Preliminary Evaluation of the Anti-Emetic Activity of Crude Methanol Extract and Fractions of Ocimum gratissimum

Chibueze Peter Ihekwereme1*, Chukwusom Maureen Aniezue1, Earnest O Erhirhie2,3 and Uche Gabriel Okafor2

1Department of Pharmacology and Toxicology, Nnamdi Azikiwe University, Awka, Nigeria
2Department of Pharmaceutical and Medicinal Chemistry, Nnamdi Azikiwe University, Awka, Nigeria
3Department of Pharmacology and Therapeutics, Delta State University, Abraka, Delta State, Nigeria

Abstract

Background: There is a need for the development of safer, anti-emetic agents effective in several conditions such as in cancer chemotherapy where vomiting is a worrisome feature.

Aim: The present study was carried out to evaluate the anti-emetic potential of crude methanol leaf extract and fractions of Ocimum gratissimum.

Method: The anti-emetic activities of the fractions were carried out following the chick emetic model. Test samples and the negative control were administered at a single oral dose of 150 mg/kg to the respective groups (n = 5). Tween 80 (5%, 10 mL/kg) and chlorpromazine (i.p) served as negative and positive controls, respectively. The number of retches each animal produced was counted for 20 minutes and recorded. Anti-emetic activity was determined by calculating percentage reduction in number of retches relative to negative control.

Results: The anti-emetic activity decreased in the following order; Chlorpromazine (98.76%) > butanol fraction (92.16%) > aqueous fraction (86.80%) > crude methanol fraction (65.15%) > N-hexane (63.09%) > Ethyl-acetate fraction (5.98%). Butanol fraction elicited the highest activity among the tested fractions, while ethyl-acetate fraction produced the least activity.

Conclusion: This study showed that the butanol extract has better anti-emetic properties than other fractions. Supplementary studies are required to isolate the active principles in butanol fraction of O. gratissimum responsible for the anti-emetic activity and also elucidate its mechanism of action.

Keywords: Anti-emetic, Ocimum gratissimum fractions, chick emetic model, Chlorpromazine.

Introduction

There is a need for the development of safer, anti-emetic agents effective in several conditions where vomiting is a worrisome feature. Even though the arrest of vomiting is not desired in several disease conditions, effective anti-emesis improves the health outcome in some other health conditions such as pregnancy and cancer chemotherapy [1]. Post-coital contraceptive hormones are often administered with other health conditions such as pregnancy and cancer chemotherapy [1]. Although an earlier study has described the anti-emetic property of Ocimum gratissimum, commonly called “Sweat Basil” or “Scent leaf”, belongs to the family of Labiaceae. It is an annual herbaceous plant popularly consumed as a vegetable in Nigeria and the entire West Africa sub-region. The plant has demonstrated a number of pharmacological properties, which include the following viz; antifungal [3], antimalarial [4], antidiarrheal [5], anti-inflammatory [6], genotoxic [7], and hypoglycemic [8] activities.

Materials and Methods

Plant material

Fresh leaves of O. gratissimum were collected from Awka in the...
month of February, and were authenticated by the Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, and a voucher specimen was deposited at the herbarium for future reference.

Animals

Young chicks of either sex (aged 11-14 days), weighing from 87-110 g were obtained from a local poultry store and kept in the animal house of the Department of Pharmacology and Toxicology, Nnamdi Azikiwe University. All chicks were kept under laboratory conditions at room temperature with 12 h light and dark cycles. They were fed with standard pellet diet and had unrestricted access to water. This research was approved by the committee on animal research and publication ethics of the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, and Awka, Nigeria.

Method

Extraction and fractionation of plant material: Fresh leaves of *O. gratissimum* were dried at ambient temperature (32°C) under a shade for 14 days and pulverized. The powdered leaves were macerated in methanol for 3 days at room temperature. Filtrate from the macerate was evaporated to dryness using a water bath at 40°C. The resulting crude extract was refrigerated prior to use. Some crude methanol leaf extract were mixed with silica gel and ground into a homogenous mixture using mortar and pestle. A solid-liquid fractionation process was carried out. The mixture was fractionated with n-hexane, water, butanol, and ethyl acetate. For each solvent, the filtrate obtained was concentrated to a constant weight using a water bath at 40°C.

Determination of Anti-emetic Activity: The anti-emetic activities of the crude and fractions were carried out following the chick emetic model [10]. The chicks were randomly divided into groups (n = 5) and used for the study. The methanol crude extract and each of the 4 fractions (aqueous, ethyl acetate, butanol, and n-hexane) were administered at a single dose of 150 mg/kg p.o. to the respective groups. Tween 80 (5%, 10 mL/kg p.o.) and chlorpromazine (150 mg/kg p.o) served as negative and positive controls, respectively. One hour after treatment, emesis was induced in all the animals by single oral administration of copper sulfate pentahydrate ([LABTECH]®, 50 mg/kg). Then the number of retches each animal produced was counted for 20 minutes and recorded. Anti-emetic activity was determined by calculating percentage reduction in number of retches relative to negative control as follows:

\[
\text{Percentage reduction} = \frac{A - B}{A} \times 100
\]

Where A = Mean number of retches in negative control group

B = Mean number of retches in test groups.

Data Analysis

Data is presented as mean ± Standard error of mean (SEM). One-way ANOVA with Dunnett’s post test was performed using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com. P < 0.05 was considered to be statistically significant.

Results

The mean numbers of retches were 12.80 ± 5.39, 35.80 ± 8.47, 91.20 ± 51.24 and 7.60 ± 3.59 for aqueous, n-hexane, ethyl acetate, and butanol, fractions respectively (Figure 1). The value for the crude extract was 33.80 ± 18.74. On analysis, only the mean value of the positive control (chlorpromazine) was statistically significant.

When compared with the negative control, the percentage reductions were 65.15%, 86.80%, 63.09%, 5.98% and 92.16% for crude extract, aqueous fraction, n-hexane fraction, ethyl acetate fraction, and butanol fraction, respectively. On the other hand, chlorpromazine (positive control) reduced the retches by 98.97% (Table 1).

The result shows that butanol fraction had the highest percentage reduction of retches (92.16%).

Discussion

The discovery of more efficacious and safer candidate molecules from natural remedies usually follows a bioassay-guided screening of crude and fractions. This present study investigated the anti-emetic activities of *O. gratissimum* fractions. This plant was chosen for further study since a previous comparative anti-emetic screening of five selected plants (*Portulaca coelestis*, *Mesua ferrea*, *Murraya koenigii*, *Fagonia cretica* and *Ocimum gratissimum*) showed that *O. gratissimum* elicited the highest anti-emetic activity (95.02%) [9].

The mean values of our test samples were not statistically significant possibly due to the dose administered. It is possible that the values will be significant at higher doses. The dose of 150 mg/kg body weight *O. gratissimum* was chosen since the previous study had reported a 95.02% inhibition of emesis at this dose. That study also showed that 150 mg/kg of chlorpromazine (30.98 %) was less effective than 150 mg/kg of crude methanol extract of *O. gratissimum* extract (95.02 %). This is at variance with the results of our study.

The difference between the earlier report [9] and our result may be due to ecological factors. Medicinal plants grown in different environments produce different contents of secondary metabolites, resulting in differences in their pharmacological activities. The primary ecological factors affecting the active ingredient contents include annual average precipitation, temperature variation, frost-free period, annual sunshine duration, soil pH, organic matter, and rapidly available soil elements [11].

**Table 1:** The percentage reduction in number of retches in the chick emetic model.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percentage Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control (Tween 80)</td>
<td>0.90</td>
</tr>
<tr>
<td>Positive control (Chlorpromazine)</td>
<td>98.97</td>
</tr>
<tr>
<td>Crude extract</td>
<td>65.15</td>
</tr>
<tr>
<td>Aqueous fraction</td>
<td>86.80</td>
</tr>
<tr>
<td>N-hexane fraction</td>
<td>63.09</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>5.98</td>
</tr>
<tr>
<td>Butanol fraction</td>
<td>92.16</td>
</tr>
</tbody>
</table>

**Figure 1:** Antiemetic activity of the crude extract and fractions of *Ocimum gratissimum*. 

Available data from our study shows that the anti-emetic ingredients are concentrated in the butanol and aqueous fractions. Consequently, there may be a need to screen these 2 fractions at higher doses for statistically significant activity. Phytochemical constituents present in *O. gratissimum* are flavonoids, alkaloid, tannin, essential oils, phenols, and saponins [12]. The class of compounds identified with anti-emetic activities so far include cannabinoids, chalcones, diarylheptanoids, flavonoids, glucosides, hydroxycinnamic acids, lignans, phenylpropanoids, polysaccharides, saponins and terpenes (sesqui & triterpenes) [10].

**Conclusion**

This study showed that butanol fraction possessed the most potent anti-emetic compounds. These compounds are more potent than the crude extract and other fractions. Supplementary studies are further required to isolate the active principles in butanol fraction responsible for the anti-emetic activity as well to also establish its specific molecular mechanism of action.

**References**