

ANTIMICROBIAL CAPACITY, ANTIOXIDANT AND FREE RADICAL SCAVENGING ACTIVITY OF EXTRACT FROM THE LEAF OF *ERIGERON FLORIBUNDUS*

ABSTRACT

Fresh leaves of *Erigeron floribundus* were obtained, and its ethanolic extract was assessed for antimicrobial, ~~antioxidant~~-free radical scavenging activity as well as phytochemical constituents. Haemolytic activities were also screened to know whether the extract will lyse the blood agar-used. Six pathogenic bacteria; the ~~gram-gram~~-positive; *Bacillus cereus*, *Clostridium* species, *Staphylococcus aureus*, and the ~~gram-gram~~-negative: *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli*, were screened for susceptibilities to the antimicrobial activity of the leaf extract. Five fungi, Yeast, *Penicillium* sp, *Aspergillus niger*, *Aspergillus flavus*, and *Aspergillus fumigatus*, were also screened for antifungal properties of the extract. The result indicated that the plant extract showed antimicrobial action but to a varying ~~the~~ degree. The extract was screened for its antioxidant property and free radical scavenging capacity. The result indicated that it has high activity in reducing iron chloride solution and ~~it also has high activities~~ high activity in (DPPH) radicals scavenging test. The phytochemical screening of the extracts showed some bioactive ingredient of pharmacological importance like Tannin, Cardiac glycosides, and Saponin. Haemolytic assay of the extract showed that it ~~is able to~~ could lyse blood cells.

KEYWORDS: Antimicrobial, Antioxidant, *Erigeron Floribundus*, Scavengers, Ekiti.

INTRODUCTION

Traditional medicines are widely spread throughout the world. It is the total combination of knowledge and practice, whether explicable or not, used in diagnosing, preventing, or eliminating a physical, mental or social disease, which may rely exclusively on past experience and observation handed down from generation to generation, verbally or in writing, while bearing in mind the original concept of nature which includes the material world, the sociological environment, whether living or dead and metaphysical forces of the universe (Sofowora, 2000).

The genus *Erigeron Floribundus* belongs to the family of Asteraceae. The plant is found growing in almost every part of the world. In the United States of America, it can be found from Washington East to North Dakota, South of Kansas—Kansas, Southwest to Arizona, and West to California and Oregon, but generally in dry valleys, in dry, rocky habitats including grasslands, open ~~panderosapine~~-ponderosa pine forests on plants, slopes and hills in Africa particularly in Nigeria (Lee *et al.*, 2003).

Erigeron Floribundus is a perennial shrub ~~of~~ about 1ft (30cm) tall and grow ~~from a taproot giving rises from a taproot~~ to a woody stem base and rhizomes. The stem has long grey hairs and a branch at the top with a light blue ray flower. The basal leaves are narrow and lance-shaped with grey hairs, and the leaves lessen in number and size further from the stem. The species usually flowers from early May to June, flowers that have petals that form an ~~elongated-elongation~~ (Rios and Recio, 2005).

The trend in traditional medicine has been progressive, ~~it seems to have given birth to the modern convectional medicine and according to Hassan and Atta(2005), most of the modern plant derived drugs were originally discovered through the study of tradition. It seems to have given birth to modern convectional medicine. According to Hassan and Atta(2005), most modern plant-derived drugs were originally discovered through the study of traditional~~ cures. Over 60% of Nigeria's rural population ~~rely-relies~~ on traditional or indigenous forms of medicine because of a shortage of hospitals and health centers ~~as well asnd~~ the medical and paramedical staff needed to man orthodox health care delivery systems (FAO, 1999).

However, the- growth in knowledge of free radicals and reactive oxygen species (ROS) in medicinal plants is producing a medical revolution that promises a new age of health. ~~In fact, t~~The discovery of the role of free radicals in chronic degenerative diseases, retinal degeneration, ischemia, dementia, and other neurodegenerative disorders and aging ~~are-is~~ as important as the discovery of the role of micro-organisms in infectious diseases (Bray, 1999). It is well known that ROS is generated spontaneously in the living cell during several metabolic pathways. These comprise components of the biological electron transport system (photosynthetic, mitochondria, microosomal), including various enzymes and biomolecules: neutrophil, xanthine oxidase cyclooxygenase, ~~lipoxigenaselipoxygenase~~, autooxidation of catecholamines (Halliwell and Gutteridge, 1989). Although, known as very harmful, there is at least one case ~~however, in which organism employ radicals in a controlled way to achieved in which organisms employ radicals in a controlled way to achieve~~ a ~~useful~~ practical purpose, the action of the phagocytic cell (neutrophils and monocytes, macrophages). Free radicals are necessary ~~in-for~~ the immune system, prostaglandin biosynthesis, and antibacterial cell activities. Besides the ~~above-mentioned~~

physiological actions, ROS generation is induced by several exogenous factors such as pollution, smoke, radiation, pesticide, [and](#) drug consumption (Noguchi *et al.*, 2000).

Antioxidants are phytochemicals, vitamins, and other nutrients that protect our cells from damage caused by free radicals. In vitro and in vivo studies have shown that antioxidants help prevent the free radical damage ~~that is~~ associated with cancer and heart disease. Antioxidants can be found in most fruits and vegetables, but ~~also in~~ culinary herbs and medicinal herbs can contain [a](#) high level of antioxidants. Dragland and colleagues showed in their study entitled Several Culinary and Medicinal Herbs are important sources of Dietary Antioxidants and published in the journal of Nutrition (Lee *et al.*, 2003) that herbs contain antioxidant levels of up to 465mmol per 100g (Lee *et al.*, 2003) without ~~become~~ [becoming](#) a free radical themselves. When antioxidants neutralize free radicals by receiving or donating ~~an~~ electron, they do not become antioxidants themselves because they are stable in both forms. In other words, antioxidants are chemicals that offer ~~up their own electrons to the free radicals, thus preventing cellular damage radicals, it becomes~~ [their electrons to the free radicals, thus preventing cellular damage radicals from becoming](#) inactive. Antioxidants act as radical scavengers, hydrogen donors, electron donors, peroxide decomposers, singlet oxygen quenchers, enzyme inhibitors, synergists, and metal-chelating agents. Both enzymatic and non-enzymatic antioxidants exist in the intracellular and extracellular environment ~~al~~ [to](#) detoxify ROS (Hassan and Atta, 2005). However, in this study, the antimicrobial activities of the crude extracts of *Erigeron Floribundus* leaves, its free radical scavenging ability and ~~haemolytic property of the extract was determined~~ [the extract's hemolytic property was determined](#).

METHODOLOGY

Sources of the Test Organisms

The test organisms used for this study were obtained from the stock culture in the Microbiology Department of the Federal University of Technology Akure, Ondo State, Nigeria. The organism includes: Bacteria *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Clostridium botulinum*, *Bacillus cereus*, *Staphylococcus aureus*, and Fungi: Yeast, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigates* and *Penicillium notatum*. The pure isolates of these organisms were inoculated on Nutrient Agar (NA) slant and Potato Dextrose Agar (PDA) stock for bacteria and fungi, respectively, and stored at 4°C.

Source of chemical

The following chemicals were used for this work. About 200mM sodium phosphate buffer (pH 6.6), 1% potassium ferricyanide, 10% perchloric acid, 0.1% ferric chloride, DPPH (1,1-diphenyl 1-2picrylhydrazyl), 0.4mM methanolic solution, ethanol solution. The chemicals were obtained from the Biochemistry and Chemistry Department of the Federal University of Technology Akure, Ondo State, Nigeria.

Source of plant sample and extraction

The leaves of the plant *Erigeron floribundus* were collected from an uncompleted building along stadium road, Akure, in Ondo State, Nigeria. ~~Both~~ The local name (ewe ilori) and taxonomical identification of the plant material ~~was~~ were confirmed by Mr. Arannilewa of the department of Biology storage, Federal University of Technology Akure, Ondo State, Nigeria. The leaves were thinly spread on a clean floor in an ~~open~~ open-air to air-dry at atmospheric temperature. The dried sample of the plant was grinded into the powder using a milling machine and then carefully packed into a clean polythene bag. A 60% ethanol was ~~the solvent~~ used for extraction in which 200g of the powdered sample was weighed ~~and poured into the solvent,~~ poured into the solvent, and left for 72hours. The ~~resultant~~ mixture was agitated and sieved with muslin cloth into a clean container. The sieved extract was concentrated to dryness using a rotary evaporator. It was weighed and kept at room temperature for ~~both~~ antimicrobial, haemolytic, phytochemical, antioxidant and free-radical scavenger studies.

Determination of the minimum inhibitory concentration (MIC)

In determining the Minimum inhibitory concentration (MIC), the method described by Ilesanmi *et al.* (1986) was employed. Different concentration of the extract was prepared at 200mg/ml, 100mg/ml, 50mg/ml and 25mg/ml. About 24 hours old, the nutrient broth culture of organisms was prepared, and 5ml of the organism was pipette into their test tubes ~~respectively,~~ already containing 4ml fresh sterile nutrient broth. With the aid of a sterile syringe, 1ml of different ~~concentration of extract~~ extract concentrations was poured into the corresponding broth culture. The absorbance of each test tubes ~~was~~ taken on the 1st day of preparation before they were incubated at 37°C, ~~it~~ It was recorded as 0 days. After 24 hours, the absorbance was also taken, and also after 48hours of incubation using cuvet tubes for the absorbance. 1ml from each tubes ~~was~~ dispensed on a prepared sterile plate of nutrient agar and incubated to check for their growth. ~~Comparison~~ The comparison was also carried out. Growth was indicated by turbidity, which was confirmed with the aid of a spectrophotometer. The last concentration at which growth inhibition was noticed was taken as minimum inhibitory concentration (MIC).

Antifungal Property of the Extract

A sterile Potato Dextrose Agar plate was prepared. It was allowed to cool and ~~solidified~~solidify. Four wells were bored into each plate using a cork borer of 5 diameters. Different concentrations of 200mg/ml, 100mg/ml, 50mg/ml and 25m/ml from the extract was prepared. Each concentration of extract was put into a corresponding labeled well in each plate, and five different fungi isolates from the stock ~~was~~were put at the center of each plate, covered and incubated for 78hours.

Determination of the Haemolytic activity of the extract

The haemolytic activity of the extract was determined using the disc diffusion method as ~~described by~~ Annapinna *et al.*; (2003). On the blood agar plate, about 3.7g of nutrient agar was prepared and sterilized together with the other equipment to be used. 4ml of blood was added at 50°C, allowed to cool, and then pour plating was done. The medium was allowed to solidify, and two wells were bored in a plate using a cork borer of 5mm, where the different ~~of the~~ extract was dispensed under aseptic conditions. The plates were ~~then incubated at 37°C for 24hours which was incubated at 37oC for 24 hours and were~~ checked ~~thereafter after that~~ for the clear zones. The concentration of the extract used are 200mg/ml, 150mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml, 3.125mg/ml. Clear zones of haemolysis indicate ~~the a~~ positive result.

~~Antioxidant~~ The antioxidant property of the Extract

The reducing property of the extract was determined by assessing the ability of the extract to reduce (FeCl₃) ferric chloride solution as described by Pulido *et al.*; (2000). One gram (1g) of the extract was dissolved in 20mls of distilled water, 2.5mls of the extract was mixed with 2.5ml, 200mM sodium phosphate buffer (pH 6.6) and 2.5ml of 1% potassium ferricyanide. The mixture- was then incubated at 500c for 20 minutes. ~~Thereafter~~ After that, 2.5ml of 10% perchloride acid was added and centrifuged at 650rpm for 10 minutes. 5mls of the supernatant was mixed with an equal amount of water. About 1ml of 0.1% ferric chloride was added, and absorbance was measured with a spectrophotometer at 700nm.

Free Radical Scavenging ability of Extract

The free radical scavenging ability of the extract against DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical was evaluated (Ursini *et al.*, 1994). The approximate dilution of 1g of the extract was diluted with 200ml of distilled water. 1ml of the diluted extract was then taken into the test tube, ~~;~~ it was ~~them~~ then mixed with 1ml of 0.4nM methanolic

solution containing 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals. The mixture was then left in the dark for 30 minutes before measuring. The measuring absorbance was taken at 516 nm.

Phytochemical Screening of The Extract

Phytochemical analysis of the extract was carried out to determine the bioactive ingredient present in the extract. The method described by Harbone (1984) was used for the determination of the phytochemical. The following were tested for:

i. Test for Saponins

The ability of saponins to produce frothing aqueous solution, was used as a screening test for saponins. About 0.5g of the plant extract was shaken with distilled water in test tube frothing, which persists on warming, was taken as preliminary evidence for the presence of saponins.

ii. Test for tannins

About 5g of the extract was stirred with 100ml of distilled water, filtered, and ferric chloride reagent was added to the filtrate. A blue-black green or blue-green precipitation indicates the presence of tannins (Trease and Evans, 1978).

iii. Test for anthraquinones

Borntrager's test was used for the detection of anthraquinones. 5g of the plant extract was shaken with 10ml of benzene, filtered, and 5ml of 10 percent (10%) ammonia solution was added to the filtrate. The mixture was shaken, and the presence of a pink, red, or violet colour in the ammonia (lower) phase indicated the presence of free anthraquinones.

iv. Test for cardiac glycosides

The following tests were carried out to test for cardiac glycosides.

1. Lieberman's test:- About 0.5g of the extract was dissolved in 2ml of acetic anhydride and cooled well in ice. Sulphuric acid was then carefully added. A colour change from violet to blue-green indicated the presence of steroids nucleus (i.e., glycone portion of the cardiac glycosides).
2. Keller-Killani test:- About 0.5g of the extract was dissolved in 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was then underlayed with 1ml of

concentrated sulphuric acid. A brown ring obtained at the interface indicated the presence of a desoxy sugar characteristic of cardenolides. A violet ring below the brown ring or in the acetic layer, a greenish ring layer is also a positive result (Trease and Evans, 1978).

v. Test for phylobatannis

The deposition of a red precipitate, when an aqueous extract of the plant was boiled with 1% aqueous hydrochloric acid, indicates the presence of phlobatannis (Trease and Evans, 1978).

RESULTS

Percentage Recovery of The Plant Extract

The percentage recovery or yield of the plant extract was calculated by dividing the final weight of the extract obtained by the initial weight and multiplying by 100. The value obtained was 23.25%

The result obtained from the ~~minimum inhibitory concentration of the extract is shown on the extract's~~ minimum inhibitory concentration is shown in Table 1. It was observed that the higher the concentration of the extract, the lower the absorbance. The higher the ~~concentration of the extract the lower the number of colony~~ extract concentration, the lower the colony number obtained after the incubation. The absorbance increased with the period of incubation. The extract has ~~the inhibitory effect on Klebsiella pneumoniae~~ most inhibitory effect on *Klebsiella pneumoniae*, followed by *Escherichia coli*, then *Pseudomonas aeruginosa* for ~~gram~~-negative bacteria. For ~~gram~~-positive bacteria also, the higher the concentration of the extract, the lower the absorbance. The absorbance increased with the incubation period except for *Pseudomonas aeruginosa*, which remained constant after 24 hours ~~after of~~ incubation. The higher the concentration of the extract, the lower the number of ~~the~~ colony. ~~For the fungi growth, the extract was not active on the all the tested fungi culture~~ The extract was not active on all the tested fungi cultures for fungi growth.

The result of ~~the~~ haemolytic activity of the extract is shown in table 2 and plate 1. The highest value of 42mm was recorded for the ~~highest concentration of the extract i.e 200mg/ml while the lowest value of 200mm was recorded for the lowest concentration of the extract~~ extract's highest concentration, i.e. 200mg/ml, while the lowest value of 200mm was recorded for the extract's lowest concentration, i.e 3.125mg/ml.

~~Antioxidant~~ The antioxidant effect of the ethanol extract was assessed by the assay for ferric reducing antioxidant power shown in Table 3.

~~Free~~ The free radical scavenging ability of the extract against DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical was reported in Table 4. ~~A strong antiradical activity was undoubtedly confirmed by the DPPH assay~~ The DPPH assay undoubtedly confirmed a strong antiradical activity.

Phytochemical screening revealed that *Erigeron floribundus* contains antinutritional constituents like saponins and tannins, and cardiac glycosides, such as killer killani and phlobatannis. This is shown ~~on-in table~~ Table 5.

Table 1: Antimicrobial activity result ~~of gram negative and gram positive organisms of the leaf extracts from the leaf extract's gram negative and gram positive organisms.~~

Organism	Extract concentration (mg/ml)	Absorbance			No of colony
		Day 0	Day 1	Day 2	
<i>Klebsiella pneumoniae</i>	200	0.54	0.74	1.00	30
	100	0.77	1.00	2.00	70
	50	2.30	2.40	2.50	104
	25	2.60	2.80	2.80	136
<i>Pseudomonas aeruginosa</i>	200	1.70	2.00	2.00	44
	100	2.30	2.30	2.50	52
	50	2.40	2.40	2.50	60
	25	2.50	2.60	2.60	192
<i>Escherichia coli</i>	200	0.72	1.20	1.60	76
	100	0.97	1.49	1.80	95
	50	2.50	1.60	2.40	173
	25	2.70	4.20	4.70	240
<i>Bacillus cereus</i>	200	0.90	2.00	2.20	40
	100	2.00	2.60	3.40	84
	50	2.70	2.80	5.30	192
	25	2.80	2.90	5.40	200
<i>Clostridium botulium</i>	200	0.95	2.00	2.40	98
	100	2.00	2.90	3.70	124
	50	2.70	3.20	5.00	176
	25	2.80	5.50	5.80	280
<i>Staphylococcus aureus</i>	200	0.70	0.95	2.30	68
	100	1.60	2.60	3.10	86

	50	2.60	3.90	5.30	184
	25	2.80	4.00	6.60	220

Table 2: The diameter zones of Haemolytic activity of the extract of *Erigeron floribundus*

Concentration of Extract (mg/ml)	Haemolytic Zones (nm)
200	42
150	40
100	35
50	30
25	30
12.5	25
6.25	23
3.125	20

Table 3: Free Radical-Scavenging effect and reducing power (Antioxidant effect) of the Ethanol Extract of *Erigeron floribundus*.

Materials	DPPH	Reducing power (Absorbance at 700nm)
Extract	1.93	5.85
T3	-	1.53

DPPH = 1,1-diphenyl-2-picrylhydrazyl

T3 = tocotrienol of Tocovoid Supra™ Bio drug

Table 5: Phytochemical constituents of the Extract of *Erigeron floribundus*

Chemical Constituents	Result
Saponins	+
Tannins	+
Phlobatannis	+
Anthraquinones	-
Cardiac glycosides	
i. Killer killani Test	+
ii. Lieberman's test	-

Keys:-
+ve = Positive
-ve = Negative

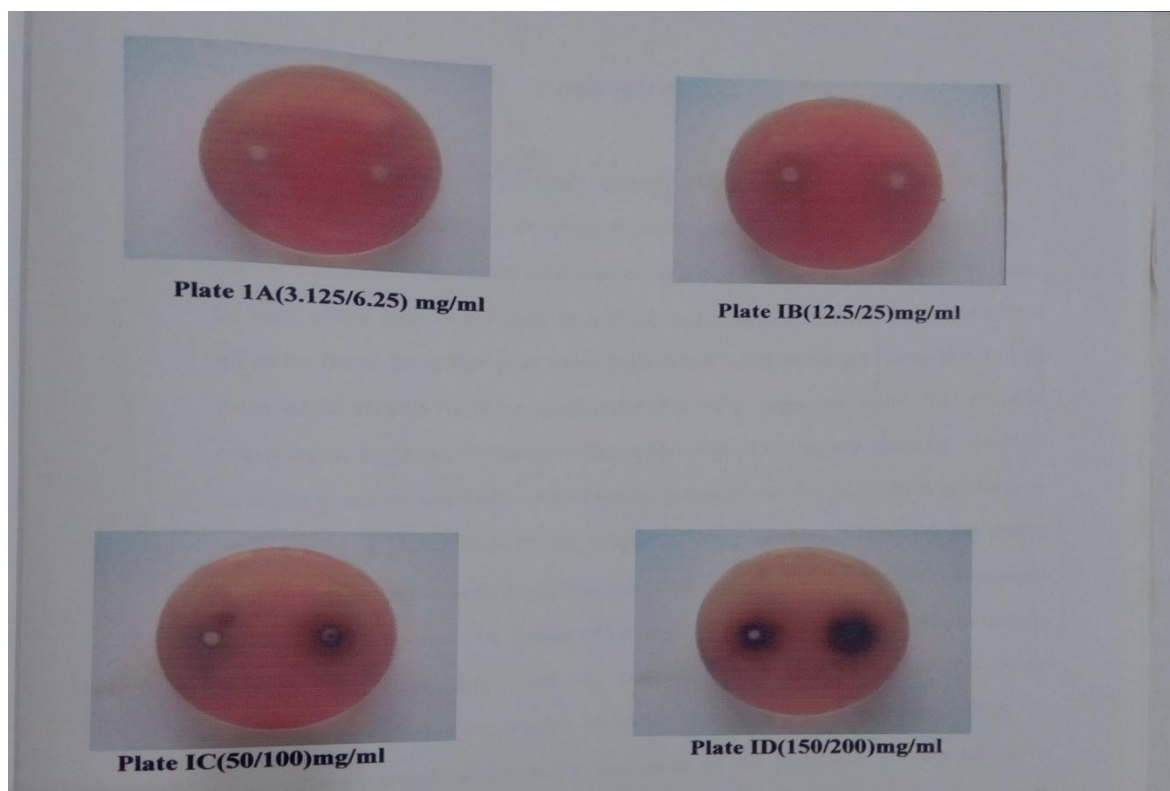


Plate 1: Haemolytic property of Leaf Extract of *Erigeron floribundus* on blood agar at a concentration of (a) 3.125 and 6.25 (b) 12.5 and 25 (c) 50 and 100 (d) 150 and 200.

DISCUSSION

The findings ~~obtained from this work shows that the leaf extract of *Erigeron floribundus* have inhibitory effect on the six bacteria used for antimicrobial test and found to hav~~ from this work show that the leaf extract of *Erigeron floribundus* has an inhibitory effect on the six bacteria used for antimicrobial tests and was found to be very effective on the test organisms. Although, it could be very active than this if another leaf extract would have been combine with it so as to boost its active principles than when used in single form, but it could be more active than this if another leaf extract had been combined with it to boost its active principles than when used in single form, it still ~~have~~ has equal volume for the suppression of the test organism used. The plant in focus here *Erigeron floribundus* has been one of the many species common in ~~various~~ urban and non-urban areas, generally in dry habitats, according to Wagner *et al.* (1999).

Due to its medicinal importance, its one of the many plants whose use, needs to be harmonized with traditional medicine practices rather than distained. It's ~~being~~ being effect against *Bacillus cereus*, *Pseudomonas aeruginosa*, *Escherichia coli*, ~~to mention and a~~ few conforms with the result obtained. Although the absence of Anthraquinones and one of the cardiac

glycosides named ~~Lieberman's~~ Lieberman's test lowered its microbial activity ~~but other phytochemical constituent present justified their medicinal use as stated by Gordian (2005), other phytochemical constituent present justified their medicinal use, as Gordian (2005) stated.~~ Phytochemical constituents like tannins, phlobatannins, saponins, killer killani of cardiac glycosides compounds has most important bioactive constituent of leaf extract Edeoga *et al.* (2005) and these compounds has antimicrobial properties. The low antimicrobial activity observed is traceable to the concentration of the extract obtained during extraction; about 200g was soaked in 1,500ml of water which makes the percentage recovering of the extract from *Erigeron floribundus* posses ~~haemolytic~~ hemolytic activities. The ~~zones of haemolytic~~ hemolytic zones were directly proportional to the concentration of the extract used. The ability of the extract to lyse the blood cell can be linked with the antimicrobial factors like the saponins, tannins, etc., which ~~has~~ have been shown to be widely distributed in *Erigeron floribundus*. The investigated extract expressed a significant capacity to reduce ferric chloride, and this shows that the ethanolic extract from *Erigeron floribundus* has a powerful antioxidant potential, and thus, had the ability to donate electrons, which ~~suggested~~ suggests that it may act as a free radical scavenger. ~~A~~ The DPPH assay undoubtedly confirmed strong antiradical activity ~~strong antiradical activity was undoubtedly confirmed by the DPPH assay;~~ therefore, the extract has been proven able to prevent the initiation of free radical-mediated chain reaction.

Conclusions

The ethanolic extract was found to inhibit the growth of microorganisms. *Erigeron floribundus* also posses ~~haemolytic activities and can thus be used in treatment of infectious diseases that is caused by intracellular parasites~~ hemolytic activities and can thus be used in treating infectious diseases caused by intracellular parasites in the blood. However, when ~~the~~ such extract ~~are~~ is used in phyto-medicine ~~there is a need for the patients to be taking, the patients need to take~~ along with its blood builders and vitamin supplement (Oladunjoye, 2007). ~~The need to study medicinal plants, according to WHO(2000)~~ According to WHO(2000), the need to study medicinal plants cannot be ~~over-over~~ emphasized for a vista of reasons, including inter alia wide-spread, use of plants in folks medicine, rescuing traditional plants and knowledge about them from imminent loss as well as the need for health for all. Phototherapy, with time, will gain attraction and may well be complete or ~~rather~~ rather harmonized with orthodox medicine. Narranjo (1995) also advocated an urgent need to supply these medicinal plants with the note that there is ~~an~~ an abject neglect of highly endangered but cheap alternative health care resources.

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