ChemSearch Journal 13(2): 14 – 20, December, 2022
Publication of Chemical Society of Nigeria, Kano Chapter

Received: 29/05/2022 Accepted: 31/08/2022
http://www.ajol.info/index.php/csj

Phytochemical and Antiulcer Investigations of Combined *Emilia coccinea and Ocimum gratissimum* Leaves Aqueous Extract

Ogbeide, O. K. and *Uadia, J. O.
Department of Chemistry, Faculty of Physical Sciences, University of Benin, Benin City, Nigeria
***Correspondence Email:** uadiajeremiah@gmail.com

ABSTRACT

Emilia coccinea and *Ocimum gratissimum* are medicinal plants that are widely used traditionally for the management of various diseases. This work aimed at evaluating the phytochemical constituents and antiulcer activity of the aqueous extract of combined *Emilia coccinea* and *Ocimum gratissimum* leaves. The phytochemical screening was conducted on the extract using standard methods. The anti-ulcerogenic effect was determined using ranitidine-induced gastric ulcer model. The phytochemical investigation revealed the presence of alkaloids, tannins, terpenes, saponins and flavonoids. At a dose of 200 mg/kg, the extract showed the highest percentage inhibition of ulcer index (UI), being 100% from the anti-ulcer evaluation. This research showed that *E. coccinea* and *O. gratissimum* are rich in medicinal compounds that could be active against many diseases including ulcer.

Keywords: antiulcer, *Emila coccinea*, *Ocimum gratissimum*, phytochemical

INTRODUCTION

Medicinal plants have been used over generations as single or combined herbal extract or formulation in the management of diverse disease conditions. Combined herbal extract also known as polyherbal extract is the use of more than one herb in a medicinal preparation. The concept is found in Ayurvedic and many other traditional systems of medicine where more than one herb in a particular ratio are used in the treatment of several diseases ranging from ulcer, diabetes, fever, cough and so on (Subramani *et al.*, 2014). In this regard, numerous wholesome products are produced from plants and have remained in use as long-established cures for various human sicknesses for many decades (Iyasele *et al.*, 2022; Lichterman *et al.*, 2004). This medicinal value of different plant products and extracts is as a result of the active phytochemicals which are present in different parts of the plant and this indicates great assurance in the treatment of intractable infectious diseases (Ighodaro and Ogbeide, 2020; Idu *et al.*, 2007). Nowadays, there is an increasing failure of chemotherapeutics and antibiotic resistance exhibited by microorganisms which has now led to the screening of several medicinal plants. (Colombo and Bosisio, 1996). Although, millions of chemical compounds with therapeutic values are currently being synthesized in the laboratory, but plant extracts remain the greatest sources of new drugs (Ogbeide *et al.*, 2018).

Peptic ulcer is one of the major prevalent diseases around the world affecting about four million people each year. Peptic ulcer is the term which refers to acid peptic injury of the digestive tract, and it gives rise to mucosal break reaching the submucosa (Jain, 2016).The disease involves an imbalance between offensive and defensive factors along with weakness of the mucosal barrier. The major offensive factors include pepsin, acid and *Helicobacter pylori*, and defensive factors include bicarbonates, prostaglandins, mucin, nitric oxide and growth factors (Appavoo *et al.*, 2019). *H. pylori* infection is a very common cause of primary peptic ulcers. It is associated with 70% of gastric ulcers and 95% of duodenal ulcers (Napolitano, 2009). Other risk factors responsible to produce peptic ulcer disease include cocaine, alcohol consumption, tobacco and amphetamine use, chronic administration of non-steroidal anti-inflammatory drugs (NSAIDs), fasting and Zollinger–Ellison syndrome (Kempenich, 2018; Lim *et al.*, 2014). In this concern, the drugs of natural origin can be used for the management of gastric ulcers and other diseases as a better alternative to synthetic drugs (Zinatloo-Ajabshir and Zinatloo-Ajabshir, 2019).

Emilia coccinea commonly known as scarlet tassel flower, belong to the family of *Compositae*. The leaves are eaten raw or chewed. In herbal medicine, the leaves and the root are used in the management of craw-craw, abscesses of the breast, yaws, lice, cough jaundice and snakebite. The ethnomedicinal report shows that**,** *E. coccinea* leaf has been proven effective in treating ulcer, ringworm, gonorrhea, measles, and convulsion in children (Odugbemi and Akinsulire, 2006). *Ocimum gratissimum* (labiatae) generally known as

scent leaf is widely distributed in tropical and warm temperate regions. The plant is commonly used in folk medicine to treat different diseases such as upper respiratory tract infections, diarrhoea, headache, ophthalmic, skin diseases, pneumonia, cough, fever and conjunctivitis (Onajobi, 1986). Some of the bioactivities of *E. coccinea* have been confirmed in the laboratory. These include anti-diarrhoea, antimicrobial and fungicidal activity (Edeoga *et al.,* 2005). Phytochemical screening has revealed the presence of alkaloids, tannin, saponin, steroids, terpeniods flavonoids and cardiac glycosides. Thymol was identified as the major ingredient responsible for the antibacterial activity of the essential oil of this plant. But the essential oils of this same plant from Europe contain eugenol as the dominant component as well as traces of ocimene and myrcene (Effraim *et al.,* 2003). The essential oils of *O. gratissimum* leaf consist of Z-tert-butyl-4-hydroxy anisole (13.93%), γ-terpinene (52.86%), caryophyllene (10.37%) and p-cymene (7.16%) as the major compounds while the essential oil of the seeds yielded α-pinene (48.19%), caryophyllene (10.71%), and 3-tert-butyl-4-hydroxyanisol (11.14%) as major compounds (Ayeni and Yahaya, 2010).This study investigated the phytochemicals, antimicrobial and antiulcer activities of combined *E. coccinea and O. gratissimum* leaves aqueous extract.

MATERIALS AND METHODS

Collection of plant samples

The fresh leaves of *E. coccinea* and *O. gratissimum* were collected from Ekosodi, Benin City, Edo State, Nigeria. They were identified and authenticated by the taxonomist at the Herbarium section of Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Edo State, Nigeria.

Sample Preparation and Extraction

The leaves were rinsed with tap water and air-dried for 14 days after which they were pulverized using British milling machine. Five Hundred gramms (500 g) of the two pulverized leaves (ratio 1:1) were co-macerated in 500 ml of distilled water. The extraction was followed with continuous stirring and shaking for 72 hours. The extract was filtered and the filtrate was concentrated using evaporation dish in a thermostated water bath at 45^{o}C. The extract was weighed and stored in the refrigerator at 4^{o}C for further use.

Phytochemical Screening

The secondary metabolites present in the concentrated extract were qualitatively investigated based on standard methods as described by Ayeni and Yahaya (2010).

Evaluation for Anti-Ulcer

Experimental Animals

Forty-five (45) healthy Wistar rats both male and female weighing between 165-250 g were obtained from the animal breeding unit of the Department of Biochemistry, University of Benin. The animals were housed in the Animal House of the Department of Animal and Environmental Biology, University of Benin and were acclimatized for two weeks. They were maintained under the standard environmental conditions; 12 hours-day/night cycle and fed with standard mash grower feeds and water *ad libitum*. They were handled according to standard protocols throughout the experimental period (Tripathi *et al.,* 2021).

Experimental Design

Exactly thirty (30) rats weighing between 165 and 250 g were distributed randomly into six groups containing five rats each. The animals were grouped as follows: **Group 1**: Positive Control received 1 ml of 75% Ethanol and treated with 20mg/kg Ranitidine(standard drug); **Group 2**: Normal control received 1 ml of 75% Ethanol and given Distilled water; **Group 3**: Received 1 ml of 75% Ethanol and treated with 50 mg/kg of aqueous combined leaves extract; **Group 4**: Received 1 ml of 75% Ethanol and treated with 100 mg/kg of aqueous combined leaves extract; **Group 5**: Received 1 ml of 75% Ethanol and treated with 200 mg/kg of aqueous combined leaves extract.

Induction of ulcer

1 ml of 75% Ethanol was administered 60 minutes after the administration of the extract in volume according to the body weight orally, alongside the standard drug (Ranitidine) in group 1(positive control) and the plants extract in group 3, 4 and 5 using oral gastric tube once per day for 14 days. At the end of the 14 days, the animals were sacrificed and the blood sample, stomach lining, liver and kidney were analysed as described by Effraim *et al.* (2003) and other researchers. The ulcer index was calculated using equation 1;

$$\text{Ulcer index (UI)} = \frac{\text{Total area of ulcer (mm 2)}}{\text{Total area of stomach (mm 2)}} \quad (1)$$

Collection of Blood Sample

The method described by Yakubu *et al.* (2005) was used for the collection of blood sample. The animals were under chloroform anesthesia for few minutes and were sacrificed by a sharply cut with a sterile scalpel blade and about 3 ml of the blood sample was collected from ventral coccygeal vein and transferred into an EDTA bottle for analysis.

Determination of Body Mass Index Weight

Body weight of the rats in their respective groups were weighed using manual digital weighing balance from the beginning of the study

and subsequently for every seven days all through the study to check for any difference in weight.

Haematological Study

Automated sysmex-KX-21 haematology analyser (Sysmex Corporation, Kobe, Japan) was used for the determination of the red blood cells (RBC), haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW), white blood cell (WBC), monocytes (MO), lymphocytes (LY), platelets (PLT), platelets crit (PCT), platelets density width (PDW), mean platelets volume (MPV) and granulocytes (GR).

Histopathological Study

The various organs (stomach, liver and kidney) which were fixed in 10%(vol/vol) formaldehyde cleaned up in xylene and embedded in a paraffin wax (Drury and Wallinton 1973).Tissue sections were prepared according to the method described by Dina *et al.* (2003) and stained with eosin and haematoxylin. Photomicrographs were taken at x400 magnifications using a digital camera.

Statistical Analysis

All the results were analysed as Mean ± SEM. Analysis of variance (ANOVA) was done by one way ANOVA using graph pad prism version 6.

RESULTS AND DISCUSSION

Phytochemical screening of the aqueous combined extract revealed the presence of flavonoids, tannins, saponins, alkaloids and phenolic compounds as shown in Table 1.

Table 1: Phytochemical constituents of combined *E. coccinea and O. grattisimum* aqueous extract

Phytochemicals	Aqueous Extract
Flavonoids	+
Tannins	+
Saponins	+
Alkaloids	+
Phenolic compounds	+

+ = Present

Alkaloids such as ambinine are known to possess antithrombotic and anticoagulant activities, while tannins are known to inhibit platelet activation and thrombus formation (Chang *et al.*, 2018; Zhu, 2018). Flavonoids, phenolics and saponins are influential secondary phyto-constituents that are potential health promoting compounds because of their antioxidant properties (Oluwafemi *et al.,* 2015); hence, they play a role in preventing oxidative damage of the cell. Therapeutic potentials of antioxidants in controlling degenerative diseases with marked oxidative damage from reactive oxygen species or free radicals could be ameliorated by flavonoids (Anyasor *et al.,* 2010).

The antiulcer evaluation results from Table 2 showed some level of significant difference between the control groups and treated groups. The reference drug (20 mg/kg Ranitidine) has a highly significant difference with 98.79% ulcer inhibition when compared with the negative control 0.0% ulcer inhibition. The treated group precisely at a dose of 200 mg/kg is more effective, resulting to 100% ulcer inhibition. 50 mg/kg and 100 mg/kg showed a significant difference when compared with the negative control (P-value<0.05) resulting to showing 76.84 and 80.50% ulcer inhibition respectively.

Table 2: Effect of combined *E. coccinea and O. gratisimum* aqueous crude extract on mean ulcer index and ulcer percentage inhibition

Parameter/Group	Doses (mg/kg)	Mean±SEM Ulcer Index (UI)	Percentage Inhibition of Ulcer Index (UI) %
Distilled water(normal control)	0.5ml	27.33 ± 11.35	0.00
Ranitidine(positive control)	20	0.33 ± 0.33 [b]	98.79
Aqueous extract	50	6.33 ± 3.18 [a]	76.84
Aqueous extract	100	5.33 ± 5.33 [a]	80.50
Aqueous extract	200	0.00 ± 0.00 [b]	100.00

Keys**:** a= P-value<0.05**,** b=P- value < 0.01

In plate I, antiulcer on the stomach lining gives clearer pictures of the mucosa membrane in positive control by showing a well-defined healthy stomach. The same observation was noted on the normal control and graded doses of the treated groups specifically at 100 and 200 mg/kg.

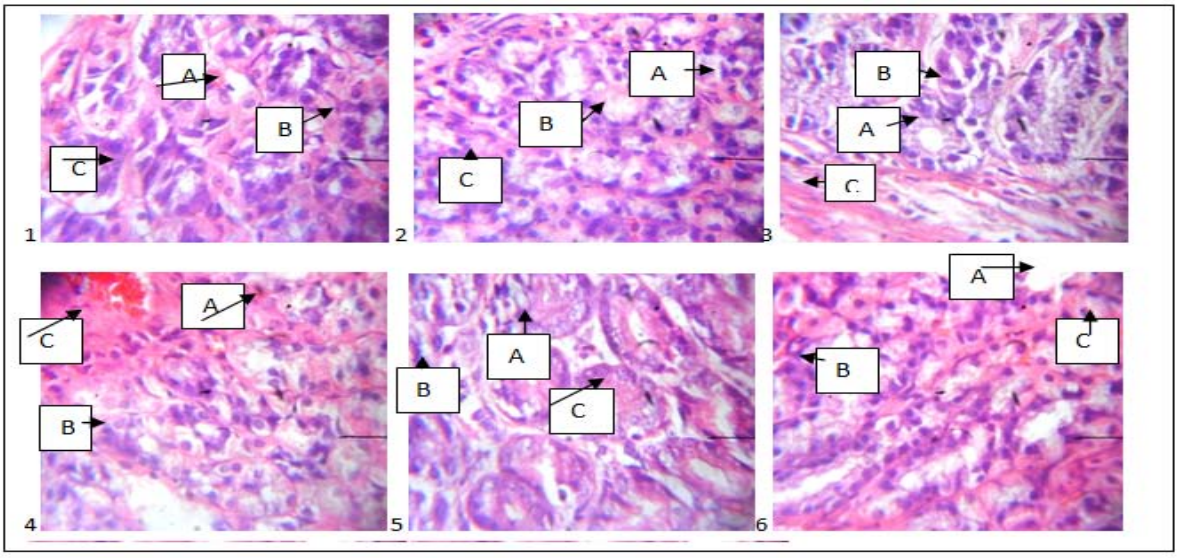

Plate I: Stomach histology - (**1)** Received 20 mg/kg Ranitidine. **A-** Stomach shows mild focal mucosal erosion. **B-** Normal mucosa. **C-**Muscularis mucosa. (**2**) Normal group. **A-** Stomach shows normal focal mucosal. **B-** Normal mucosa. **C-**Muscularis mucosa. (**3**) Received 50 mg/kg combined aqueous extract. **A-** Stomach showing slight mucosal devitalisation. **B-** Mild ulceration. **C-** Thickened muscularis mucosa. (**4**) Received 100 mg/kg combined aqueous extract. **A-** Stomach shows mild focal mucosal erosion. **B-** Normal mucosa. **C-**Muscularis mucosa. (**5**) Received 200 mg/kg combined aqueous extract. **A-** Stomach showing mild mucosal devitalisation. **B-** Normal mucosa. **C-** Thickened muscularis mucosa.

In plate II, the picture of the hepatocytes indicated no disease condition associated to the positive control. The same observation was observed from the normal control and graded doses of the treated groups.

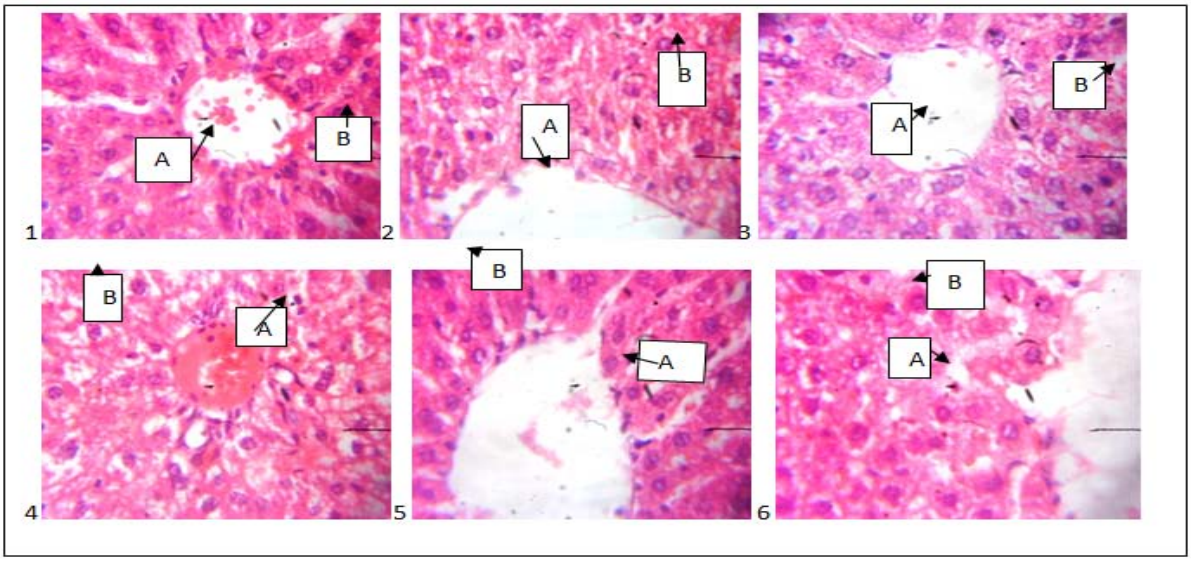

Plate II: Liver histology- (**1)** Received 20 mg/kg Ranitidine. **A-** Visible centrioles with well fenestrated sinusoidal space. The hepatocytes appear distinct with well differentiated nucleus. **B-** Mild inflammatory cells.(**2**) Normal group. **A-** Visible centriole that appear thickened surrounded by mild inflammatory cells. **B-** The hepatocytes however are distinct with visible nucleus. (**3**) Received 50 mg/kg combined aqueous extracts. **A-** Clear centrioles with radiating hepatocytes with visible nucleus. **B-**However there are mild fatty changes. (**4**) Received 100 mg/kg combined aqueous extracts. **A-** Clear and distinct centrioles with radiating hepatocytes with visible nucleus. **B-** However there are mild fatty changes. (**5**) Received 200 mg/kg combined aqueous extract. **A-** Liver histology revealed visible centriole that appears thickened surrounded by mild inflammatory cells. **B-** The hepatocytes however are distinct with visible nucleus.

In plate III, the picture of the kidney is an indication of no disease condition associated to the positive control. The same observation goes to the normal control and graded doses of the treated groups.

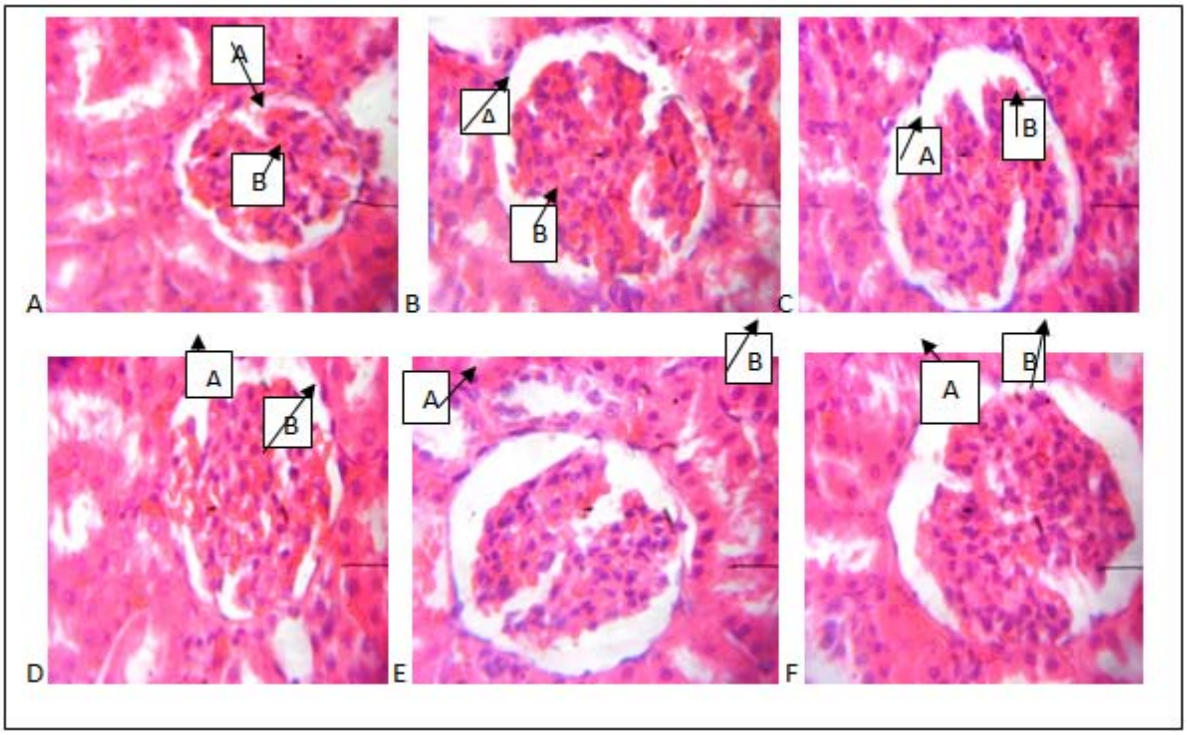

Plate III: Kidney histology – (**1**) Received 20 mg/kg Ranitidine. **A**- The renal corpuscles appeared as dense rounded structures. **B**- Kidney section show detailed cortical parenchyma. (**2**) Normal group**. A**- Kidney section showed normal histological features, renal tubules and the renal corpuscles appeared as dense rounded structures. **B**- Section indicated a detailed cortical parenchyma. (**3**) Received 50 mg/kg combined aqueous extracts. **A**- Kidney histology reveals renal corpuscle with prominent glomerulus. **B**- Mild focal tubular necrosis. (**4**) Received 100 mg/kg combined aqueous extracts. **A**- Kidney histology reveals renal corpuscle with prominent glomerulus. **B**- Mild focal tubular necrosis. (**5**) Received 200 mg/kg combined aqueous extract. **A**- Kidney histology revealed renal corpuscle with prominent glomerulus. **B**- Mild focal tubular necrosis.

For the haematological study, the WBC lymphocytes in all groups showed an insignificant decrease ($P<0.05$) compared to the normal control. Similar trend was observed for the WBC derivatives (LY and MO) with only the 200 mg/kg group and the same with normal control. The observed increase for GR in all extract treatment groups was not significant.

The RBC for all extracts treated groups were appreciably normal in comparison with all controls. The red blood cell derivatives (MCH and MCHC) were observed to follow the same trend. Although, in the other RBC derivatives like the Hgb and HCT, there was a slight increase in 50 and 100 mg/kg groups respectively in comparison with the positive control (Ranitidine). This insignificant increase was same for MCH in all treated groups.

The values for the Platelet for positive control were somewhat same with the treated groups of 50 and 100 mg/kg respectively, except for the slight increase observed in 200 mg/kg, similar trend was observed for PCT. The other platelets derivative (PDW) value was slightly higher in 50 and 100 mg/kg groups respectively compared with the positive control, while the MPV values were normal in all extract treated groups.

Medicinal plants can cause high alterations in total blood count parameters (red and white blood cells, haemoglobin, haemocrit, platelets, etc.) which may be detrimental to vital tissues or organs in the body and lead to disease conditions of the kidney and liver (Ogbeide *et al.*,2021) hence, the importance of haematological assessments of medicinal plant extracts. Generally, from the haematological assay in this study, the extract combination showed no toxic abnormalities or any risk of anaemia. The concentration of extract possibly has lesser toxic effects as shown in the work reported by Dina *et al.* (2003) that aqueous leaves extract of *O. gratissimum* has haematinic effect.

Table 3: Effect of combined aqueous extract on the various haematological parameters across the groups

Parameters	Normal control	Ranitidine	Distilled water(5ml)	Aqueous extract		
				50 mg/kg	100 mg/kg	200 mg/kg
WBC (10^3/ µL)	17.40±1.89	11.97±1.64	12.20±2.10	12.77±3.12	14.17±0.67	14.00±4.92
LY (10^3/ µL)	12.60±1.14	8.53±1.18	8.53±1.51	6.90±0.47	6.93±0.66	8.43±2.74
MO (10^3/µL)	2.07±0.33	1.63±0.32	1.43±0.29	1.40±0.31	1.70±0.00	2.03±0.70
GR (10^3/ µL)	2.80±0.53	2.00±0.21	2.23±0.38	4.57±2.52	5.53±1.19	4.23±1.13
RBC(10^6/µL)	8.37±0.39	8.92±0.83	9.99±0.10	10.06±0.32	10.31±0.46	8.65±0.34
Hgb (g/d L)	13.63±0.52	14.33±1.39	16.13±0.17	17.50±0.30	18.10±1.66	14.63±0.64
HCT (%)	42.80±1.30	41.13±3.92	45.17±1.35	50.97±1.25	52.70±5.35	43.30±1.37
MCV (fl)	51.37±3.81	46.03±0.18	45.03±1.21	50.80±2.66	50.90±3.35	50.10±0.95
MCH (pg)	16.23±0.52	16.03±0.23	16.27±0.23	17.37±0.89	17.43±0.98	16.87±0.33
MCHC(g/dL)	31.87±1.62	34.84±0.57	35.70±0.72	34.30±0.40	34.37±0.33	33.70±0.42
RDW (%)	17.10±1.27	15.03±0.12	15.10±0.27	16.27±0.68	17.00±0.60	15.80±0.10
PLT (10^3/µL)	462.0±33.25	458.0±51.73	652.3±48.26	468.3±18.77	476.7±49.71	502.3±102.1
PCT (%)	0.28±0.02	0.26±0.03	0.38±0.02	0.28±0.01	0.28±0.03	0.36±0.07
MPV (fl)	6.03±0.03	5.60±0.10	5.80±0.12	5.97±0.13	5.93±0.09	5.87±0.09
PDW (%)	6.87±0.09	6.07±0.23	6.07±0.17	7.23±0.35	7.37±0.49	6.67±0.15

Values are presented as mean ± standard error at $p<0.05$; WBC, white blood cells; LY, lymphocytes; MO, monocytes; GR, granulocytes; RBC, red blood cells; Hbg, haemoglobin; HCT, haematocrit; LY, lymphocytes; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; RDW, red cell distribution width; PLT, platelets; PCT, platelet crit; MPV, mean platelet volume; PDW, platelet distribution width.

CONCLUSION

The result of the present study revealed that the combined aqueous extract of *O. gratissimum and E. coccinea* leaves possesses potent antiulcer activity against chemicals induced ulcer. Graded doses of 100 and 200 mg/kg of aqueous extract showed effectiveness by increased preventive ulcer index and also from histopathological studies.These effects could be attributed to the phytoconstituents present in the extract and therefore could be considered as a potential therapeutic candidate in managing many ailments including ulcer. However, the antiulcer bioactive compounds present in the extract, should be isolated, purified and characterized.

ACKNOWLEDGEMENT

The authors are sincerely indebted to Dr. G.O. Benjamin of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin.

REFERENCES

Anyasor, G. N., Ogunwenmo, K. O., Olatunji, A. O. and Blessing, E. A. (2010). Phytochemical, proximate and mineral element composition of stem of *Costusafer* (Bush cane).*Asian J. Plant Sci.*, 2(5):607–612.

Appavoo, K., Ram, M. and Rao, K. (2019). Informatics in medicine unlocked treatment of peptic ulcers with a Siddha medicine, "*SirucinniUppu*" and prediction with regression models. *Inform Med Unlocked*, 15:1–4.

Ayeni, K. E. and Yahaya S. A (2010). Phytochemical screening of medicinal plants. *Cont J. Pharm. Sci.*, 4:47-50.

Chang, S., Yang, Z., Han, N., Liu, Z. and Yin, J. (2018). The antithrombotic, anticoagulant activity and toxicity research of ambinine, an alkaloid from the tuber of *Corydalis ambiguavaramurensis*. *Regul Toxicol Pharmacol*, 95: 175-181.

Colombo, M. L. and Bosisio, E. (1996). Pharmacological activities of *Chelidoniummajus L (Papaveraceae)*. *Pharmacol. Res*, 33:127-134.

Dina, O. A., Adedapo, A. A., Oyinloye, A. A. and Saba, A. B. (2003). Haematologocal effect of aqueous extract of *Telfairia Occidentalis*. *African journal of biomedical research*, 3: 181- 188.

Drury, R. A. B. and Wallinton, E. A. (1973). Carletons Histological Technique, 16th edn. Oxford University Press, London, pp124-136.

Edeoga, H. O., Okwu, D. E. and Mbaebie, B. O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *Afr. J. of Biotech*, 4(7): 685 – 688.

Effraim, K. D., Jacks, T. W. and Sodipo, O. A. (2003). Histopathological studies on the toxicity of *Ocimum gratissimum* leaves extract on some organs of rabbit. *African Journal of biomedical Research*, 6:21-5.

Idu, M., Omonigho, S. E. and Igeleke, C. L. (2007). Preliminary investigation on the phytochemical and antimicrobial activity of *Sennaalata L.* flower. *Parkistan J. of Biol. Sc.* 10(5): 806-809.

Ighodaro, A. and Ogbeide, O. K. (2020). Antioxidant Activity and Assessment of Total Phenolic and Flavonoid Contents of Caesalpinia pulcherrima leaf Extract and Fractions. *J. Chem. Soc. Nigeria,* 45(5): 945 – 950.

Iyasele, J. U., Uadia, J. O., Akhigbe, I. U., Jacob, J. N. and Ogbeide, O. K. (2022). Physico-Chemical Properties, Chemical Composition and Antimicrobial Activity of *Adonidia merrillii* Kernel Seed Oil. *Trop J Nat Prod Res*. 6(4): 50-58.

Jain, P. (2016). Secondary metabolites for antiulcer activity. *Nat Prod Res*, 30(6):640–656.

Kempenich, J. W. (2018). Acid peptic disease. *SurgClin N Am*, 98(5):933–944.

Lichterman, B. L. (2004). Aspirin: The Story of a Wonder Drug. *British Med. J* 329 (7479): 1408.

Lim, W. Y., Subramaniam, M., Abdin, E., Vaingankar, J. and Ann, S. (2014). Peptic ulcer disease and mental illnesses. *Gen Hosp Psychiatry*, 36(1):63–67.

Napolitano, L. (2009). Refractory peptic ulcer disease. *Gastroenterol Clin N Am*, 38(2):267–288.

Odugbemi, T. and Akinsulire, O. (2006). Medicinal plants by species names. In: Odugbemi .T. (Ed). Outlines and Pictures of Medicinal Plants from Nigeria, University of Lagos Press, Nigeria, p.73-116

Ogbeide, O. K., Dickson, V. O., Jebba, R. D., Owhiroro, D. A. Olaoluwa, M. O., Imieje, V. O., Erharuyi, O., Owolabi, B.J. Fasinu, P. and Falodun, A. (2018).Antiplasmodial and Acute Toxicity Studies of Fractions and Cassane-Type Diterpenoids from the Stem Bark of *Caesalpiniapulcherrima (L.)* Sw. *Trop J Nat Prod Res*, 2(4):179-184.

Ogbeide, O.K., Akhigbe, I.U., Unuigbe, C.A., Erharuyi, O., Imieje, V., Benjamin, G.O., Ikeke, K., Ayeni, B., Irabor, E., Owolabi J.B. and Falodun, A. (2021). Haematological and Biochemical Examination of Pulcherrimin A isolated from *Caesalpinia pulcherrima* stem bark. *Trop J Nat Prod Res.* 5(11): 2011-2015.

Oluwafemi, K. A., Jesumoroti, O. J., Tinubu, B. E. and Uadia, J. O. (2015). Antioxidant Activities, Total Flavonoid and Total Phenolic Contents of Whole Plant of *Kyllingaerecta Shumach*. *J. Food and Nut. Res* 3(8): 489-494.

Onajobi, F. D. (1986). Smooth muscle contracting lipidic soluble principle in chromatographic functions of *Ocimum gratissimum. J. Ethnopharmacol*, 18: 3-11.

Subramani, P., GanSiaw, T. and Sokkalingam, A. D. (2014). Polyherbal formulation: Concept of Ayurveda. *Pharmacogn. Rev*. 8(16): 73-80.

Tripathi, A., Singh, S. and Mukerjee,A. (2021).Antiulcer activity of ethanolic leaf extract of *Capparis*zeylanicaagainst chemically inducedulcers. *Futur J Pharm Sci*,7:211

Yakubu, M. T., Akanji, M. A. and Oladiji, A. T. (2005). Aphrodisiac potentials of aqueous extract of *Fadogia agrestis* (Schweinf. Ex Heim) stem in male albino rats.*Asian J Androl*, 7:399-404.

Zhu, L. (2018). Tannic acid inhibits protein disulfide isomerase, platelet activation and thrombus formation. *Blood*, 132(1): 2418.

Zinatloo-Ajabshir, Z. and Zinatloo-Ajabshir, S. (2019). Preparation and characterization of curcuminniosomal nanoparticles via a simple and eco-friendly route.*J Nanostruct*, 9(4):784–790.