Journal of Forestry Research and Management. Vol. 18(1).107-113; 2021, ISSN 0189-8418
www.jfrm.org.ng

PHYTOCHEMICAL, PROXIMATE ANALYSIS, TOXICITY STUDY AND PARTIAL FRACTIONATION OF THE AERIAL PART OF YELLOW TASSEL FLOWER (*Emila coccinea*(Sims) G. Don) EXTRACT

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ABSTRACT

Emilia coccinea is used in folkloric medicine in the treatment of ulcers, ringworm, gonorrhoea and measles, while its anti-diarrhoeal and antimicrobial activities have been evaluated. The phytochemical content, proximate parameters and study profile of the aerial parts of the plant are yet to be determine. Thus, the aim of this study was to determine the phytochemical constituents, proximate analysis and toxicity activities of the aerial parts of *E. coccinea* and fractionation of the methanol extract. The aerial parts was collected, identified, extracted with methanol (99.8 %) and concentrated *invacuo* using rotary evaporator at 60 ^{o}C. The phytochemical screening and proximate analysis was done using standard methods.Fractionation of the crude extract was done in n-hexane, dichloromethane and ethylacetate, while acute and chronic toxicity testing were done using Swiss albino mice. Student t-test was used to check the level of significant ($P<0.05$) of the weight of the mice.Some of the results were presented in mean ± standard deviation. Saponins, tannins, alkaloids and flavonoids were observed. Moisture content (10.50 ± 0.50%), total ash value (8.00 ± 1.75%), crude fibre content (10.75 ± 2.04%), fat content (4.50 ± 0.54%) and crude protein content (5.60 ± 0.67%) were obtained. n-hexane fractionation had the highest value of 5.34g. Oral administration showed no treatment-related abnormalities and mortality in the mice up to the dose of 8000mg/kg when compared to negative controls and there was no significant change in the weight of the mice. The aerial part of *E.coccinea*is rich in phytochemicals that aresafe base on the acute toxicity profile.

Keywords: Aerial *Emilia coccinea*, phytochemicals, proximate analysis, acute toxicity.

Introduction

Herb is an ancient form of medication known to man and used by many traditions throughout history (Petrovska, 2012). It is an important part of contemporary advancement and healthcare, thus many of the commonly used drugs today have herbal origin (Mosihuzzaman, 2012). Indiscriminate and irrational collection of herbs could lead to misidentification and adulteration of the actual herbs and change the standardisation of the herbal drug. It is an important fact that most herbs are not standardized and usually contain many ingredients, hence the need for standardization of herbal product, this will enable proper dosing during administration (Fokunang *et al*.,2011).

Another key drawback in the use of herbs is the lack of important clinical and scientific information in support of proper understanding of the efficacy and safety of the phytochemicals in these herbs. This is mainly due to the neglect of the determination of the toxicity profile of herbal preparations, as they are mistakenly considered natural and safe. Many extracts from plant could be inherently unsafe, due to the presence of toxins, that may be cytotoxic or carcinogenic (Humphrey and

McKenna, 1997; Seth and Sharma, 2004). It is thus appropriate to conduct a toxicity study in order to ensure safety in the preparation of these herbs.

Acute toxicity study evaluates the untoward effects of a substance that result either from a single dose or from repeated doses within a short time (less than 24 hours). The untoward effects should be noted within 14 days of administration of the substance (IUPAC, 1997). The unethical nature of using human subjects may limit the information from such a study, however data from animal models can be extrapolated in human to determine comparative probable danger and assist in determining the dose level for effective treatment (Sofowora, 1993; Van Norman, 2019).

Emilia coccinea (Sims) G. Don (EC)of family Asteraceae is an annual, erect bushy herb that is known as yellow tassel flower with maximum height of about 1.20m. Found mostly in waste and fallow land, it is well distributed in the plain of Central and Eastern Africa in dry area of almost 2000m altitude. Over 50 species have been identified in Africa from this genus (Bosch, 2004). Different parts of EChave been used for the treatment of fever, epilepsy, seizures in children (Agoha *et al*., 1981), sore, sinusitis, ulcer, ringworm, jaundice, hernia, syphilis (Burkill, 1985), measles and cough (Edeoga *et al*., 2005). Some of these claims have been evaluated pharmacologically, they include antioxidant, anti-inflammatory, antidiarrheal (Okei *et al*., 2009), antibacterial (Ogbebor *et al*., 2005), anti-fungi (Edeoga *et al*., 2005), anti-ovulatory and estrogenic properties (Elvis-Offiah *et al*., 2016), antidiabetic and antidepressant activities (Foyet *et al*., 2014).These effects are due to the presence of phytochemicals in ECwhich include flavonoid, phenolic compounds (Okei *et al*., 2009), alkaloids, terpenoids, tannins and steroids (Viola *et al*., 2017).

The aqueous and methanol extract of ECleaves have been evaluated for the phytochemical contents (Obinna *et al*., 2017; Idu *et al*., 2010), while the acute toxicity study have also been previously studied but there is no record of the aerial part of EC. This study intends to provide some of the standardizing parameter (proximate) for the aerial parts of EC. Thus, the aim of this study was to determine the phytochemical constituents, proximate parameter and evaluate the toxicity activity of the aerial part of EC.

Materials and Methods

Collection and preparation of plant materials

Fresh aerial samples of *Emilia coccinea* were collected in September, between 9-11 a.m within the locality of the University of Benin, Benin City, Edo State, Nigeria. It was authenticated and identified at Faculty of Life Science herbarium, University of Benin, Benin City, Nigeria. The herbarium sample with voucher numberUBHE363was prepared and deposited for future references.

The aerial part of *Emilia coccinea* was carefully selected excluding sand from the root, air dried devoid from direct sunlight for 3 weeks (21 days) at ambient temperature. It was crunched to powder by the use of electrical milling machine and then stored in an airtight container until further work was done.

Extraction and Fractionation

To 500 g of the aerial part of *Emilia coccinea* powder was added 800 ml of methanol in an extractive jar, this was allowed to stand for 72 hours after initial shaking to allow for proper mixing. This was then passed through filter paper and the filtrate was concentrated *invacuo* using a

rotary evaporator at 60 ^{o}C, the weight was noted and the crude extract was kept in the refrigerator at a temperature of 4 ^{o}C until when used.

Twenty grams (20 g) of the crude extract was dissolved in 50 ml of methanol and 200 ml of distilled water was then added. The mixture was properly stirred and poured into the separating funnel, before 100 ml of n-hexane was added to mixture in the funnel and its content was swirled and allowed to stand for 20 minutes, then decanted into a separate container. Another 100 ml n-hexane was added to the content in the separating funnel and the process of swirling and decanting was repeated until the n-hexane fraction that is collected appears colourless. The collected n-hexane fractions were put together and concentrated *invacuo* using rotary evaporator at 60 ^{o}C. This procedure was repeated for dichloromethane and ethylacetate fractions, these fractions were then stored in the refrigerator at 4^{o}C until used.

Phytochemical Screening and Proximate Analysis

The phytochemical screening of the aerial parts of *Emilia coccinea* was evaluated using already established methods by Sofowora1992 and Trease and Evans, 2002. This involves test for alkaloids, tannins, saponins, flavonoids, anthraquinone glycosides and cardiac glycosides.

The proximate analysis was determined according to the methods described in monograph of Association of Official Analytical Chemists(AOAC, 2010). This analysis includes moisture content determination, total ash content, crude fibre content, protein and fat content.

Acute Toxicity Profile of the Methanol Extract of *Emilia coccinea*

Adult Swiss albino mice (both sexes)aged 3-4 months, weighing between 20-33 g were acquired from the Department of Pharmacology and Toxicology Animal House Faculty of Pharmacy, University of Benin, Benin City, Nigeria., They were feed with standard rodent pellet and water as approved by Faculty of Pharmacy Ethnical Committee and the mice were grouped into five groups of five mice for each test, the crude extract was administered orally at doses of 1, 2, 4, 6 and 8 g/kg to group II to VI by means of orogastric tubes attached to a syringe while the control group (I) received 2ml of distilled water. The animals were observed for symptoms of toxicity and mortality within 24 hours for autonomic, behavioural and neurological symptoms or death. These symptoms include change in appearance of faeces, movement, response to noise, and tail action. Four hours after observation the animals were given freedom to food and water and those that survived after 24 hours were observed for 2 weeks for signs of toxicity (Locke, 1983).

Statistical Analysis

Some of the results were presented in Mean±SEM (standard error of mean) and student's t-test was used to determine the level of significance between the mean weights of the mice. The version of the student's test used was SPSS 23 computer software package and level of significant was placed at $P<0.05$.

Results and Discussions

It was observed in table 1that the 500 g powdered EC yielded 30.48 g of the crude extract following it extraction with methanol. Methanol though classified as a polar solvent have a lipophilic and hydrophilic part in its molecular structure, thus it is able to extract both polar and non-polar compounds present in the powder of EC. N- hexane fraction had the highest value of 5.34gwhen compared to other fractions. The use of n-hexane solvent in partitioning the crude extract, enable the fractionation of the non-polar compounds into the n-hexane solvent, while the semi-

polar constituents are retained in the dichloromethane fraction and the polar fractions are held in the ethylacetate fraction. The process of fractionation has the advantage of partially purifying or grouping the chemical constituents of EC and separating the phytochemicals into different solvents base on their polarity.

Table1: Weight of crude extract and fractions of the powdered aerial part of EC

Extract/ Fraction	Weight (g)
Methanol	30.48
n-Hexane	5.34
Dichloromethane	2.30
Ethylacetate	1.90

The result in Table 2 showed the presence of phytochemicals which include alkaloids, steroids, triterpenoid, flavonoids, saponins, tannins and cardiac glycosides. These phytochemicals are distributed into the various solvents differently base on their general chemical formular or properties. Steroids are likely to be seen in the n-hexane fraction while flavonoids in ethylacetate fraction respectively (Snehlata *et al*., 2018). The presences of these phytochemicals in EC could be responsible for its numerous ethno-medicinal uses which include abdominal pains, gastritis and gonococcal infection (Burkill, 1985). The presence of flavonoids in the powdered of EC has been previously confirmed in its leaves (Okei*etal*., 2009; Edeoga *et al*., 2015; Mensah *et al*., 2013). These could be responsible for the antioxidant and neuro protective effect that have been observed with EC (Foyet*etal*., 2014).Flavonoids and alkaloids act in plant as a metabolic defence mechanism. Many pharmacological activities have been ascribed to the presence of phytochemicals in the plant (Ogbebor and Adekunle, 2005; Teke *et al*., 2007; Okiei *et al*., 2009). These phytochemicals have been used as a lead in drug discovery, production of pesticides (phytotoxins), as food additives (colour, flavours and sweeteners), fragrances, and even as precursors for the synthesis of plastics (Breitling *et al*., 2013).The use of EC as herb, makes it necessary for the proximate parameter to be evaluated, in order to determine the moisture, material and anti-toxin contents and level of fat and protein in the powder.

Table 2: Phytochemical screening of the powdered aerial parts of *Emiliacoccinea*

Phytochemical	Inference
Alkaloids	+
Flavonoids	+
Saponins	+
Cardiac glycosides	+
Anthraquinone glycosides	–
Tannins	+
Steroid	+
Triterpinods	+

Present (+)/Absent (-)

Result presented in Table 3 showed a high moisture value of (10.50±0.5) %, implying high water content in aerial parts of EC. Thus, the powdered sample could be prone to microbial degradation. The total ash value of (8.00±1.75) % is an indication of high level of macro nutrient in EC. A crude fibre content of (16.75±2.04) % showed

high level of cellulose, hemicellulose and lignin. These have been reported to aid the removal of toxin from the body (Viola *et al*., 2017). The fat and protein contents for the aerial part of EC were reported to be (4.50±0.54) % and (5.60±0.67) %, indicating low level of these nutrients and thus EC is not a good source for fat and protein (Omoregie and Osagie, 2011; Abulude *et al*., 2006).

Table 3: Proximate analysis of the powdered aerial parts of EC

Proximate parameter	Values (Mean±SD)%
Moisture content	10.50 ± 0.5
Total ash	8.00 ± 1.75
Crude fibre	16.75 ± 2.04
Crude protein	5.60 ± 0.67
Fat content	4.50 ± 0.54

Analysis was done in triplicate (n=3)

Herbal preparations are believed to be safe and thought to be free from side effects, thus there is need to evaluate the toxicity in mice by oral administration since EC extract are taken by this route. Result presented in Table 4 showed no visible signs of toxicity and mortalityin the mice after 24 hours and 14 days of administering 8 g/kg body weight of the crude extract of EC. Thus implying that the extract lethal dose 50 (LD50) of EC is above 8 g/kg and thus it is rationally safe at this dose following oral administration within 24 hours and 14 days of study. Idu and co-wokers, (2010) have reported zero death for the leaves of ECat similar doses. Initial toxicity study is an integral part of drug development, necessary to produce relevant data of likely harm a plant material or herb may possess in human (Elvis-Offiah *et al*., 2016).The statistical analysis showed no significant difference in the weight of the mice post administration, implying that the mice maintained normal appetite during the study period (Idu *et al*., 2010).

Table 4: Acute toxicity of study in mice of the aerial parts of EC.

Group	Dose (mg/kg)	Weight on day 1 (Mean ±SEM)	Weight on day 14 (Mean ±SEM)	Percentage mortality
I	Control	21.30±0.40	23.75±0.57	0
II	1000	23.50±0.65	25.75±0.48	0
III	2000	26.50±0.60	28.75±0.05	0
IV	4000	23.50±0.55	25.75±0.80	0
V	6000	22.50±0.65	24.75±0.85	0
VI	8000	23.50±0.56	23.95±0.86	0

Conclusion and recommendation

The aerial part of EC contain phytochemicals that could be responsible for the folkloric claims. The toxicity study showed the aerial part of EC to be orally safe when administered within two weeks in mice while the proximate parameters show the need for proper storage of the powdered sample. However, there is need for long-term determination of the toxicity profile of the aerial part of EC and monitoring of the effect it will have on the internal organs.

Acknowledgement

We wish to appreciate the effort of the members of the laboratory staff of the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin,

Benin City, Nigeria, for providing the technical assistance during this study.

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