Original Research Article

Identification and Quantification of phytochemicals in ethanol Leaf extract of *Emilia praetermissa*

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Abstract

Emilia pratermissa, an erect annual medicinal plant from the family *Asteracea* used in treatment of different illnesses by the local populacewas worked on determine the phytocompounds responsible for its therapeutic properties. Fresh leaveswas harvested from uncultivated farm land at Prefab Aladinma, Owerri Municipal Imo state,washed and dried at room temperature for two weeks, the dried leaf was pulverized using mechanical grinder, 400g of the pulverized leaves were extracted with ethanol using maceration method and concentrated in a rotary evaporator. The extract was subjected to phytochemical analysis using standard methods, the qualitative phytochemical analysis result revealed the presence of alkaloids, flavonoids, terpenoids, tannins, saponins, phenol and oxalate, confirmation of the phytochemical analysis result using Gas Chromatography Flame Ionization Detector revealed the presence of these phytocompounds with their concentration: Ammodendrine(5.95%), Phytate (14.50%), Hydroxylupanine (6.74%) Sapogenin (15.65%) Tannin (2.55%), Cardiac glycoside (1.00%), Epihedrine (9.05%), Anthocyanin (9.17%), Flavones (7.97%), Flavonone (5.25%), Proanthocyanidin (5.71%), Cyanogenic glycoside (3.96%) and Narigenin (8.39%). The presence of these phyto- compounds on the leaf extract of *Emilia pratermissa* proves the efficacy of this plant as acclaimed ethnomedically.

Keywords: *Emilia pratemissa*, phytochemicals, ethnomedicine,medicinal plant.

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INTRODUCTION

A medicinal plant is any plant which in one or more of its organs contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs[1]. Our earliest human ancestors found plants to heal wounds, cure diseases, and ease troubled minds. People on all continents have long used hundreds, if not thousands, of indigenous plants, for treatment of various ailments dating back to prehistory. Knowledge about the healing properties or poisonous effects of plants, mineral salts, and herbs accumulated from

these earliest times to provide health and predates all other medical treatment[2]. Medicinal plants have been used in healthcare since time immemorial,Plant resources have remained an integral part of human society throughout history. After filling the primary needs like food and shelter, man has sought for a suitable remedy among plants for curing various diseases and illness [3].

Traditional medicine includes diverse health practices, approaches, knowledge and beliefs incorporating plants, animal and/or mineral based medicines, spiritual therapies, manual techniques and exercises applied singularly or in combination to maintain well-being as well as to treat, diagnose or prevent illness. The use of traditional medicine has expanded globally and gained popularity in the last few decades. Specifically, these practices have not only continued to be used for primary care of the poor in developing countries, but have also been used in other countries where conventional medicines are predominant in national health care system. In the last decades traditional medicines has become popular in developing countries, partly due to long unsustainable economic situation in the countries. The high cost for drugs and increase in drug resistance to common diseases like malaria, bacterial infection and other sexually transmitted diseases has caused the therapeutic approach to alternative traditional medicine as an option for concerted search for new chemical entities [4]. Natural medicine is still in use in modern day Africa after hundreds of years of its existence without much reported cases of adverse effects [4].

Among such species or plant use for their beneficial medicinal effect is *Emilia Praetermissa* from the family of *Asteraceac*[5]. The plant is documented in ethnomedicine to possess medicinal benefits in treating diarrhoea, night blindness and sore throat , rashes, measles, inflammatory diseases, fever , stomach tumor , malaria , asthma, liver diseases , eye inflammation , earache and chest pain . In China, the leaves are used for the treatment of dysentery and roundworm infestations, wounds and abscesses, influenza, burns and snake bites. The leaf paste in doses of one spoonful once a day at bed time for about 2-3 months is recommended to treat night blindness. The crushed leaves are used externally to treat breast abscesses among tribal women[6,7]. The leaves are rubbed on the forehead to relieve headache. The Africans consume the leaves as vegetable for its laxative property. The plant has been documented in the Nigerian folk medicine for the treatment of epilepsy in infants[7].The use of plants for treating diseases is as old as the human species,popular observations on the use and efficacy of medicinal plant significantly contribute to the disclosure of their therapeutic properties.Medicinal plants have provided mankind a large variety of potent drugs to alleviate or eradicate infections and suffering from diseases in spite

of advancement in synthetic drugs, some of the plant-derived drugs still retained their importance and relevance. The use of plant-based drugs all over world is increasing [8].

Fig. 1 :***Emilia praetermissa*plants**

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MATERIALS AND METHODS

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Plant Collection and Identification

Fresh leaf of *Emilia praetermissa* was harvested from uncultivated farm land located in Prefab Aladinma in Owerri Municipal Local Government Area of Imo State Nigeria and was identified by Professor F.N Mbagwu of the Department of Botany Imo State University, Owerri as *Emilia praetermissa* from the family of *Asteraceae.*

Preparation of the Sample for Analysis

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The harvested leaf of *Emilia praetermissa* was washed with water to remove sand and dirt, and then dried at room temperature for two weeks. The plant material after complete dryness was pulverized with new corona mechanical grinder 2013 model, weighed and stored in amber coloured Winchester bottle for analysis.

2.4 Ethanol Extract

300g of the pulverised sample was percolated with 500ml of redistiiled ethanol, allowed to stand for 24 hours, filtered using sterile whatmann no 1 filter paper and used for phytochemical analysisusing standard procedure by Harborne, (1998)to identify the constituents present in the plant[9].

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Qualitative Phytochemical Analysis

Qualitative phytochemical analysis was carried out to identify the phytocompounds present in ethanol leaf extract of *Emilia praetermissa* using standard methods as shown below.

Test for Alkaloid

Preparation of Wagner's Reagent:

Wagner's Reagent: 1.3g of iodine crystals and 2.0g of KI were dissolved in 100ml volumetric flask made up to the mark with distilled water. Reddish brown colour on the addition of the reagent to the extract indicated the presence of alkaloid [9,10].

Test for Saponins :

0.5g of dried plant powdered sample was extracted with 5ml of 50% aqueous methanol solution. The filtrate was transferred into a test tube and shake vigorously. Observation of persistent froth for 10 minutes was an indication of saponin presence[11].

Test for Tannin

0.1g of plant material was measured in a test tube and 3ml of butanol-HCl reagent (95ml of n-butanol and 5ml of concentrated HCl) were added to it. The test tube was plugged with cotton and was heated on boiling water bath for an hour. Appearance of pink colour indicated the presence of tannin [11].

Test for Phenol

Ferric chloride test: 2ml of plant extract was measured in a test tube, 2ml of distilled water added followed by addition of 10% $FeCl_3$solution. Bluish black colour indicates the presence of phenol [11].

Test for Terpenoids(Salkowski test)

Sulphuric acid Test: 5ml of the crude extract was dissolved in 2ml of chloroform and 2ml of conc. H_2SO_4 was added carefully to form a layer, a reddish-brown coloration at the interphase was formed to show positive results for the presence of terpenoids [12].

Test for Steroids

Sulphuric acid Test: To the plant extract 2ml of chloroform was added. 2ml of conc. $H_2SO_4$was added by the sides of the test tube and red colour at the lower chloroform layer indicates the presence of steroids [12].

Test for Flavonoid

To 4ml of extract in a test tube, add 2ml of 50% methanol and warm. 1g of magnesium metal was added, followed by the addition of 5 to 6 drops of concentrated hydrochloric acid. Red coloration confirmed the presence of flavonoids [12].

The result of qualitative phytochemical analysis were recorded in the table below.

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Table 1: Result of Qualitative Phytochemical Analysis of *Emilia Praetermissa* Leaves.

Phytochemicals	**Results**
Phenol	+

Flavonoids	+
Saponin	+
Terpenoids	+
Alkaloids	+
Tannins	+
Steroids	+
Oxalate	+

Present = +

Phytochemical analysis result revealed the presence of phenol, flavonoids, saponins, terpenoids, alkaloids, tannins, steroid and oxalate which are known to exhibit medicinal as well as physiological activities. Alkaloids and flavonoids have been linked with antibacterial and antiviral activity, medicinally alkaloid are pharmacologically active compounds used as local anesthetic, stimulants, psychedelics, analgesics, antibacterials, anticancer drugs, antihypertensive agents, cholinomimerics, spasmolysis agents, vasodilators, antiarrhythmia, antiasthma therapeutics, antimalarials, etc.[13,14].These compounds are known to show curative activity against several diseases and therefore could explain the plants traditional use for the treatment of wide array of illnesses[16]. Mamta,(2012) reported that flavonoids are responsible for the antimicrobial activity associated with some ethnomedicinal plants.These flavanoids have also been reported to possess antioxidant and antiradical properties [15,17]. As antioxidants, it can prevent the oxidative damage caused by the free radicals, interfere with the extraction process by reacting with free radicals, chelating catalytic metals and act as oxygen scavengers. Phenolic and flavonoids compound are widely distributed in plants and have been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory, anticarcinogenic etc[18].The result also revealed the presence of terpenoid which was reported to have a broad range biological properties which include cancer chemo-preventive effects, anti tumour, antimicrobial, antifungal, antiviral, anti-hyperglycemic, anti-inflammatory, anti-parasitics activities and memory enhancers[19].Antimicrobial activities of tannins are well documented, the growth of many fungi, yeasts, bacteria, and viruses were inhibited by tannins [20,21] .Even though their concentrations and classes differ in the different fractions of plants, tannins seem to have similar properties such as antioxidant, antimicrobial or predator-deterrent (i.e., against helminths or herbivores) itpossess anti-inflammatory, immunomodulatory, antimicrobial, antiviral, and antifungal properties [22,23]. Saponin are reported to have Antimicrobial and anti-inflammatory activity, significantly, certain saponins have been identified to drastically

enhance the efficacy of many chemotherapeutic agents, including cisplatin, paclitaxel, doxorubicin, docetaxel, mitoxantrone, and cyclophosphamide. Moreover, saponins used in combination therapy enhance the sensitivity of chemoresistant tumor cells to clinically used chemotherapeutic agents [20].Over 50% of all modern clinical drugs are of natural product origin and natural products plays an important role in drug development programme of the pharmaceutical industry. In the continuation of this strategy of new drug discovery, emphasis has been laid on the aerial parts of most plants for their antibacterial and anti-oxidant properties[24].These pytocompounds might be responsible for the efficacy of the plant.

Table 2:Result of Quantitative phytochemical Screening of ethanol leaf extract of *Emilia praetermissa*.

Phytochemicals	Structure	External Units	Percentage % Concentration
Ammodendrine		10.8252 ppm	5.95
Sparteine		7.3667 ug/ml	4.05
Phytate		26.3637 ug/ml	14.50
Hydroxylupanine		12.2504 ppm	6.74
Sapogenin		28.4607 ug/ml	15.65

Tannin		4.6310 ppm	2.55
Cardiac Glycoside		1.8250 ug/ml	1.00
Epihedrine		16.4623 ug/ml	9.05
Anthocyanin		16.6731 ug/ml	9.17
Flavone		14.5350 ug/ml	7.97
Catechin		0.0000 ppm	0
Flavonones		9.5471 ppm	5.25
Proanthocyanidin		10.3853 ug/ml	5.71

Cyanogenic glycoside		7.2091 ppm	3.96
Narigenin		15.2624 ug/ml	8.39

Discussion

Results of quantitative phytochemical screening of *Emilia praetermissa* ethanol leaf extract using Gas Chromatography Flame Ionization Detector (GC-FID) revealed the presence of the following compounds in different concentrations

1 AMMODENDRINE $C_{12}H_{20}N_2O$

This is a piperidine alkaloid,Ammodendrine has a role as a plant metabolite and as a teratogenic agent, a N- acylpiperidine and a member of acetamides[26].

2 SPARTEINE $C_{15}H_{26}N_2$

Sparteine is a lupin alkaloid, an antiarrhythmic agent and a sodium channel blocker. It is the predominant alkaloid in *Lupinus mutabilis* and is thought to chelate the bivalent cations of calcium and magnesium.it has been reported to reduced cardiac conductivity, stimulate uterine motility, circulatory collapse and respiratory arrest, World Health Organization have approved some drugs that contains sparteine like Abatacept, Acebutolol for the treatment of various illness[27].

3. PHYTATE $C_6H_{18}O_{24}P_6$

Phytate represents the major storage form of phosphorus and inositol in plants and has also been suggested as a storage of trace elements in plants. Phytate has antinutritional activity in human and monogastric animal diets through its strong chelation of Ca, Fe, and Zn. It has a positive nutritional role as an anti-oxidant, through the suppression of Fe-mediated. OH formation by Fe, which is complexed by phytate and acts as an anticancer agent [28].

4. HYDROXYLUPANINE $C_{15}H_{24}N_2O_2$

Hydroxylupanine is a bioactive alkaloid that acts as ganglionic blocker, it inhibits autonomic activity by interfering with neutrotransmission within autonomic ganglia. Hydroxylupaine reduces sympathetic outflow to the heart thereby decreasing cardiac output by decreasing heart rate and conductivity [29].

5. SAPOGENIN$C_{30}H_{50}O_5$

Sapogenins are lipophilic triterpene derivatives which, similarly to phenol protects plants against microbes, fungi and other hostile organisms.it has several health benefits that is, antioxidant, antitumor and antidiabetic effect [30]. Sapogenins are important mainly because of their steroid structure. They are precursors for hormones, like corticosteroids. According to findings, steroidal sapogenins are effective candidate for treating fungal and yeast infection in humans and animals. They are reported to be used in the treatment of some disorder like, cardiovascular, urological, respiratory, neurological and other disorder. They reduce the risk of heart disorder in humans who consume a diet rich in legume food [31].

6. TANNIN $C_{76}H_{52}O_{46}$

Medically tannins help prevent urinary tract infections in women by reducing the ability of *E.coli* bacteria from adhering to cells lining the urinary tract. Its anti-adhesive property may reduce the ability of H. pylori, to cause stomach ulcers. Because of its styptic and astringent properties, tannins have been used to treat tonsillitis, pharyngitis, haemorrhoids, and skin eruptions. It is used as an antidote for metallic, alkaloidal and glycosidic poisons, with which it forms insoluble precipitate [32].

7. CARDIAC GLYCOSIDE

Cardiac glycosidesare steroids having the ability to exert specific powerful action on the cardiac muscles. They are chemical compounds responsible for the treatment of congestive heart failure and help to slow down heartbeats that are fast. Cardiac glycoside inhibits the influx of Na^+ and outflow of K^+ ATPase enzyme in the heart, and consequently increase the flow of myocardial contraction. They also perform antitumor activity. It is used to treat patients with atrial fibrillation and atrial flutter [33].

8.EPIHEDRINE $C_{10}H_{15}NO$

Epihedrineis a central nervous system stimulant that is often used to prevent low blood pressure during anesthesia,They are used to treat mild hypertension and bradycardia associated with general or regional anasthetia. In patients with prostatic hypertrophy, epihedrine can produce urinary retention [34].

9. ANTHOCYANIN $C_{15}H_{11}O^{+}$

Anthocyanins are important classes of flavonoids that represent a large group of plant secondary metabolites. They play a role in reducing chronic and degenerative diseases. Anthocyanidin are responsible for the blue, purple, red and intermediate colours of many flowers, leaves, vegetables and fruits. It has been recorded to contain anticancer properties, show induce cell apoptosis thus eliminating damaged cells or tumour cells [35].

10. FLAVONES $C_{15}H_{10}O_2$

Flavones are classes of flavonoids thatexhibit inhibitory activity against various human viruses. It can act as a natural pesticide in plants, providing protection against insects and fungi diseases. Flavone helps to resume cardiac functional parameters after ischemia and was associated with lower oxidative injury [36].

11. CATECHIN $C_{15}H_{14}0_6$

Catechin belongs to a group of polyphenol present in green tea called flavonoid which ascribed a potent antioxidant activity, it prevent inflammation, reduced platelate clumping, help to reduce the risk of blood clots and heart attack. Its beneficial effect to human health is due to its antioxidant property [37,38].

12. FLAVONONES $C_{15}H_{12}O_2$

FLavonones is an aromatic flavonoid that often occur in plants as glycosides,
Flavonone is used as an antioxidant, cholesterol lowering and anti-carcinogenic agents. It can be is used as an anti-ulcer agent, antihypertensive and immune system modulator [49].They have beneficial effect as antihypertensive, lipid-reducing, insulin- sensitizing, anti-oxidant and anti-infammatoryproperties[39].

13. PROANTHOCYANIDIN $C_{31}H_{28}O_{12}$

They are chemical compound responsible for fruit and flower colour of many plants. They are group of compounds called polyphenol. They have strong antioxidant activity and many contribute to reduction in chronic diseases in human.They inhibit the development of pathogens, provide protection against UV radiation and oxidative stress. Proanthocyanidins contain antioxidative, vasodilatory, anticarcinogenic,antiallegic, anti-inflammatory, cardioprotective and estrongic activities [40].

14. NARINGENIN $C_{15}H_{12}O_5$

Naringeninis a chemical compound responsible for fruit and flowers of many plants, contributing to their red, blue or purple colours, they help to prevent cancer, they are in group of compounds called polyphenols. Naringenin has many beneficial effects on the human body such as aiding metabolism, acting as an antioxidant, anti-tumour, and anti-inflammatory, as well as preventing cardiovascular diseases, it has been observed in humans that the consumption of narigenin reduces the incidence of brain vascular diseases[41].

Conclusion

Phytochemical analysis of ethanol leaf extract of *Emilia pratermessa*revealed that it contain different alkaloids which include Ammodendrine, sparteine, hydroxylupanine and epihedrin, flavonoids present include phytate, tannins, anthocyanins, flavone, catechin, flavonones,

proanthocyanidin and narigenin. Other phytocompounds present include sapogenin, cardiac glycoside and cyanogenic glycoside in different concentrations. The use of this plant in treatment of wide range of illnesses can be attributed to the different phytocompound present in it thus; the use of the *Emilia pratermissa* in treatment of cardiovascular diseases and as anti-inflammatory agent is due to the presence ofcardiac glycoside, naringin, sparteine and catechin.The results of these study proved that the plant *Emilia praetermissa* leaf can be used in treatment of various illness which include inflammation, cough, rheumatism, fever, dysentery, wounds,tumour, heart related issue and preventing miscarriage as acclaimed by the local populaceethnomedicallymay be due to the presence of these secondary metabolites.

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