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Regular Article

# Evaluation of phytochemical contents of *Emilia coccinea* leaves

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**Abstract**
*Emilia coccinea* is widely used in folkloric medicine for eye and ear ailments as well as for fever. This present study evaluated the preliminary and quantitative phytochemical properties of *E. coccinea* leaves using standard procedures. The results revealed the following bioactive compounds Flavonoids (0.90 ± 0.02), Alkaloids (0.94 ± 0.03), Tannins (10.36 ± 0.02), Saponins (2.34 ± 0.02), Oxalate (1.62 ± 0.01), Phenols (0.89 ± 0.02), Terpenoids (0.11 ± 0.01). The high concentration of tannin and moderate concentration of other phytochemical proved that *E. coccinea* can serve as a vital medicinal plant that could be used for pharmaceutical formulations.

**Key words:**
*Emilia coccinea*, phytochemical, medicinal plant, metabolites

## Introduction

The usage of plants in the medical systems is of great significance in almost all countries and the medicinal plants became part of many modern medicines. There are many phytochemicals and metabolites isolated from plants including steroids, terpenoids, carotenoids, flavonoids, alkaloids, tannins and cardiac glycosides (Ajibesin, 2011).

The usage of some of the secondary metaoblites from plants are not well established due to the lack of knowledge and techniques. There are many antibiotic principles in plants like anthraquinones, alkaloids etc. Now a days, the usage of medicinal plants in allopathy, herbal and many other medical systems are very common. Most of the medicinal plants are used as spices and food plants as well (Akinpelu and Onakoya, 2006). These medicinal systems are popular especially in the developing countries due to ease of obtaining and also cheaper comparing to synthetic medicine (Michael, 2002). These reasons might account for their worldwide attention and use. The medicinal properties of plants have been documented by some researchers (Michael, 2002; Akinpelu and Onakoya, 2006; Ajibesin, 2011).

*Emilia coccinea*, being a folkloric medicine is traditionally used as remedy for eye and ear ailments, for fever, convulsion in children, ulcer, craw-craw ringworm (Edeoga et al., 2005), rashes, measles and other forms of inflammatory diseases (Sofawara, 1996). The aim of this work is to screen for the bioactive and pharmacological potential of *Emilia coccinea* leaf.

## Materials and method

### Collection and identification of *Emilia coccinea* leaf

*Emilia coccinea* was collected in the month of September, 2016 from bushes around and within FUTO, Ihiagwa in Imo State, Nigeria, during

rainy season when weeds were in their maximum densities, and was identified by Botanist (Mr.) Duru C.M. of FUTO.

**Sample preparation and extraction**

The sample preparation and extraction of sample using maceration method were done by following the method described by Farooq (2013).

**Phytochemical screening of *Emilia coccinea* leaves**

**Mayer's test for alkaloid**

1ml of Iodine and 1ml of potassium Iodide was used to form Mayer's reagent by mixing in a test tube. Few drops of the methanolic extract were added to the mixture in the test tube and was shaken. A reddish-brown precipitate was formed. The same procedure was repeated for the aqueous extract, and a reddish-brown precipitate also, was formed.

**Flavonoid test**

Few drops of the reagent ($H_2SO_4$) were added to a small amount of the mathanolic extract. Immediate development of orange color indicated the presence of flavonoid in the methanolic extract. The same was done for the aqueous extract and an orange color was also gotten.

**Saponin test**

About 0.5ml of the extract was shaken with 5ml of distilled water.

**Test for steroid**

5ml of the extract (Methanolic) was added with 2ml of acetic anhydride, each with 2ml of $H_2SO_4$. The color was changed from violet to green. Same was done and gotten for the aqueous extract.

**Test for terpenoid**

5ml of the extract (Methanolic) was mixed with 2ml of chloroform and 3ml concentrated $H_2SO_4$. A layer was formed, and a reddish-brown color appeared in the inner face, indicating the presence of terpenoid. Same was done and gotten for the aqueous extract.

**Test for tannins (ferric chloride test)**

0.5ml of each extract was dissolved in 10ml of distilled water, and then filtered.

Two drops of Ferric chloride solution were added to the filtrates, and a blue-black precipitate was formed, indicating the presence of tannins.

**Test for phenol**

0.5g of the extracts (Methanolic and aqueous) were dissolved in 5ml of distilled water. Few drops of neutral 5% ferric chloride solution were added. A dark green color was formed, indicating the presence of phenolic compounds.

**Quantitative test**

**Flavonoid determination**

2g of sample was weighed into a beaker. 100ml of 2M HCl solution was added and was boiled for 30 minutes. It was then cooled and filtered. Titrate was treated with drop wise solution of 5ml ethyl acetate. Ethyl acetate layer containing flavonoid was collected and the aqueous layer was discarded. A pre-weighed filter paper was used to filter the ethyl acetate extract, which was then dried in an oven at $100^0$C for 30 minutes. It was cooled in the dessicator, and was re-weighed, and calculated using the formula

$$\% \text{ flavonoid} = \frac{W2-W1}{Wt\ of\ sample} \times \frac{100}{1}$$

**Alkaloids determination**

2g of the sample was weighed. It was then soaked in 20mls of 10% Ethanolic acetic acid. It was allowed to stand for 4 hours at room temperature. The concentrated filtrate was filtered. It was then extracted by evaporation over a steam bath to $^1/_4$ its original volume. Concentrated ammonia hydroxide was added in drops until in excess to extract alkaloid precipitate. Alkaloid precipitate was recovered by filtration, through a pre-weighed filter paper. The alkaloid precipitate was then washed with1% ammonia solution and was dried in the oven at $60^0$C for 30minutes. It was cooled in a dessicator and was re-weighed. The % Alkaloid was calculated using the formula;

$$\% \text{ Alkaloids} = \frac{W2-W1}{Weight\ of\ sample} \times \frac{100}{1}$$

**Saponin determination**

2g of the sample was weighed. 50mls of 20% aqueous ethanol solution was added. The solution was heated with periodic agitation for 90min $55^0$ C in a water bath. It was then filtered, and the residue was extracted with 50mls of 20% ethanol. Both extracts were re-pooled together and the combined extract was reduced to 40mls

at $90^0$C. It was then transferred to a separating funnel and 40mls of diethyl was added and it was shaken vigorously. It was then separated by partition. Re-extraction by partition was done repeatedly until the aqueous layer was cleared. Saponin was then extracted with 60ml of N-butanol. Extracts were washed with 10ml 5% aqueous NaCl solution. It was evaporated to dryness in a pre-weighed evaporating dish after retaining upper part. It was then dried in the oven at $60^0$C, and was re-weighed. % saponin was calculated thus;

$$\% \text{ saponin} = \frac{W2-W1}{Weight\ of\ sample} \times \frac{100}{1}$$

**Tannin determination**

1g of the sample was weighed into a beaker. 25ml of distilled water was added. It was shaken and was allowed to stand for 30minutes, but with 10minutes interval shake. It was then filtered into a clean beaker. 5ml of the filtrate was taken, and 1ml of $FeCl_3$ and potassium farrocyanide was added. It was made up to 50ml with distilled water. Absorbance was read at a wave length of 760nm, and % tannin was calculated thus;

$$\% \text{ Tannin} = \frac{100}{w} \times \frac{Au}{As} \times \frac{C}{1000} \times \frac{Vf}{Va} \times D$$

**Phenol determination**

0.2 of sample was dispensed into a test tube. 10ml of methanol was added, and it was shaken thoroughly. The mixture was allowed to stand for 5minutes and was filtered. 1ml of the filtrate was measured out into a test tube. 1ml of follins reagent, and 5ml distilled water was added. The color was allowed to develop for about 3 to 4 hours at room temperature. The absorbance was then taken at 760nm, and % phenol was calculated thus;

$$\% \text{ phenol} = \frac{100}{w} \times \frac{Au}{As} \times \frac{C}{1000} \times \frac{Vf}{Va} \times D$$

**Oxalates determination**

2g of sample flour was suspended in 190ml of $H_2O$ in a 250ml volumetric flask. 10ml of 6M HCL was then added The mixture was heated at $100^0$C for 1hour. It was cooled and was made up to 250ml, and was after which Filtered. Duplicate portion of 125ml of the filtrate were measured into beakers. 4 drops of methyl red indicator was added. Conc. $NH_4OH$ was added in drops until test solution changed from pink color to a faint yellow. Each portion was heated to $90^0$C. They were cooled and then, filtered. The filtrate was heated at $90^0$C and 10ml of 5%$CaCl_2$ was added and was constantly stirred. It was cooled and was left over night at $50^0$C. It was then centrifuged at 25000rpm for 5minutes. The supernatant was decanted and was dissolved in 100ml of 20% $H_2SO_4$. The filtrate resulting from digestion of 2g of flour was made up to 300ml. Aliquot of 125ml was heated until near boiling. It was then titrated against 0.05M $KMnO_4$ solution to a faint pink color which persisted for 30secs, and was calculated using the formular;

$$\frac{Tx\,(Vms)(Df)\times}{(ME)\times Mf}\ 105(\text{mg}/100\text{g})$$

**Terpenoid test**

2g of the plant leaf powder was weighed and soaked in 50ml of 95% ethanol for 24hours. The extract was then filtered, and the filtrate was extracted with petroleum ether at $60^0$C/ It was then concentrated to dryness, and the dried ether extract was treated as total terpenoids. Thus;

$$\% \text{ Terpenoid} = \frac{W2-W1}{Weight\ of\ sample} \times \frac{100}{1}$$

**Result and discussion**

The results of the phytochemical screening of the extracts revealed that flavonoids, alkaloids, terpenoids, saponins, steroid, steroid, phenol. Terpenoids, were present in the extracts from methanol and water as shown in table 1 below.

Table 1. Qualitative phytochemical constituent of the extracts.

| Constituents | Extracts | |
|---|---|---|
| | Aqueous | Methanol |
| Flavonoids | + | + |
| Alkaloids | + | + |
| Tannins | ++ | + |
| Saponins | + | - |
| Steroids | + | + |
| Phenols | + | + |
| Terpenoids | + | + |

+: Indicate presence of phytochemicals.

Table 2. Quantitative determination of phytochemical constituents of *E. coccinea* leaf.

| Sample | Constituents | Value (Mg/100g) |
|---|---|---|
| | Flavonoids | 0.90 ± 0.02 |
| | Alkaloids | 0.94 ± 0.03 |
| | Tanninss | 10.36 ± 0.02 |
| | Saponins | 2.34 ± 0.02 |
| | Oxalate | 1.62 ± 0.01 |
| | Phenol | 0.89 ± 0.02 |
| | Terpenoids | 0.11 ± 0.01 |

Values are mean ± standard deviation of triplicate determination.

The present study carried out on *Emilia coccinea* revealed the presence of medicinal active constituents. The phytochemical compounds of *Emilia coccinea* analyzed are presented in Table 1 and 2 above.

From this study, the preliminary phytochemical content of *E. coccinea* leaf reviewed the presence of alkaloids, flavonoids, tannins, saponnins, phenols, cardiac glycoside, terpenoids, steriods. The quantitative phytochemical contents were found to be, Flavonoid 0.90 ± 1.64 mg/100g, Alkaloid 0.94 ± 1.77 mg/100g, Tannins was relatively high in the *E. coccinea* leaf, with 10.36 ± 214.45 mg/100g, Sapnninss 2.34 ± 10.99 mg/100g, Cardiac glycoside 0.27 ± 0.15 mg/100g, oxalate 1.62 ± 5.25 mg/100g, Phenol 0.89 ± 1.57 mg/100, and Terpenoids 0.11 ± 0.02 mg/100.

There is significant action of phytochemicals in treating various diseases (Kilani, 2006; Kilonde et al., 2004). Steroids, are important part of modern medicine as their relationship with such compounds as sex hormones (Benjamin, 2005). Steroids compounds are good for breast feeding and expectant mothers, as they could serve as potent starting material in synthesis of hormones (Benjamin, 2005).

Phenolic and flavonoids are major phytochemicals with significant role in medicine (Azaizeh et al., 2003). They have many significant actions likes antiapoptosis, anti-aging, anti-carcinogenesis, etc. (Falodun et al., 2006; El-Mahmood et al., 2010).

## Conclusion

The extract of *E. coccinea* leaf showed significant phytochemical constituents, which supports the traditional use of this plant in various diseases and/or ailments amelioration.

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