak and will be identified with the help of "Flora of Pakistan" and available literature. The plant sample will be preserved, given voucher number, and will be deposited in Herbarium for further reference [112].

4.2. Extraction procedure

The collected plant specimen will be washed, dried, and grinded to form fine powder. The powder will be transferred to an airtight container. The powdered plant specimens will be processed to obtain an extract by dissolving them in methanol. After vigorous shaking for 72 hours, the extract will undergo separation from the solvent using a rotary evaporator. Next, the extract will be subjected to a water bath to eliminate any remaining solvent and subsequently dried [113].

4.3. GC-MS Analysis

The identification of various phyto-compounds in the selected plant extract will be carried out using the GC-MS technique. The analysis will involve utilizing electron ionization energy systems in conjunction with a carrier gas (constant flow rate of 1.50 ml/min) and an injection volume of 2 µl. The GC process will run for a duration of 50-55 minutes, generating mass spectra and chromatograms through dedicated software. Identification of compounds will be based on factors such as their molecular structure, mass, and calculated fragments. The interpretation of GC-MS mass spectra data will be accomplished using the NIST (National Institute Standard and Technology) and Wiley library databases. Correlation of sample component names, molecular weights, and structures with the library will facilitate the identification process. Furthermore, the relative percentage of each component will be determined by comparing its average peak area with the total areas, and the outcomes will be meticulously documented [114].

4.4. Elemental Analysis

Following the addition of 0.5 g of powdered leaves, roots, and stem to 10 mL of nitric acid (HNO_3), the mixture was allowed to remain for 24 hours before being analyzed. Following that, 4 mL of perchloric acid ($HClO_4$) was added, and the mixture was cooked in a fume hood using a hot plate. After the boiling process, a transformation from yellow to white was seen in the vapors. After that, one hundred milliliters of distilled water were added to it. After passing through filter paper, the resulting filtrates were placed in plastic bottles and given appropriate labels. With the assistance of an atomic absorption spectrometer, an investigation into the presence of a number of different elements was carried out on these solutions [115].

4.5. Proximate/Nutritional Analysis

The nutritional composition, including crude fat, ash, fiber, moisture content, carbohydrates, protein, and gross energy, of the stem, leaves, and roots of Fingerhuthia africana, will be determined following a modified procedure based on the method described by [116].

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4.5.1. Determination of Moisture Content

To prepare the plant samples for analysis, individual samples weighing two grams each were measured and placed into sterile Petri dishes. The weight of each sample was recorded as variable A. After that, the Petri dishes were heated to 105°C for 4 to 6 hours, covered with a lid, and kept there until a steady weight was achieved. To preserve their quality, the samples were subsequently transferred to a desiccator and allowed to cool gradually for a duration of thirty minutes. The qualities of the samples may be lost as a result of sudden temperature fluctuations. The Petri dishes were weighed once more after cooling, and the result was noted as B [117]. The moisture percentage was calculated according to the AOAC 2000 method.

$$\textbf{Moisture content}\ (\%) = \frac{\mathbf{X}}{\textbf{weight of sample}}\mathbf{x\ 100}$$
$$\textbf{Where X} = \textbf{Weight of the sample (after heating)} = \mathbf{B} - \mathbf{A}$$
$$\mathbf{B} = \textbf{weight of the empty Petri dish} + \textbf{sample (after heating)} \quad (1)$$

4.5.2. Determination of Ash Content

The methodology used for this experiment involved several steps. Firstly, a flat-bottomed silica crucible was washed thoroughly and then dried in a microwave oven for 30 minutes at a temperature of 700°C. The crucible was then treated by flame burning and tarring, allowed to cool in desiccators, and weighed to obtain the initial weight (A). Two grams (2 g) of each plant sample were added equally to the crucible, which was gradually heated on a Bunsen burner and then transferred to a stifled incinerator (furnace). The samples were heated for several hours at a temperature of 600°C until the carbon content had evaporated, and the samples turned white. Once complete, the samples were moved to desiccators, allowed to cool, and reweighed to obtain the final weight (B). Finally, the ash percentage and absolute ash values were calculated using the AOAC 2000 method described by Ullah et al. [118].

$$\textbf{Weight of blank China Dish} = \mathbf{A}$$
$$\textbf{Weight of China Dish with ash} = \mathbf{B}$$
$$\textbf{Total ash (mg/g)} = \mathbf{B} - \textbf{A of the sample} = \textbf{mg/g} \quad (2)$$
$$\%\ \textbf{Ash} = \frac{\mathbf{B} - \mathbf{A}}{\textbf{Weight of plant material}}\ \mathbf{x\ 100}$$

4.5.3. Determination of Crude lipid

For the determination of crude lipid, 5g of plant powder was taken, and an extract was prepared using petroleum ether (40-60°C) for 3 hours. The extract was then allowed to evaporate, and the flask was reweighed.

$$\%\ \textbf{lipid} = \frac{\textbf{weight of lipids}}{\textbf{weight of sample}}\ \mathbf{x\ 100} \quad (3)$$

4.5.4. Determination of Crude protein

0.5 g dried plant material were taken in an indigestion flask and mixed with a digestion mixture of copper sulphate and potassium sulphate in specific ratios. H_2SO_4 was added, and the flask was heated for 1 hour and 15 minutes. The solution was made alkaline by adding solid alkali and a solution of H_3BO_3, NaOH, and water. The mixture was then titrated using 0.1N HCl, and the protein percentage was measured using the AOAC 2000 formula, which utilizes the Macro Kjeldahl method to determine protein percentages based on nitrogen content multiplied by 6.25.

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$$\textbf{Percentage Nitrogen} = \frac{(\textbf{Sample reading} - \textbf{Blank reading})\ \textbf{x}\ 14.01\ \textbf{x}\ 0.5\ \textbf{x}\ 100}{(\textbf{Sample in Mg})}$$

$$\textbf{V1} = \textbf{Titration reading of sample}$$

$$\textbf{V2} = \textbf{Titration reading of blank} \quad (4)$$

$$\textbf{14.01} = \textbf{Atomic weight of nitrogen (N)}$$

$$\textbf{Protein}\ (\%) = \%\ \textbf{Nitrogen x 6.25}$$

4.5.5. Determination of Crude Fiber

In this method, samples are first weighed and a comparable quantity is produced by removing crude lipids with petroleum ether. The resulting filtrate is then boiled with a combination of asbestos and H_2SO_4 for 30 minutes. The contents are filtered and the residue is washed, cleaned, and added to a boiling NaOH absorption flask. The mixture is heated to a boiling point and maintained for a duration of 30 minutes. Subsequently, the mixture is filtered using a Gooch crucible along with an asbestos mat. The crucible is washed and baked until a steady weight is achieved. The resulting residue is then burnt in a muffle furnace and crude fibers are estimated.

$$\%\ \textbf{Crude fibers} = \frac{\textbf{X}}{\textbf{weight of sample}}\ \textbf{x 100}$$

$$\textbf{Where X} = \textbf{W2} - \textbf{W1}; \quad (5)$$

$$\textbf{W2 - W1} = \textbf{Crude fiber}$$

4.5.6. Determination of carbohydrates contents and gross energy

To estimate the carbohydrate content, the total weight of protein, crude fiber, fats, ash, and moisture contents were subtracted from 100 [119].

4.6. Genotoxic Activity

The Genotoxic Activity is performed through comet assay as described by Singh et al. [120] with some changes. Firstly, cells are cultured and treated with the test compound, followed by harvesting and suspension in a suitable buffer. The cells are then embedded in a layer of low melting point agarose on a microscope slide, lysed to remove cellular components, and subjected to electrophoresis to induce DNA migration. Subsequently, the slide is stained with a DNA-specific fluorescent dye and analyzed using fluorescence microscopy and image analysis software to quantify parameters such as tail length, tail intensity, and tail moment. By comparing these parameters between treatment groups and controls, genotoxic activity can be determined.

4.7. Antioxidant Activity via DPPH

The DPPH test was used to investigate the degree to which the methanolic extract of *Fingerhuthia africana* has antioxidant properties. The methodology for the experiment came from [121] with a few minor adjustments made. After making a solution of 0.135 mM DPPH in methanol, 1.0 ml of this solution was combined with 1.0 ml of a solution of plant extract with different concentrations (100, 200, and 300 g/ml). The final product was analyzed for its ability to inhibit the formation of reactive oxygen species. The reaction mixture was left at room temperature and in the dark for a period of thirty minutes. The ascorbic acid concentration was employed as the benchmark for the experiment. As a control, we utilized a combination that included 1 milliliter of methanol and 1 milliliter of DPPH solution. The reaction was performed three times, and the reduction in absorbance was measured at

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517 nm using a UV-V is spectrophotometer at 30, 60, and 90 minutes following the beginning of the reaction. Using the following formula, we were able to get the inhibition percentage.

$$\text{Inhibition \%} = \text{Ac-As/Ac}\times 100 \quad (6)$$

where 'Ac' is the absorbance of the control and 'As' is the absorbance of the sample.

4.8. Antidiarrheal Activity

To assess the antispasmodic activity of methanolic extracts from medicinal plants, we adopted the method described by Mascolo et al. [122]. We conducted the experiment using twentyfive albino mice, divided into five groups, each comprising five mice. To induce diarrhea, we orally administered 1 ml of castor oil to all mice in each group. After one hour, the control group (Group I) received 10 ml/kg of saline orally. Meanwhile, Group II was treated with the standard drug Atropine sulphate (10 mg/kg b. wt. i.p), and Groups III to V were given methanolic extracts of plants at doses of 100, 200, and 300 mg/kg b. wt. i.p., respectively. An hour later, all mice were orally administered 1 ml of charcoal meal (consisting of a 10% charcoal suspension in 5% gum acacia). After an additional hour, we measured the distance covered by the charcoal meal in the intestine, from the pylorus to the caecum. This distance was expressed as a percentage of the total intestinal length, calculated using the formula:

$$\text{Intestinal transit (\%)} = (D/L) \times 100 \quad (7)$$

where D represents the distance covered by the charcoal (in meters) and L is the intestinal length (in meters).

To analyze the data, we performed statistical tests using Dunnet's t-test, considering a p-value below 0.05 as statistically significant.

4.9. Statistical Analysis

Data was examined with SPSS Version 20.0. Results were determined as Mean ± SE of three repeat determinations.

5. Conclusions

The current research highlights that the plant contains a substantial quantity of phytochemicals and micro and macro nutrients which is evident that this plant serves as a significant natural source of vital mineral elements at reasonable concentrations, which play a crucial role in the treatment of numerous diseases. Nutritional value indicates the presence of ash, fats, fibers, carbohydrates, proteins, moisture contents and gross energy. Due to the presence of these nutritional components, this plant has pharmacological and pharmaceutical value. The plant also exhibits pharmacological activities like genotoxic, antioxidant and antidiarrheal activities. These properties render the plant suitable for developing remedies to treat various therapeutic diseases**.**

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Data Availability Statement: The data such as the source file associated with these findings are available from the corresponding author upon request.

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