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GC-MS and Antioxidative Effects of Poly Herbal Formulation of *Persea americana* Seed Against Ethanol-Induced Gastric Ulcer in Rats

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ABSTRACT

Background and Objective: Plant-derived natural products have considerable significant attention due to their various pharmacological properties and functions. Avocado (*Persea americana* Mill.), is a fruit that is native to Central America. GC-MS analyses of avocado samples showed α-tocopherol, squalene, cycloartenol acetate, β-sitosterol, campesterol and stigmasterol. This study aims to check the gastroprotective and antioxidant effect of avocado seed extract on gastric ulcer. **Materials and Methods:** A total of 60 experimental rats were used and divided into a group of six in two experimental designs and administered treatments with the crude extract of virgin avocado. **Results:** Results were expressed as Mean±Standard Error of the Mean (SEM). Statistical significance was determined using a One-way Analysis of Variance (ANOVA) followed by Tukey's *post hoc* Test. Values of $p<0.05$ were considered to be significant. The result of the GC-MS profile of the ethanol crude extract of avocado seed extract was found to compose of 30 chemical constituents which can be used to manage different kinds of ailments. **Conclusion:** Avocado seed extract has potential antioxidant activity *in vitro* and *in vivo*. Oral administration of Avocado seed extract once daily for 7 days alleviates gastric ulcers induced by ethanol, but more significant was observed with the formulation.

KEYWORDS

Avocado, ethanol, *in vivo, in vitro,* gastric ulcer, antioxidants, virgin, GC-MS

INTRODUCTION

Due to their numerous pharmacological qualities, such as their anti-inflammatory[1], anti-hyperglycemic[1], gastroprotective[1] and hepatoprotective[2] activities, plant-derived natural products have received a great deal of interest in recent years. Avocados are regarded as a very important plant due to their significant economic and medicinal benefits. The avocado, scientific name *Persea americana* Mill., is a fruit that originates from Central America. It has also been called the alligator pear, midshipman's butter, vegetable butter and even the butter pear. It is primarily grown in warm climates and is known by the Spanish names

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aguacate, cura, cupandra, or palta, abacate in Portuguese, and avocatier in French[3]. The Lauraceae, or family of Laurels, only has one significant edible fruit[3]. Vitamins A, B, G and E were abundant in the seed's crude extract. A 93.8% digestibility coefficient was recorded for it[3]. In terms of pharmacological effects, it was analgesic[4], hypotensive[5], antiviral[6], wound healing[7] and anti-inflammatory. Avocado pulp oil's bioactive component composition is regulated by the extraction and drying techniques[8]. Lyophilization and soxhlet extraction yielded the most oil, but lyophilization and cold pressing generated oils with higher concentrations of antioxidants and other beneficial components[8]. Avocado samples subjected to GC/MS analysis revealed the presence of -tocopherol, squalene, cycloartenol acetate, -sitosterol, campesterol and stigmasterol[8].

One of the most prevalent conditions affecting the digestive tract is gastric ulcers. The pathophysiology of this illness is a multifactorial process that is triggered by infection, smoking, stress, the prolonged use of Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) and excessive alcohol consumption. It is brought on by an imbalance of factors that protect and destroy the gastric mucosa (acid and pepsin)[8]. Alcohol is a dangerous substance linked to numerous pathologies and can be given orally to laboratory animals to cause acute stomach ulcers[9]. Disruption of the stomach mucosa, which raises mucosal permeability and bleeding, causes ethanol-induced gastric ulcers.

Reactive Oxygen Species (ROS) and other inflammatory mediators are overproduced at the site of stomach injury by white blood cells such as neutrophils, which also cause oxidative damage and cell damage[8,9]. Superoxide Dismutase (SOD) and Catalase (CAT), two antioxidant enzymes, as well as the non-enzymatic antioxidant Glutathione (GSH), are less active when Reactive Oxygen Species (ROS) are present[10]. These are some of the factors that contribute to the development of acute gastric ulcers brought on by ethanol and cause cell membrane damage.

Additionally, oxidative stress during ethanol-induced gastric inflammation induces lipid peroxidation, as shown by higher Malondialdehyde (MDA) levels in gastric tissues, according to a prior study[11]. When gastric ulcer illness causes mucosal inflammation, Nuclear Factor kappa B (NF-B) is activated. According to studies, NF-B regulates the production of proinflammatory cytokines that are implicated in inflammatory responses, including interleukin 1beta (IL-1beta), interleukin-6 (IL-6), Tumour Necrosis Factor-alpha (TNF-alpha) and Inducible Nitric Oxide Synthase (iNOS)[12,13]. Antacids, antibiotics, H2 receptor antagonists and Proton-Pump Inhibitors (PPIs) are a few contemporary medications that are frequently used to treat gastritis[14]. Numerous studies have been published that claim that PPI Omeprazole (OMZ) has a gastroprotective effect[14-18]. However, using contemporary medications long-term can have negative effects[19]. PPIs can have two different kinds of negative effects: those unrelated to acid inhibition and those connected to acid inhibition. Acute interstitial nephritis, chronic kidney disease and collagenous colitis are examples of adverse outcomes unrelated to acid inhibition.

The unfavourable effects of acid inhibition include stomach carcinoid tumours, gastric fundic mucosal hypertrophy, alterations in the gut microbiome, small intestine bacterial overgrowth, gastric fundic gland polyps and gastric cancer[20]. Alternative methods of treating stomach ulcers are required due to the side effects of contemporary medications.

Due to their various gastroprotective mechanisms, such as the promotion of mucosal proliferation, prevention of acid formation and antioxidant capabilities, herbal medicines have thus emerged as an alternative to conventional medications[21,22]. Since ancient times, traditional Higgi Kamue plants and other components have been used for medical purposes. Due to their antioxidant, anti-gastric-ulcer and anti-inflammatory qualities, some traditional Higgi Kamwe plants are employed[23]. A traditional Higgi Kamwe polyherbal that has a high amount of total phenolics and flavonoids and displays good antioxidant activity

is virgin avocado (*Persea americana*) seed with honey[24]. Higgi Kamwe's herbal composition, however, has neither an offensive flavour nor odour. It was learned that local users gave it very good sensory acceptance ratings. The herb's first preparation received the best marks. The objective of this study is to evaluate the GC-MS, gastroprotective and antioxidative effects of the traditional Higgi Kamwe poly herbal formulation virgin avocado (*Persea americana*) seed against ethanol-induced gastric ulcers in rats.

MATERIALS AND METHODS

Study area: This study was carried out in the Central Laboratory complex, Federal University, Wukari, Nigeria, from March, 2021 to July, 2022.

Spectrophotometric analysis: Spectrophotometric assessment of DPPH Radical Scavenging Activity, the control solution was made by combining 2.0 mL of 95% ethanol and 2 mL of a 60 M DPPH solution in a VMX-S vortex mixer (Bioevopeak Co, Jinan City, Shandong Province, China) Additionally, the blank solution could be made by completely combining 2.0 mL of the test sample solution and 2.0 mL of 50% ethanol in the vortex mixer. Additionally, 2.0 mL of 60 M DPPH solution and 2.0 mL of test sample solution were gently combined using a vortex mixer to create the prepared standard ascorbic acid solutions and the test sample. After that, the solutions were allowed to stand for 30 min at room temperature. Then, the absorbance value of the each solution at 517 nm was measured by a T60 UV spectrophotometer (Pg Instruments, Alma Park, Wibtoft Leicestershire, Le17 5bh, United Kingdom). The absorbance values obtained were applied to calculate percent inhibition by the following formula[24]:

$$\text{Inhibition (\%)} = \frac{\text{DPPHalone} - (\text{Sample} - \text{Black})}{\text{DPPH}} \times 100$$

Inhibition (%) = Inhibition (%) of test sample
Sample = Absorbance of test sample solution
DPPH = Absorbance of control solution
Blank = Absorbance of blank solution

Control solution:

- 2.0 mL (60 μM DPPH of EtOH)+2.0 mL (95% EtOH) →30 min R.T

Blank solution:

- 2.0 mL (Sample)+2.0 mL (50% EtOH)→30 min R.T
- Measure absorbance at 570 nm

Sample solution:

- 2.0 mL (60 μM DPPH of EtOH)+2.0 mL (Sample)→30 min R.T

Preparation of extra virgin avocado seed crude extract: Ten (40) mature, high-quality avocado fruits were bought from Takum Local Government Area of Taraba State, Nigeria. They were washed and kept until soft. The pulp was removed and the seed was mashed and spread on a large surface open container kept under room temperature. During this period, the seed mash was turned occasionally for faster drying. After about 4 days, the mashed dry seed powder was soaked in ethanol and allowed to standard for 7 days. The filter was subjected to evaporation in the rotary evaporator and the crude was allowed to be stored in the refrigerator under 4 for onward use.

Experimental animals: A total of 60 rats of both sexes, weighing (180-200 g) were obtained from the animal house of the Natural Product Research Laboratory, Federal Housing Estate No: 10 Sanitation Rd

Bajabure Gerie Adamawa State. They were maintained on standard animal pellets and water *ad libitum*, housed in cages to acclimatize to the animal house and maintained under standard conditions (25-28°C) with 12 hrs dark/light cycles.

Experimental design I: The experimental rats were divided into 6 groups consisting of 5 rats per group. Rats of each group were orally treated as follows:

Group 1 (Negative control) : Given distilled water (10 mL kg^{-1}) for 7 days
Group 2 (Positive control) : Given Omeprazole (30 mg kg^{-1}) for 7 days
Group 3 (Treatment group) : Given extra virgin avocado ethanol crude extract 50 mg per 200 g body weight for 7 days
Group 4 (Treatment group) : Given extra virgin avocado ethanol crude extract 100 mg per 200 g body weight for 7 days
Group 5 (Treatment group) : Given extra virgin avocado ethanol crude extract 200 mg per 250 g body weight for 7 days
Group 6 (Treatment group) : Given extra virgin avocado ethanol crude extract 400 mg per 200 g body weight for 7 days

Experimental design II: The experimental rats were divided into six groups consisting of five rats per group. Rats of each group were orally treated as follows:

Group 1 (Negative control) : Given distilled water (10 mL kg^{-1}) for 7 days
Group 2 (Positive control) : Given Omeprazole (30 mg kg^{-1}) for 7 days
Group 3 (Treatment group) : Given extra virgin avocado ethanol crude extract 50 mg+50 mg per 200 g body weight for 7 days
Group 4 (Treatment group) : Given extra virgin avocado ethanol crude extract 100 mg+50 mg per 200 g body weight for 7 days
Group 5 (Treatment group) : Given extra virgin avocado ethanol crude extract 200 mg+50 mg per 200 g body weight for 7 days
Group 6 (Treatment group) : Given extra virgin avocado ethanol crude extract 400 mg+50 mg per 200 g body weight for 7 days[25]

Ethanol-induced ulcer model: Rats in each group were treated for 7 days and after fasted for 24 hrs. The ulcer was induced through the administration of ethanol (2 mL kg^{-1}) 1 hr after administration of distilled water (group 1), omeprazole (group 2) and avocado seed crude extract and Avocado crude extract plus honey (group 3 -6 for an experiment I and II), respectively. The animals were kept further for 4 hrs for the ethanol to take effect. After 4 hrs, animals were euthanized by cervical dislocation under chloroform anaesthesia. The stomachs were excised and gently rinsed with normal saline, then inflated with 1% formalin solution (10 mL) and immersed in the same solution to fix the outer layer of the stomach[26,27]. After about 10 min, each stomach was opened along the greater curvature, rinsed with normal saline to remove gastric contents and examined by using a 10× magnifier lens to assess the formation of ulcers, then scored by using the Kulkarni Method (0 = no ulcer, 0.5 = red colouration, 1 = spot ulcers, 2 = deep ulcers and 3 = perforations)[28]. This procedure was performed by an expert in the identification of ulcer types.

Ulcer index and percentage of ulcer inhibition were determined as follows[28]:

$$\text{Ulcer Index (UI)} = UN+US+UP\times 10^{-1}$$

Where:

UN = Average number of ulcers per animal
US = Average severity score
UP = Percentage of animals with ulcers

$$\text{Ulcer inhibition (\%)} = \frac{\text{UI control} - \text{UI test}}{\text{UI control}} \times 100$$

Histopathology: The stomach tissues which were preserved in 10% formalin pending histopathological studies were dehydrated serially through progressive concentrations of alcohol and cleared using xylene. After clearing, the tissues were embedded in paraffin wax and thin sections of about 5 µm were made using the microtome. Each section was mounted on a clean glass slide and stained with Haematoxylin and Eosin. Later, a mounting medium (Canada balsam) was dropped on each tissue section and a cover slip was placed on it and allowed to dry[28-30]. They were examined with a light microscope and photomicrographs were captured using moticam images plus 2.0 (Motic China Group Ltd.) The digital camera is attached to the microscope.

Ethical issues: The procedures were performed according to the guidelines on the use of animals and approved by the Institutional Animal Ethical Committee of the quality control unit at Federal University Wukari, Nigeria.

Statistical analysis: Results were expressed as Mean±Standard Error of the Mean (SEM). Statistical analysis was carried out using SPSS Version 23 Statistical Software. Statistical significance was determined using a One-way Analysis of Variance (ANOVA) followed by Tukey's *post hoc* Test. Values of $p<0.05$ were considered to be significant.

RESULTS

In Fig. 1, the GC-MS profile of ethanol crude extract of avocado seed shows peaks of the different compounds. In Table 1, the various phytochemical constituents of ethanol crude extract of avocado seed

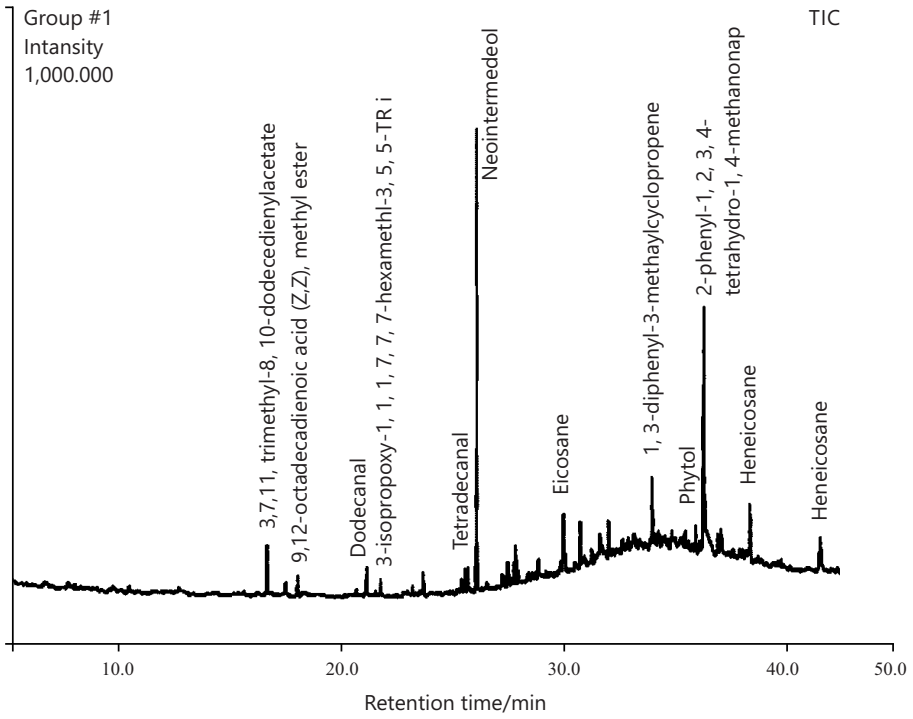

Fig. 1: Showing the GC MS profile of ethanol crude extract of avocado seed

Table 1: Phytochemical constituents of ethanol crude extract of avocado seed peak report TIC

R. time	Area	Height	Names
16.753	238485	76595	3,7,11, Trimethyl-8,10-dodecedienylacetate
18.158	78459	32830	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
21.303	94947	40577	Dodecanal
21.941	60421	25081	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5r-ti
23.880	88251	35366	Tridecanal
25.629	60030	19913	4,9:5,8-Dimethano-1H-benz[f]indene, 3a,
25.810	86980	37060	Heptadecane, 7-methyl-
25.923	108650	36421	Diethyl Phthalate
26.320	1849659	758605	Tetradecanal
27.743	175616	39140	Neointermedeol
28.005	83788	32154	1,1'-Biphenyl, 2,2',5,5'-tetramethyl-
28.104	184183	61856	Heptadecane, 2,6,10,15-tetramethyl-
28.238	57844	20446	1,1'-Biphenyl, 3,4-diethyl-
29.138	85980	28026	1,4-Dimethyl-2-phenoxybenzene
30.279	254504	93654	Heneicosane
30.380	118273	25692	Eicosane
31.051	178301	72600	Neophytadiene
31.954	98600	29101	Neophytadiene
32.349	149187	57771	Heneicosane
33.002	121222	19313	1-Tetracosene
34.220	54965	15132	cis-1-Chloro-9-octadecene
34.326	288668	109605	Heneicosane
34.901	107309	14522	3-Eicosyne
35.839	91891	26224	1,3-Diphenyl-3-methylcycopropene
36.323	110320	38683	Heneicosane
36.685	1428714	383496	Phytol
37.318	113367	30918	2-Phenyl-1,2,3,4-tetrahydro-1,4-methanonap
37.470	214162	37711	Z-(13,14-Epoxy) tetradec-11-en-1-ol acetate
38.790	318110	86326	Heineicosane
42.003	257617	47391	Heneicosane
	7158503	2332209	

Table 2: Antioxidant activity absorbance and inhibition (%) of ethanol crude extract of avocado seed

Control	Ascorbic acid (517 nm)	Crude extract	Inhibition (%)
2.413±0.02	0.023±0.01	1.42±0.02	99.78
2.367±0.03	0.025±0.01	1.02±0.01	98.88
2.375±0.04	0.027±0.01	1.67±0.02	99.97
2.382±0.06	0.029±0.01	0.36±0.04	99.99
2.394±0.07	0.03±0.01	0.42±0.02	99.89
2.399±0.07	0.034±0.01	0.22±0.03	99.99

Table 3: Effects of ethanol crude extract of virgin avocado seed

Groups	Treatments	Ulcer index	Inhibition (%)
Normal Control	Distilled water 10 mL kg^{-1}	3.94±0.46	-
Negative control	Indomethacin-induced	66.36±0.45	0.00
Positive control	Omeprazole 30 mg kg^{-1}	2.12±0.29	88.8
Crude extract mg kg^{-1}	Avocado 50 mg kg^{-1}	55.48±0.13	12.26
Crude extract mg kg^{-1}	Avocado 100 mg kg^{-1}	45.11±0.43	23.44
Crude extract mg kg^{-1}	Avocado 200 mg kg^{-1}	24.36±0.19	36.12
Crude extract mg kg^{-1}	Avocado 400 mg kg^{-1}	14.07±0.34	47.16

peak report TIC. In Table 2, the antioxidant activity absorbance and inhibition (%) of ethanol crude extract of avocado seed are represented. In Table 3, the effects of ethanol crude extract of virgin avocado seed on the rats are shown. In Table 4, the effects of ethanol crude extract of virgin avocado seed+honey 50 mg kg^{-1} on the rats are shown.

DISCUSSION

The main and secondary metabolite composition of plant extracts has been implicated in the majority of the biological effects that have been ascribed to them. Figure 1 and Table 2 display the GC-MS

Table 4: Effects of ethanol crude extract of virgin avocado seed+honey 50 mg kg^{-1}

Groups	Treatments	Ulcer index	Inhibition (%)
Normal control	Distilled water 10 mL kg^{-1}	3.94±0.47	-
Negative control	Indomethacin-induced	66.40±0.33	0.00
Positive control	Omeprazole 30 mg kg^{-1}	2.15±0.36	78.9
Treatment extract mg kg^{-1}	Avocado 50+Honey 50 mg kg^{-1}	21.11±0.48	27.08
Treatment extract mg kg^{-1}	Avocado 100+Honey 50 mg kg^{-1}	24.00±0.11	44.12
Treatment extract mg kg^{-1}	Avocado 200+Honey 50 mg kg^{-1}	12.03±0.13	56.87
Treatment extract mg kg^{-1}	Avocado 400+Honey 50 mg kg^{-1}	5.47±0.34	74.79

profile of the ethanol crude extract of avocado seed results and antioxidant activity absorbance and inhibition (%) of ethanol crude extract of avocado seed respectively. Table 1 shows the phytochemical constituents of ethanol crude extract of avocado seed. Table 3 and 4 show the effects of ethanol crude extract of virgin avocado seed and effects of ethanol crude extract of virgin avocado seed+honey 50 mg kg^{-1}, respectively.

It was discovered that the seed extract contains 30 different chemical components, including dodecanal, 3,7,11,-trimethyl-8,10-dodecedienylacetate, 9,12-octadecadienoic acid (Z,Z) and methyl ester. Tridecanal 3,5,5-tr I, isopropoxy-1,1,1,7,7,7-hexamethyl-4,9:5,8-dimethano-1H-benz[f] Heptadecane, indene, 3a, 4 and Heneicosane, Eicosane, Neophytadiene, Tetradecanal, 2,2',5,5'-tetramethyl-1,1'-Biphenyl, 3,4-diethyl-1,4-dimethyl-2-phenoxybenzene, 1,4-dimethyl-2-phenoxybenzoic acid, 1,1'-Biphenyl, 2,6,10,15-tetramethyl-1,1'-Bi Heneicosane, 1,3-diphenyl-3-methylcyclopropene, 1-Tetracosene, cis-1-Chloro-9-octadecene, 3-Eicosyne, Phytol Heneicosane, Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate and 2-phenyl-1,2,3,4-tetrahydro-1,4-methanonap as shown in Table 1. According to reports by Velderrain-Rodríguez *et al.*[31] the seed extract may also be a source of lupeol, β-sitosterol and hexacosanol. Because of its chemical makeup, it is utilized in the treatment of cancer, hypertension and other diseases[32].

Table 1 and 2, the formulation and avocado seed extract both have a protective effect against ethanol-induced stomach ulcers. The inhibition (%) of ascorbic acid which is an antioxidant is high as shown in Table 2. This result might be due to the ROS scavenging ability of antioxidants resulting in low levels of lipid oxidation, contributing to the reduction of oxidative gastric injury caused by oxygen radicals as reported by Athaydes *et al.*[33]. One strategy of cell membrane protection is to increase the activity of intracellular enzymatic antioxidants, such as SOD, since this enzyme catalyzes the dismutation of the superoxide radical. This effect is probably resultant of antioxidant properties, including transition metal ions chelation, free-radical scavenging and inhibition of oxidizing enzymes, besides increased mucus production[34]. SOD and CAT play a vital role in the detoxification of superoxide anion and H_2O_2, respectively, thus shielding cells against damage. The reactive superoxide radicals are first converted to H_2O_2 by SOD. Later these H_2O_2 radicals are scavenged by CAT to prevent the lipid peroxidation resulting due to the generation of hydroxyl radicals.

The imbalance of the gastric mucosa-protecting (pepsin) and gastric mucosa-destroying (acid) components is what leads to this disease's multifactorial pathophysiology[35], which is triggered by stress, illness, smoking, extended use of NSAIDs and excessive alcohol use and in this case ethanol which was used to induce the ulcer. The antioxidant assay result, therefore, showed a considerable scavenging activity[36] with a dosage-dependent pattern, i.e., increasing in activity with the rise in concentration. The inhibition (%) of ethanol crude extract of avocado seed's antioxidant activity increased by 1.420.02, 1.020.01, 1.670.02, 0.360.04 and 1.420.02.

The effects of the formulation of avocado seed extract on ethanol-induced stomach ulcers are displayed in Table 3 and 4. The antisecretory action from flavonoids plus astringent action and vasoconstriction effects from some of the phytochemicals shown in Table 1 could be the reason for the higher

inhibition (%) in the treatment with avocado seed extract+honey (44.12%)[37]. The wound healing effects of *Persea americana* have also been reported[38,39], which may also play a role in gastroprotection.

The result of the research shows that gastric ulcers induced by ethanol can be treated by traditional Higgi Kamwe poly herbal formulation virgin avocado (*Persea americana*) seed. It can also be applied in folk medicine especially in traditional Africa in the management of gastric ulcers. This plant is recommended for trials in higher animals in the management of gastric ulcers, however, its gastroprotective effects in humans are to be studied extensively.

CONCLUSION

In vitro and *in vivo* antioxidant activity of avocado seed extract was shown in this work. Gastric ulcers brought on by ethanol may be treated with oral administration of avocado seed extract once daily for seven days, but the formulation showed more substantial results. In addition to significantly reducing oxidative stress, avocado seed extract's antiulcer actions also boosted GSH, SOD and CAT activity, which decreased MDA lipid peroxidase. Additionally, the formulation therapy and avocado seed extract lowered iNOS levels, which may lessen inflammation in stomach ulcers. The decrease of oxidative stress and an increase in antioxidant activity may therefore play a role in the gastroprotective benefits of avocado seed extract.

SIGNIFICANCE STATEMENT

This study discovers the gastroprotective activity of Higgi Kamwe poly herbal formulation virgin avocado (*Persea americana*) seed against ethanol-induced gastric ulcers in rats that can be beneficial for the management of gastric ulcers particularly in folk medicine. This study will also help researchers to uncover the critical areas of medicinal plants, particularly *Persea americana* that many researchers were not able to explore in the management of gastric ulcers. Thus a new theory on the treatment and management of gastric ulcers may be arrived at.

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