

## PRELIMINARY PHYTOCHEMICAL AND ANTIBACTERIAL ACTIVITY SCREENING OF THE LEAF EXTRACT OF *EMILIA PRATERMISSA*

### ABSTRACT

Fresh leaves of *Emilia pratermissa* were extracted using four different solvents ~~such as including~~ hexane, chloroform, methanol and distilled water. Phytochemical analysis of the leaf extracts showed the presence of ~~alkaloid~~ alkaloids, glycosides, flavonoids, steroids, saponins, and tannins in methanol extract, water extract showed these same phytochemicals except steroids, Hexane extract showed the same except saponin and glycoside while chloroform extract showed alkaloid, glycosides, and flavonoids. The antibacterial activity test of chloroform, methanol, and hexane extracts showed some zones of inhibition against ~~Escherichia~~ *Escherichia coli* and *Staphylococcus aureus*. Alkaloids ~~where were~~ further isolated from the leaves with Rf ~~value values~~ of 0.43, and 0.87 in methanol and  $\text{NH}_4\text{OH}$  (solvent). The infrared spectra (FTIR) for the alkaloid showed the presence of OH, N=N, C-O, N-H, C=O attached to the aromatic rings. Therefore, the presence of these phytochemicals justified the use of *Emilia pratermissa* as a medicinal plant.

*Keyword:* *Emilia pratermissa*, phytochemicals, antimicrobial, alkaloids

### INTRODUCTION

History recorded that earliest humans used various plants to treat illnesses without knowing the actual components<sup>[1]</sup>. Before the 20<sup>th</sup> century, crude and semi-pure extracts of plants, ~~were used to treat~~ microbes and animal ~~, were these plants were~~ the only medication available to treat human and domestic animal illnesses<sup>[2]</sup>.

As a product from nature medicinal plants exert all their curative and preventive power when used in combination with other health improving natural products like sun, water, fresh air, healthy food and mental balance. They should not be used only when looking for a curative action as we do in pharmaceutical products. One of their great virtues is precisely their ability to regulate life processes and prevent diseases<sup>[3]</sup>. Studies of medicinal values of some Nigeria plant have attracted so much attention from various professionals all over the world including Nigeria and this has led the federal government of Nigeria to adopt the national policy on traditional medicine in 1997<sup>[4]</sup>.

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. These plants do not just fight the invading organism in man's body but gives the body the ability to defend itself, their use as well as adoption of healthy life style must give better result than any synthetic drugs<sup>[5]</sup>. WHO consider medicinal plant as any plant which in any or some of its organs have some therapeutic property<sup>[6]</sup>. Among many plants and herbs used by local people in Nigeria for treatment of disease is the plant under study in this research *Emilia pratermissa*. This plant is used locally in management of diabetes, fever, convulsion in children, ulcer, worms, fibroid, syphilis, eye inflammation, diarrhoea, night blindness and sore throat etc<sup>[7,8]</sup>. The leaves are eaten occasionally as vegetable either fresh in salad or cooked with pulses such as peas and beans. It serves as fodder for rabbit and guinea pigs.

#### DESCRIPTION OF THE PLANT

This plant and other *Emilia* species has been misidentified. Unlike most *Emilia* species whose chromosome numbers are  $2n = 10$ , *Emilia pratermissa* has chromosome number  $2n = 20$ . It is allotetraploid specie while others like *Emilia coccinea*, and *Emilia sonchifolia* are both diploid. It is very similar to *Emilia fosbergii* but differ mainly by the brick - coloured capitula. *Emilia fosbergii* has been hypothesized as a hybrid sibling of *Emilia pratermissa* originated from the same parental specie *Emilia sonchifolia* and *Emilia coccinea*<sup>[9,10]</sup>. *Emilia pratermissa* is an annual herb, a dicotyledon, an ornamental from the family Asteraceae. Up to 140cm tall, stem is erect or ascending, simple or branched from base, flowering and fruiting all year round.

In this present study, phytochemical and anti-bacterial screening of the leaf extracts of *Emilia pratermissa* were carried out in a view to identify various chemical components that are responsible for the therapeutic properties of the plant. Therefore this research is aimed at helping pharmacologists, microbiologists, and general public to appreciate the potential of the plant and its high medicinal values as it has been used in the management of diabetes<sup>[7]</sup>.

## EXPERIMENTAL

### Identification, Extraction and Isolation of Samples.

The fresh leaves of *Emilia pratermissa* were collected from uncultivated farm land around Chemistry department, Nnamdi Azikiwe University Awka, Anambra State Nigeria. The plant was identified by Prof. J.C. Okafor of Tree, Crops and Tropical Ecology Centre Enugu, Nigeria.

### EXTRACTION

200g of ground fresh leaves of *Emilia pratermissa* were separately extracted with four different solvents hexane, chloroform, methanol and distilled water respectively. The concentrated extracts were used for both phytochemical and antibacterial activity test using standard procedures<sup>[9,12]</sup>.

### ALKALOID EXTRACTION

Fresh leaves of *Emilia pratermissa* 250g were ground and homogenized with a mixture of methanol and water (200:50) for 5 minutes. The mixture was filtered and concentrated. The concentrated filtrate was acidified with 2ml H<sub>2</sub>SO<sub>4</sub> and extracted subsequently with chloroform. The chloroform extract was dried by evaporation giving terpenoids and phenolics, while the aqueous acid layer was basified to pH of 10 with 10cm<sup>3</sup> NH<sub>4</sub>OH, extracted with chloroform - methanol in the ratio of 3:1, dried to give basic extract (mostly alkaloid). Thin layer chromatography of this extract was carried out using methanol and NH<sub>4</sub>OH as eluting solvent in the ratio of 200:3<sup>[2]</sup>.

### ANTIBACTERIAL ACTIVITY

This was carried out in Microbiology Department Nnamdi Azikiwe University Awka. The crude leaf extracts were screened for antibacterial activity using disc diffusion method<sup>[13,14]</sup>.

### PREPARATION OF MEDIUM

**Comment [tb1]:** Please add the water and hexane extraction method

28g of nutrient agar was weighed and dissolved in 1000ml of water in a conical flask. The solution was sterilized at 121°C for 15 minutes in an autoclave, allowed to cool to 45°C. Then 20cm<sup>3</sup> was dispensed in each petri dish and was allowed to gel.

**Comment [tb2]:** If you used Mueller Hinton Agar which is more specific

### TEST ORGANISM

The test organisms were *Escherichia coli* and *Staphylococcus aureus* (pure culture) from Microbiology Department Nnamdi Azikiwe University Awka.

### PREPARATION OF DISC:

Perforated Whatmann No 1 filter paper was sterilized at 180°C for 1 hour.

### PROCEDURE

The test organisms were streaked on the gelled plates, each organism was streaked on different plates. Sterilized filter paper discs were soaked in the extracts for 24 hours, placed on the plates inoculated with the test organisms and incubated for 24-48 hours at 37°C. The plates were examined for clear zones of activity and the zones of inhibition were measured using a plastic clear ruler in mm.

### RESULTS AND DISCUSSIONS

The result of the hexane extract indicated the presence of alkaloids, steroids, tannin and flavonoids but absence of saponin and glycosides. Chloroform extract showed the presence of alkaloids, glycosides and flavonoids but absence of steroids, tannins and saponins. Methanol extracts indicated the presence of alkaloids, saponins, tannins, steroids, glycoside and flavonoids.

**TABLE 1**

#### RESULTS OF PHYTOCHEMICAL ANALYSIS OF THE FOUR SOLVENT EXTRACTS.

Plant Extracts	Hexane	Chloroform	Methanol	Distilled water
Alkaloids	+	-	+	+
Steroids	+	-	+	-

Saponins	-	-	+	+
Tannins	+	-	+	+
Glycosides	-	+	+	+
Flavonoids	+	+	+	+

Key: + (present), - (absent)

The results showed that alkaloids and flavonoids were present in all the four extracts and that the best solvent for the extraction was methanol. The presence of saponins and glycosides in water and methanol extracts could be due to higher polarity of these solvents for the sugar moiety and hydrogen bonding. The phytochemical screening showed that the leaves were rich in alkaloids, flavonoids, tannins, steroids and glycosides which has been known to show medicinal activity as well as exhibiting physiological activities<sup>[14]</sup>. It has been shown that *Emilia coccinea* and *Emilia sonchifolia* also contain steroid<sup>[3,15]</sup>.

The presence of alkaloids in the four extracts were reported to inhibit the growth of *Staphylococcus aureus*. Alkaloids are used in medicine for treatment of malaria, diabetes and motion sickness. It can be used also as anaesthetic agent and can be used in treating hypertension, snakebite, mental illness and also as a tranquilizer as in indole alkaloid<sup>[2]</sup>. Extract containing flavonoids has been used as diuretics and antibacterial activity. They are anti-oxidant, anti-allergic, anti-inflammatory, anti-cancer and also prevent cardiovascular diseases<sup>[3,16]</sup>. Saponin can be anti-carcinogenic, cholesterol decreasing, anti-inflammatory and as insect poison while extracts containing tannins cause astringent taste of wine and chocolates. They act as barrier to herbivores, anti-inflammatory and can control gastritis and irritating bowel disorders, it ensure inhibition of organism by coagulating their micro protoplasm. Steroids can reduce the risk of coronary heart diseases, cancer and high blood pressure<sup>[16]</sup>. These ingredients are the starting materials in the synthesis or production of new drugs today.

**Comment [tb3]:** Why you not listed the concentration of each chemical material that you found in each extract solution this will give more indication to your manuscript and shown how each chemical and extract solution effect bacterial

#### Antibacterial Screening of Different Leaf Extracts of *Emilia Pratermissa*

Antibacterial Screening of different leaf extracts of *Emilia pratermissa* showed that methanol extract has inhibition zone of 5mm against *E. coli* and 1mm against *S.aureus* while chloroform extract has no zone of inhibition against *E. coli* and 1 mm against *S. aureus*. Methanol and chloroform extracts were very active on *E. coli*. These results supported the fact that *Emilia pratermissa* is used traditionally in the cure of diarrhea<sup>[7]</sup>. The *S.aureus* inhibition was only 1mm on both methanol and hexane extracts but not in chloroform extracts.

Thin Layer Chromatography of the alkaloid content of the leaf using MeOH/NH<sub>4</sub>OH as eluting solvent showed two spots. The lower spot is a light brown liquid with R<sub>f</sub> of 0.43 with most prominent UV(λ max) nm peak of 218. The presence of vibrational peaks in the functional group region of (FTIR) spectra of *Emilia pratermissa* alkaloid extract was a clear indication that some particular functional groups were present in the extracted samples. The IR (Vmax) cm<sup>-1</sup> vibration peak at 1619.29 showed the presence of C=C, N-H bend and N=N of Azo groups, amine, amides and alkenes. The upper spot is also a light brown liquid with R<sub>f</sub> of 0.87 with most prominent UV (λ max) nm peak of 223.00

Its IR (Vmax)cm<sup>-1</sup> absorption at 1622.19 showed the presence of N=N,N-H, and C=C for alkenes, amines and Azo group while absorption at 3019.66 showed the presence of N-H and OH stretch of alkanols and amines. These alkaloids can be responsible for the efficacy of the plant in the management of diabetes.

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