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 assessed for antimicrobial, antioxidant free radical scavenging activity as well as phytochemical constituents. Haemolytic activities were also screened to known whether the extract will lyse the blood agar used. Six pathogenic bacteria; the gram positive; *Bacillus cereus, Clostridium* species, *Staphlococcus aureus*, and the 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 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 in (DPPH) radicals scavenging test. The phytochemical screening of the extracts showed some bioactive ingredient of pharmacological importance like Tannin, Cardiaglycosides and Saponin. Haemolytic assay of the extract showed that it is able to 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 State of America, it can be found from Washington East to North Dakota South of Kansas Southwest to Arizona and West to California and Oregon, but generally in dry valleys, in dry rocky habitat including grasslands, open panderosapine 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 rise to a woody stem base and rhizomes. The stem has long grey hairs and branch at the top with 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 stem. The species usually flowers early May to June, flowers that have petals that form an elongated (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 cures. Over 60% of Nigeria rural population rely on traditional or indigenous forms of medicine because of shortage of hospitals health centers as well as 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, the discovery of the role of free radicals in chronic degenerative diseases, rectinal degeneration, ischemia, dementia, and other neurodegenerative disorders and aging are 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 biological electron transport sytem (photosynthetic, mitochondria, mirosomal), including various enzymes and biomolecules: neutrophil, xanthine oxidase cyclooxygenase, lipoxigenase, autooxidation of catecholamines (Halliwell and Gulteridge, 1989). Although, known as very harmful, there is at least one case however, in which organism employ radicals in a controlled way to achieved a useful purpose, the action of phagocytic cell (neutrophils and monocytes, macrophage). Free radicals are necessary in the immune system, prostaglandin biosynthesis and antibacterial cell activities. Beside the above mentioned physiological actions, ROS generation is induced by several exogenous factors such are pollution, smoke, radiation, pesticide, 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 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 a free radical themselves. When antioxidants neutralize free radicals by receiving or donating and 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 inactive. Antioxidants act as radical scavenger, hydrogen donors, electron donor, peroxide decomposer, singlet oxygen quencher, enzyme inhibitor, synergist and metal-chelating agents. Both enzymatic and non-enzymatic antioxidants exist in the intracellular and extracellular environmental 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 determine.

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 include: 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^{o}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 plant *Erigeron floribundus* were collected from uncompleted building along stadium road, Akure, in Ondo State, Nigeria. Both the local name (ewe ilori) and taxonomical identification of the plant material was confirmed by Mr Arannilewa of the department of Biology storage, Federal University of Technology Akure, Ondo State, Nigeria. The leaves were thinly spread on clean floor in an open air to air-dry at atmospheric temperature. The dried sample of the plant was grinded into the powder using milling machine and then carefully packed into 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 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 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 sterile syringe, 1ml of different concentration of extract 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^{o}C , it was recorded as 0 day. After 24hours, 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 was also carried out. Growth was indicated by turbidity which was confirmed with aid of 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. 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, five different fungi isolates from the stock was 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 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^{o}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 cork borer of 5mm where different of the extract was dispensed under aseptic condition. The plates were then incubated at 37^{o}C for 24hours which was checked thereafter 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 positive result.

Antioxidant property of the Extract

The reducing property of the extract was determined by assessing the ability of the extract to reduce ($Fecl_3$) 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 20minutes. Thereafter 2.5ml of 10% perchloride acid was added and centrifuged at 650rpm for 10minutes. 5mls of the supernatant was mixed with equal amount of water. About 1ml of 0.1% ferric chloride was added and absorbance was measured with spectrophotometer at 700nm.

Free Radical Scavenging ability of Extract

The free radical scavenging ability of the extract against DPPH (1, 1-diphenyl1-2 picrylhydrazyl) free radical was evaluated (Ursini *et al*., 1994). The approximate dilution of 1g of the extract was diluted with200ml of distilled water. 1ml of the diluted extract was then taken into the test tube, it was them mixed with 1ml of 0.4nM methanolic solution containing 1,1-diphenyl1-2 picrylhydrazyl (DPPH) radicals. The mixture was then left in the dark for 30minutes 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 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 10percent (10%) ammonia solution was added to the filtrate. The mixture was shaken and the presence of a pink red or violet colour in the ammonial (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. Killer-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 characteristics 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, it 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 multiply by 100. The value obtained was 23.25%

The result obtained from the minimum inhibitory concentration of the extract is shown on the 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 obtained after the incubation. The absorbance increased with the period of incubation. The extract has inhibitory effect on *Klebsiella pneumoniae* most, followed by *Escherichia coli*, then *Pseudomonas aeroginosa* 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 *Psedomonas aeruginosa* which remained constant after 24hours after incubation. The higher the concentration of the extract, the lower the number of colony. For the fungi growth, the extract was not active on the all the tested fungi culture.

The result of 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 i.e 3.125mg/ml.

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

Free radical scavenging ability of the extract against DPPH (1,1-diphenyl1-2 picrylhydrazyl) free radical was reported in Table 4. A strong antiradical activity was undoubtedly confirmed by the DPPH assay.

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

Table 1: Antimicrobial activity result of gram negative and gram positive organisms of the leaf extract.

		Absorbance			
Organism	Extract concentration (mg/ml)	Day 0	Day 1	Day 2	No of colony
Klebsiella	200	0.54	0.74	1.00	30

pneumoniae	100	0.77	1.00	2.00	70
	50	2.30	2.40	2.50	104
	25	2.60	2.80	2.80	136
Pseudomonas aeroginosa	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
Escherichia coli	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
Bacillus cereus	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
Clostridium botulium	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
Staphylococcus aureus	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 12.5 6.25 3.125	30 25 23 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-picrythydrazyl

T3 = tocotrienol of Tocovoid Supra ^{TM} 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

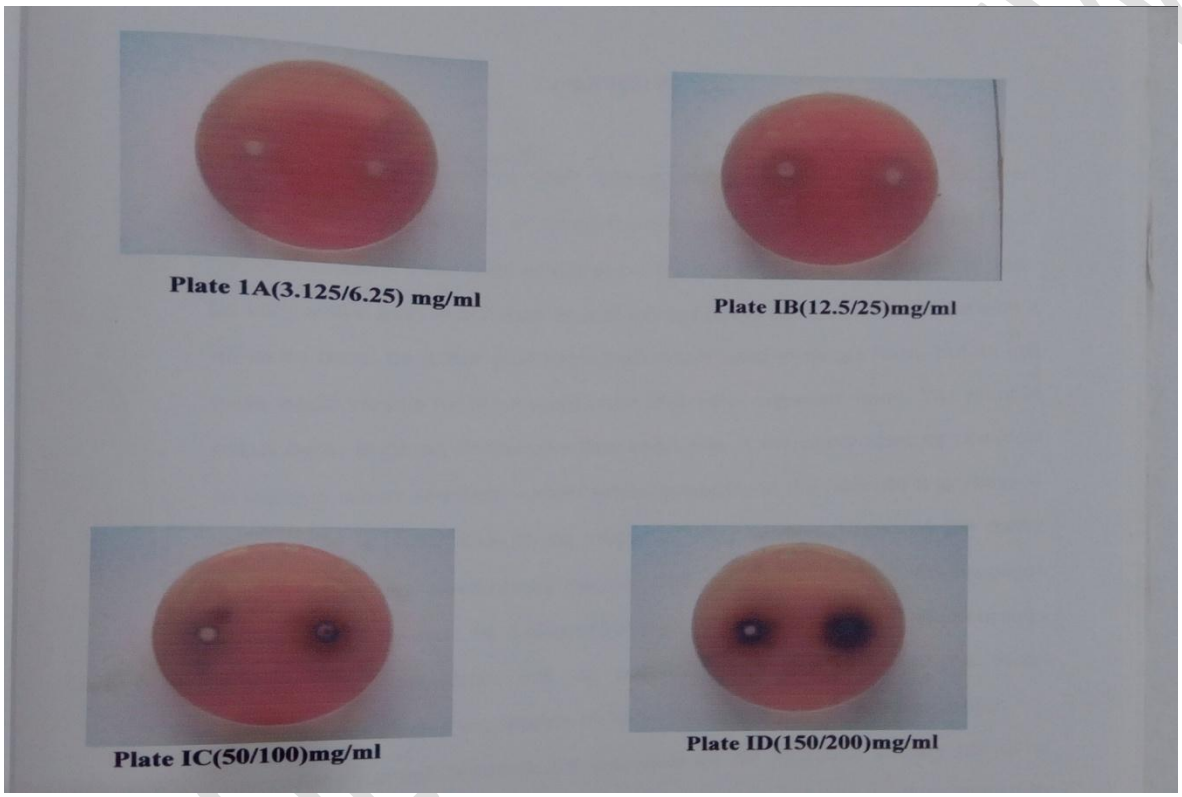

Plate 1: Haemolytic property of Leaf Extract of *Erigeron floribundus* on blood agar at concentration of (a) 3.125 and 6.25 (b) 12.5 and 25 (c) 50 and 100 (d)150 and 200.

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 have 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 still have 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. Its being effect against *Bacillus cereus*, *Pseudomonas aeruginosa*, *Escherichia coli*, to mention few conforms with the result obtained. Although the absence of Anthraquinones and one of the cardiac glycosides named lieberman's test lowered its microbial activity but other phytochemical constituent present justified their medicinal used as stated by Gordian (2005). Phytochemical constituents like tannins, phlobatannis, 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 percentage recovering of the extract from *Erigeron floribundus* posses haemolytic activities. The zones of haemolytic 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 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 that it may act as a free radical scavenger. A 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 infection diseases that is caused by intracellular parasite in the blood. However, when such extract are used in phyto-medicine there is a need for the patients to be taking along with its blood builders and vitamin supplement (Oladunjoye, 2007). The need to study medicinal plants, according to WHO(2000) cannot be over emphasized for a vista of reason 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 complete or rather harmonized with orthodox medicine. Narranjo (1995) also advocated an urgent need to supply these medicinal plants with the note that there is an abject neglect of highly endangered but cheap alternative health care resources.

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